Risk Factors for Malaria Infection Prevalence and Household Vector Density between Mass Distribution Campaigns of Long-Lasting Insecticidal Nets in Northern Tanzania

CURRENT STATUS: UNDER REVIEW

Malaria Journal  BMC

Jacklin Franklin Mosha
National Institute for Medical Research Mwanza Research Centre

jfmosha@yahoo.com Corresponding Author
ORCiD: https://orcid.org/0000-0001-7940-8903

Eliud Lukole
FBGU Naucno-issledovatel'skij institut onkologii imeni N N Petrova

J Derek Charlwood
London School Of Hygiene and Tropical Medicine Department of Disease Control

Alexandra Wright
London School Of Hygiene and Tropical Medicine Department of Disease Control

Olivia Bullock
London School Of Hygiene and Tropical Medicine Department of Disease Control

Alphaxard Manjurano
National Institute for Medical Research, Mwanza

William Kisinza
National Institute for medical research centre, Muheza

Franklin W Mosha
Kilimanjaro Christian Medical University College

Immo Kleinschmidt
London School of Hygiene and Tropical Medicine Department of Infectious Disease Epidemiology

Mark Rowland
London School Of Hygiene and Tropical Medicine Department of Disease Control
Abstract
Background Long lasting insecticide nets (LLIN) are the intervention most widely deployed in Sub-Saharan Africa to prevent malaria. Recent reports indicate selection of pyrethroid insecticide resistance is widespread in mosquito vectors. This paper explores the risk factors associated with malaria infection prevalence and vector density between mass distribution campaigns of LLIN in Northern Tanzania.

Methods A cross sectional malaria survey of 3,456 children was undertaken in 2014. Vector density was assessed using indoor light traps and outdoor tent traps. Anophelines were identified to species using PCR and tested for falciparum circumsporozoite protein. Logistic regression was used to identify household and environmental factors associated with malaria infection and vector density.

Results LLIN use was 27.7%. Only 16.9% of households had sufficient nets to cover all sleeping places. Malaria infection was independently associated with access to LLIN (OR: 0.57; 95%CI 0.34 – 0.98), age group, altitude and house construction quality. LLIN less than 2 years old were more protective than older LLIN (53% vs 65% prevalence of infection); however, there was no evidence that LLIN in good condition (hole index <65) were more protective than LLIN which were more holed. Independent risk factors for vector density were consistent with malaria outcomes and included altitude, wind, livestock, house quality, open eaves and LLIN access. Indoor collections comprised 4.6% Anopheles funestus and 95.4% An. gambiae of which 4.5% were An. arabiensis and 93.5% were An. gambiae s.s.

Conclusion Three years after the mass distribution campaign and despite top-ups, LLIN usage had fallen considerably. While children living in households with access to LLIN were at lower risk of malaria, infection prevalence remained high even among users of LLIN in good condition. While effort should be made to maintain high coverage between campaigns, distribution of just standard pyrethroid LLIN appears insufficient to prevent malaria transmission in this area of intense pyrethroid resistance.

Introduction
Long lasting insecticide nets (LLIN) and indoor residual spraying (IRS) are the main vector control
interventions deployed in Sub-Saharan Africa to control malaria [1]. According to the WHO, 69% of the estimated 663 million malaria cases averted during the 15 years after the millennium were attributed to the use of LLIN. Despite the increase in LLIN access, in recent years malaria has increased in several Sub-Saharan countries [2]. A similar trend has emerged in Tanzania [3] where malaria remains a serious public health problem responsible for 40% of all hospital outpatient visits. Insecticide treated nets that required annual re-treatment were first distributed to pregnant women and infants through antenatal clinics in 2004. In 2008 LLINs that did not require re-treatment were distributed through mass campaigns targeting children under five years of age [4]. From 2011 onwards, distribution of LLINs was scaled up to cover the population, including adults, not previously reached. Approximately 18 million LLINs were distributed during this ‘universal coverage campaign’ which led to an increase in net ownership (households owning at least one net) to 92% and net usage to 72% [5].

ITNs are the most effective when usage is high to provide community protection [6]. The challenge, therefore, is to maintain high coverage between the mass distribution that occurs every 3-4 years [7]. Between universal coverage campaigns, LLIN coverage may be sustained by routine distributions through school and health facilities services [8, 9]. The insecticidal effectiveness of WHO-recommended LLIN are shown to last for at least three years of use. However, the physical durability of the textile may be shorter than 3 years depending on usage practices and wear-and-tear. LLIN that are still insecticidal may be discarded due to accumulation of holes long before the end of the net’s insecticidal life [10].

The gains arising from increased usage of LLINs might also be undermined by the development of insecticide resistance among malaria vectors [11]. Resistance to pyrethroids is spreading across Africa, and has been reported in various districts of Tanzania [12]. While LLIN provided long term protection when mosquito vector populations in Tanzania were fully susceptible, effectiveness may become compromised in areas with high levels of pyrethroid resistance once LLIN start to deteriorate and develop holes [13, 14].

This paper explores whether LLIN distributed during and after the universal coverage campaign of
2011 provided protection in an area of intense pyrethroid resistance. It also explores other risk factors for malaria infection and vector density in NW Tanzania.

Material And Methods

1.1 Study area

Cross sectional prevalence and entomological surveys were conducted during baseline of a cluster randomised controlled trial (RCT) assessing PBO Insecticide treated Nets and IRS with Actellic (pirimiphos Methyl) [15]. The study was conducted in 40 villages, divided in 48 clusters for the purpose of the RCT, in Muleba district (1° 45’ S 31° 40’ E), North West Tanzania [15]. The study area covers 944 km², 29,000 households and an estimated population of 135,000 and is situated between 1,100 and 1,600 m above sea level [15]. There are two rainy seasons: the short rains in October-December with an average monthly rainfall of 160 mm and the long rains in March-May with an average monthly rainfall of 300 mm[16]. Malaria transmission occurs throughout the year and peaks after the rainy seasons. Malaria prevalence infection in children under 15 years of age in the study area was 37% in July 2011, higher than the overall prevalence of 23% in the district during the same period[17].

Anopheles gambiae s.s. resistant to pyrethroids and An.arabiensis were the only vectors found in 2011 [18]. Permethrin treated LLINs (Olyset Net) were distributed through universal coverage campaign in 2011. After the campaign in 2011, net ownership was 90.8% and net usage was 55.7% [16]. Bendiocarb IRS was sprayed in 2011 and 2013, with house coverage over 90% in 2011 [19]. In the years following the 2011 campaign, additional LLIN were distributed though government clinics to protect pregnant women and children under 5 years of age.

1.2 Data Collection

Cross sectional malaria parasitaemia and household surveys

The prevalence survey was carried out from September to October 2014 [15]. All households in the study area were mapped using Global Positioning System (GPS) hand-held units (Garmin Legend etrex) and Expert GPS v3.8 (TopoGrafix) software. A total of 2880 households (60 households per cluster) were randomly selected amongst the census list to participate in a household survey. Up to three children aged 6 months to 14 years per house were randomly selected.
On obtaining household consent, a questionnaire was administered and data entered into a personal digital assistant (PDA, HP IPAQ 114 Classic Handheld) on number of residents, socio economic status, house construction and quality, education, access to and use of LLIN and other malaria preventive measures, and presence of livestock.

Selected children were given a coupon and asked to present the following day for parasitological testing. Plasmodium infection was assessed by a Rapid Diagnostic Test (CareStart RDTs; HRP2/pLDH, (pf/PAN), DiasysS Workingham, UK) and haemoglobin levels measured using HemoCue Hb 201+ (Aktiebolaget Leo Diagnostics). Results were initially recorded on paper forms and double entered into a digital database and checked for consistency.

In the same households selected for cross sectional survey, 20% of LLIN were inspected for assessment of physical condition and hole index determined according to WHO guidelines [20]. Nets were draped over a frame and holes counted, classified into categories of: less than 2 cm (size 1); 2–10 cm (size 2); >10–25 cm (size 3) and > 25 cm (size 4), and proportional hole index (pHI) as an estimate of hole area was calculated. For purposes of analysis the LLIN were further categorized into three groups: Good condition (pHI 0–64), moderately damaged (pHI 65–642) and badly damaged (pHI > 643)[21].

Entomological monitoring

From November 2014 to January 2015, indoor mosquito collections were conducted using CDC light traps in 14 to 21 randomly selected houses per cluster for one night only. The light traps were installed at the foot of a bed occupied by a family member sleeping under a LLIN. Outdoor night collections were performed using an adapted furvela tent traps [22] placed near to two houses selected for indoor light trap collections. Further information on house structure, livestock, and LLIN access and usage was collected. All mosquitoes collected were morphologically identified to species [23]. Up to 20 Anophelines per house were subsequently tested for Plasmodium falciparum circumsporozoite protein (Pf-CSP) [24]. A sub-sample of An.gambiae s.l. was tested using real time PCR Taq Man assay to distinguish between the two sibling species An.gambiae s.s. and An.arabiensis [25]. Species composition of An.funestus complex was established using conventional PCR [26].
1.3 Statistical analysis

Statistical analysis was performed using STATA (version 12, College Station, TX, USA). Random effects logistic regression models were used to determine possible risk factors for malaria infection determined by RDT in children 0.5 to 14 years old adjusting for correlation of malaria infection within clusters.

Potential household and personal risk factors were explored: age, gender, individual net use, education of household head, social economic status (SES), quality of house construction and presence of open eaves, number of people per sleeping room (a proxy for crowding), LLIN ownership and access, indoor residual spraying (IRS) in 2013, presence and proximity of livestock.

The data were cleaned and coded and any variables which were nonlinearly related to the outcome were categorized. Variables were created for improved housing structure, individual net use, household net access (which is defined as number of houses with adequate LLIN to cover all sleeping places), household crowding and social economic status, detail of all variables is indicated in Table 1.
Table 1
Definition of variables

| Indicators                                | Description                                                                 |
|-------------------------------------------|-----------------------------------------------------------------------------|
| Age                                       | Age was categorized into three groups: 0.5–1 year, 2–4 years and 5–14 years. |
| Altitude                                  | Altitude was categorized into three groups with the following cutoff: Low altitude ranging (1128–1264), Medium altitude ranging (1265–1352), High altitude ranging (1353–1654) |
| Social economic status                    | SES was created as a weighted sum of data on household possessions and utilities, using principal components analysis and divided into tertiles. |
| Improved housing structure                | Is a binary variable where individuals are coded as ‘yes’ if they have at least one of the following intact ceiling, brick walls, plastered walls, full cement floors and iron roofs. |
| Household crowding                        | Number of people sleeping per room. Number of sleepers per room was dichotomized into groups with 1, and > 1 person sleeping in the room |
| Eaves of the house                        | Binary variable ‘open’ if household has open eaves or ‘closed’ if household has no open eaves |
| LLIN ownership                            | Binary variable ‘yes’ if household owns at least one LLIN |
| Individual LLIN condition                 | Physical condition of the net. The variable is categorized in. 1/ use good condition net (PHI 0–64), 2/ use moderate condition net (PHI 65–642) and 3/ use torn net (PHI > 643) |
| Individual LLIN usage                     | This is a binary variable indicating whether individual is sleeping under a net or not |
| Household net access                      | Binary variable ‘yes’ if household owns enough net to cover every sleeping place. Which is number of LLIN owned (regardless if used or not) divided by the number of sleeping places |
| Indoor residual spraying (IRS)            | Binary variable ‘yes’ if household was sprayed in 2013 |
| Livestock present                         | Binary variable ‘yes’ if livestock are owned |
| Animal kept inside the house               | Binary variable ‘yes’ if animals are sleeping inside the household, this included goats and sheep |
| Level of education of head of household   | Binary variable ‘yes’ if the head of household has at least completed primary education |
| Moderate to severe Anaemia                 | Defined as haemoglobin < 8 g/dL |

All variables were analysed individually for an association with malaria infection (outcome) using random effects logistic regression. Random effect model was used to account for village cluster and household level correlation. All potential risk factors showing evidence for a possible association with malaria infection (p < 0.1) were included in the preliminary main effect multivariate logistic regression model. A forward stepwise method was used, retaining any risk factors showing an association (LRT test p < 0.05). No a priori interaction terms were identified. Multi-collinearity was assessed for by removing parameters one by one from the model and examining the standard errors of the remaining parameters. If this caused the standard error of one of the remaining parameters to change by > 10% this was defined as multi-collinearity and one of the parameters removed.

A similar approach was adopted to assess risk factors for indoor vector density. Because vector
density count data was skewed and often over dispersed, a negative binomial regression analysis was used. The model estimated a density rate ratio (DRR) as the measure of effect. Univariate regression of each risk factor was performed initially and the forward step-wise method was used to build a multivariate model.

Results
Household and study participants
The total number of households which were selected to participate in the baseline survey was 2880. Of these 2270 (78.8%) consented to participate, 276 (9.6%) failed to meet the inclusion criteria (no children < 15 years), 13 (0.5%) refused and 11.1% were excluded for other reasons (Fig. 1). Of the 6,918 children aged between 0.5 and 14 years, 4388 (63.4%) were selected for inclusion and of these 3871 participated in the clinical survey, 10 children had missing RDT results and 517 (11.8%) children did not turn up for testing. Therefore, of the total children selected 3,861 (88%) were included in the analysis (Fig. 1).

Of the 1008 households selected for indoor entomological collection 770 participated, and of 144 selected for outdoor collection 120 participated (Fig. 1). Altitude of study households ranged from 1128 to 1654 meters above sea level. Around 76.8% of households were constructed with mud walls and floors, and 61.2% of houses had open eaves. Most households (94.3%) relied on farming or fishing and selling cash crops for income. Most individuals came from houses with livestock (73.1%) and 33.5% of these kept animals inside the household. 73.3% of heads of households had attended primary school (Table 2).

Table 2
Factors associated with malaria infection in the univariate and multivariate analysis

| Altitude | Number of Children | % with malaria infection | Univariate | Multivariate |
|----------|--------------------|--------------------------|------------|-------------|
| Low  (1128–1264) | 1,300 | 74.7% | 1 | |
| Medium  (1265–1352) | 1,291 | 68.1% | 0.38 (0.28–0.50) | 0.001 | 0.78 (0.42–1.43) | 0.416 |
| High  (1351–1654) | 1,266 | 51.0% | 0.14 (0.10– 0.20) | 0.001 | 0.27 (0.14 < 0.001) |
| Age                  | < 1 year | 2 to 4 years | 5 to 14 years | 5+ years |
|----------------------|----------|--------------|---------------|---------|
| Population          | 384 (50.5%) | 1,062 (63.0%) | 2,414 (67.7%) | 2,354 (49.1%) |
| Age                  | 1.86 (1.44-2.41, < 0.001) | 2.52 (1.99-3.20, < 0.001) | 2.52 (1.99-3.20, < 0.001) | 2.52 (1.99-3.20, < 0.001) |
| Age                  | 2.59 (1.31-5.11) | 3.82 (1.89-7.72) | 3.82 (1.89-7.72) | 3.82 (1.89-7.72) |

### Improved Housing Structure

| Improved Housing Structure | No | Yes | Risk Ratio (95% CI) |
|----------------------------|----|-----|---------------------|
| Age < 1 year               | 589 (76.9%) | 3,272 (62.5%) | 0.32 (0.22-0.48, < 0.001) |
| Age 2 to 4 years           | 1,261 (72.6%) | 101 (62.5%) | 1.07 (0.98-1.07) |
| Age 5 to 14 years          | 1,264 (64.3%) | 1,026 (67.6%) | 0.98 (0.75-1.01) |
| Age 5+ years               | 1,258 (57.6%) | 1,026 (67.6%) | 0.98 (0.75-1.01) |

### Socioeconomic Status

| Socioeconomic Status | Poorest | Middle | Least poor |
|----------------------|---------|--------|------------|
| Age < 1 year         | 1,261 (72.6%) | 1,264 (64.3%) | 1,258 (57.6%) |
| Age 2 to 4 years     | 1.07 (0.98-1.07) | 0.98 (0.75-1.01) | 0.98 (0.75-1.01) |
| Age 5 to 14 years    | 0.98 (0.75-1.01) | 0.98 (0.75-1.01) | 0.98 (0.75-1.01) |
| Age 5+ years         | 0.98 (0.75-1.01) | 0.98 (0.75-1.01) | 0.98 (0.75-1.01) |

### Adequate LLIN access

| Adequate LLIN access | No | Yes |
|----------------------|----|-----|
| Age < 1 year         | 3,167 (66.0%) | 643 (58.8%) |
| Age 2 to 4 years     | 1.01 (0.88-1.17) | 0.57 (0.42-0.98) |
| Age 5 to 14 years    | 1.01 (0.88-1.17) | 0.57 (0.42-0.98) |
| Age 5+ years         | 1.01 (0.88-1.17) | 0.57 (0.42-0.98) |

### Individual net age

| Individual net age | < 2 years | 2-3 years | 4 years and more | 5+ years |
|--------------------|----------|-----------|------------------|---------|
| Age < 1 year       | 1,869 (66.0%) | 1,870 (63.4%) | 1,870 (63.4%) | 1,870 (63.4%) |
| Age 2 to 4 years   | 1.01 (0.88-1.17) | 1.01 (0.88-1.17) | 1.01 (0.88-1.17) | 1.01 (0.88-1.17) |
| Age 5 to 14 years  | 1.01 (0.88-1.17) | 1.01 (0.88-1.17) | 1.01 (0.88-1.17) | 1.01 (0.88-1.17) |
| Age 5+ years       | 1.01 (0.88-1.17) | 1.01 (0.88-1.17) | 1.01 (0.88-1.17) | 1.01 (0.88-1.17) |

### Sex

| Sex     | Male | Female |
|---------|------|--------|
| Age < 1 year | 1,869 (66.0%) | 1,870 (63.4%) |
| Age 2 to 4 years | 1.01 (0.88-1.17) | 1.01 (0.88-1.17) |
| Age 5 to 14 years | 1.01 (0.88-1.17) | 1.01 (0.88-1.17) |
| Age 5+ years | 1.01 (0.88-1.17) | 1.01 (0.88-1.17) |

### Eaves of the house

| Eaves of the house | Open | Closed |
|--------------------|------|--------|
| Age < 1 year       | 2,334 (69.2%) | 1,478 (57.9%) |
| Age 2 to 4 years   | 1.09 (0.51-0.69, < 0.001) | 1.09 (0.51-0.69, < 0.001) |
| Age 5 to 14 years  | 1.09 (0.51-0.69, < 0.001) | 1.09 (0.51-0.69, < 0.001) |
| Age 5+ years       | 1.09 (0.51-0.69, < 0.001) | 1.09 (0.51-0.69, < 0.001) |

### Livestock present

| Livestock present | No | Yes |
|-------------------|----|-----|
| Age < 1 year       | 1,026 (67.6%) | 2,786 (63.7%) |
| Age 2 to 4 years   | 1.01 (0.81-1.17) | 0.79 (0.65-0.91) |
| Age 5 to 14 years  | 1.01 (0.81-1.17) | 0.79 (0.65-0.91) |
| Age 5+ years       | 1.01 (0.81-1.17) | 0.79 (0.65-0.91) |

### Animals kept inside the house

| Animals kept inside the house | No | Yes |
|------------------------------|----|-----|
| Age < 1 year                 | 1,851 (63.5%) | 935 (63.6%) |
| Age 2 to 4 years             | 1.07 (0.81-1.17) | 0.97 (0.81-1.17) |
| Age 5 to 14 years            | 1.07 (0.81-1.17) | 0.97 (0.81-1.17) |
| Age 5+ years                 | 1.07 (0.81-1.17) | 0.97 (0.81-1.17) |

### Head of household attended school

| Head of household attended school | No | Yes |
|----------------------------------|----|-----|
| Age < 1 year                     | 1,007 (71.4%) | 935 (63.6%) |
| Age 2 to 4 years                 | 1.07 (0.81-1.17) | 0.97 (0.81-1.17) |
| Age 5 to 14 years                | 1.07 (0.81-1.17) | 0.97 (0.81-1.17) |
| Age 5+ years                     | 1.07 (0.81-1.17) | 0.97 (0.81-1.17) |
| Yes (any schooling) | 2,791 | 62.4% | 0.74 | 0.62–0.88 | 0.001 |
|--------------------|-------|--------|------|-----------|-------|
| IRS 18 months before |       |        |      |           |       |
| No                 | 2,798 | 67.0%  | 1    | 0.70–1.01 | 0.06  |
| Yes                | 979   | 59.1%  | 0.84 |           |       |
| Household owns at least 1 net |       |        |      |           |       |
| No                 | 1,165 | 69.1%  | 1    |           |       |
| Yes                | 2,647 | 62.6%  | 0.71 | 0.60–0.83 | < 0.001 |
| Individual LLIN usage |       |        |      |           |       |
| Don’t use net Use Net | 2,276 | 66.4%  | 1.0  | 0.50–0.94 | 0.018 |
| Use Net            | 1,045 | 61.2%  | 0.69 |           |       |
| Individual LLIN Condition |       |        |      |           |       |
| Use good condition net (PHI > 64) | 154   | 68.2%  | 1    | 0.28–1.49 | 0.307 |
| Use moderate condition net (PHI 65–642) | 206   | 61.7%  | 0.65 | 0.34–1.77 | 0.544 |
| Use torn net (PHI > 643) | 221   | 63.8%  | 0.77 |           |       |
### Table 3
Risk factors associated with vector density in the univariate and multivariate models

|                     | Number of HH | Indoor vector density | Univariate | Multivariate |
|---------------------|--------------|-----------------------|------------|--------------|
|                     |              |                       | IRR        | 96%CI        | p-value      | IRR         | 96%CI        | p-value      |
| **Altitude**        |              |                       |            |              |              |            |              |              |
| 1133–1242           | 192          | 63.2                  | 1          | < 0.001      | 1            | < 0.001     |              |              |
| 1243–1302           | 191          | 22.6                  | 0.63       | 0.49–0.80    | 0.58         | 0.46–0.74   |              |              |
| 1303–1373           | 192          | 6.8                   | 0.51       | 0.40–0.66    | 0.44         | 0.33–0.58   |              |              |
| 1374–1651           | 191          | 5.7                   | 0.39       | 0.29–0.51    | 0.35         | 0.27–0.46   |              |              |
| **Wind during collection** |              |                       |            |              |              |            |              |              |
| None/light          | 433          | 28.2                  | 1          | 0.007        | 1            | < 0.001     |              |              |
| Moderate/Strong     | 335          | 19.9                  | 0.77       | 0.64–0.93    | 0.69         | 0.57–0.83   |              |              |
| **Livestock**       |              |                       |            |              |              |            |              |              |
| No                  | 267          | 17.5                  | 1          | 0.159        | 1            | 0.001       |              |              |
| Yes                 | 502          | 28.3                  | 1.13       | 0.95–1.35    | 1.35         | 1.12–1.62   |              |              |
| **House construction** |              |                       |            |              |              |            |              |              |
| Poor construction   | 579          | 29.3                  | 1          | 0.016        | 1            | 0.072       |              |              |
| Better construction | 183          | 10.1                  | 0.78       | 0.63–0.95    | 0.83         | 0.67–1.02   |              |              |
| **House using LLIN**|              |                       |            |              |              |            |              |              |
| No                  | 264          | 22.9                  | 1          | 0.006        | 1            | 0.004       |              |              |
| Yes                 | 505          | 25.4                  | 0.79       | 0.66–0.93    | 0.78         | 0.66–0.92   |              |              |
| **Number of rooms** |              |                       |            |              |              |            |              |              |
| No                  | 770          | 24.9                  | 0.89       | 0.84–0.95    | 0.90         | 0.84–0.96   | 0.001        |              |
| Yes                 |              |                       |            |              |              |            |              |              |
| **Household cooks inside the house** |              |                       |            |              |              |            |              |              |
| No                  | 620          | 23.3                  | 1          | 0.010        |              |            |              |              |
| Yes                 | 149          | 29.6                  | 1.31       | 1.07–1.59    |              |            |              |              |
| **Total time house has been sprayed** |              |                       |            |              |              |            |              |              |
| No                  | 756          | 24.9                  | 0.94       | 0.90–0.99    | 0.94         | 0.90–0.99   | 0.011        |              |
| Yes                 |              |                       |            |              |              |            |              |              |
| **Animal inside the house** |              |                       |            |              |              |            |              |              |
| No                  | 308          | 29.2                  | 1          | 0.933        |              |            |              |              |
| Yes                 | 196          | 26.5                  | 1.01       | 0.82–1.25    |              |            |              |              |
| **Rain during collection** |              |                       |            |              |              |            |              |              |
| None                | 435          | 23.7                  | 1          | 0.446        |              |            |              |              |
| Light               | 164          | 37.1                  | 0.98       | 0.78–1.24    |              |            |              |              |
| Heavy               | 169          | 14.5                  | 1.15       | 0.92–1.44    |              |            |              |              |
| **Eaves of the house** |              |                       |            |              |              |            |              |              |
| Closed              | 324          | 22.9                  | 1          | 0.071        |              |            |              |              |
| Open                | 445          | 25.7                  | 1.17       | 0.99–1.38    |              |            |              |              |

Almost all nets which were used were LLINs (96.8%). The proportion of individuals who reported using a net the night before was 27.1%. The proportions of nets declared to be less than 2 years old were
37.1%, those 2–3 years old was 31.3% and those over 3 years old was 31.4%. Many of the nets had been received via clinic-based distribution during the time since the last UCC. Only 26% of individuals slept under nets of good condition, 35% slept under nets of moderate condition and 38% under nets which were badly torn. Net access was very low; only 16.9% of individuals were residing in households with enough nets to cover all sleeping places. About 25.9% of the households reported to be sprayed with insecticide in the year before (Table 2).

Prevalence and risk factors for malaria infection

Overall malaria infection prevalence (all Plasmodium) was 64.8% (95% CI 61.8–67.8) in children aged 6 months to 14 years old. The prevalence of clinical malaria, defined as fever (ear temperature, ≥ 37.9 °C) and any Plasmodium infection by RDT was 6.8% (95% CI 5.5–8.1). Only 2.9% (114) of tested children had fever and among those who had fever 86% had a positive test result. The prevalence of moderate or severe anaemia in children under five was 19.5% (95%CI 17.9–21.4). Among anaemic children 88.9% (95%CI 85.5–92.2) had malaria infection. The odds of moderate-severe anaemia was six times higher if the child had malaria infection (OR 6.2, 95% CI 5.0–7.6).

In the univariate analysis the odds of malaria infection was lower with living at higher altitudes, in children in younger age groups, with better education, improved house structure, closed eaves, greater wealth, presence of livestock, net ownership, adequate access to LLIN, use of LLIN and with newer LLIN (Table 2).

In the multivariate model, malaria infection was independently associated with altitude, age, quality of housing structure, and adequate net access per sleeping place.

The odds of malaria infection decreased with increasing altitude. Individuals living at high altitude had much lower odds of malaria infection compared to those who were living at lower altitude (OR 0.18 95%CI; 0.13–0.26).

The odds of malaria infection increased with age. Children living in improved housing structure had lower odds of malaria infection compared to those who were living in unimproved housing (OR 0.27, 95%CI 0.13–0.54). Households living with a gap between wall and eaves showed a strong association with malaria infection compared to those individuals who were living in houses with closed eave gaps
Adequate net access was associated with decreased odds of malaria infection. Individuals who were living in households with adequate net access per sleeping place were better protected from malaria infection (59% vs 66%, OR 0.57; 95% CI 0.34–0.98, P = 0.042). LLIN access and LLIN usage were closely correlated, hence LLIN usage as a risk factor dropped out in the multivariate analysis. Age of LLIN was also significant, with only LLIN less than two years of age showing evidence of protection (53% vs 65%, P = 0.004). There was no association between malaria protection and the hole index of LLIN over the WHO LLIN categories of good condition (32% infection prevalence, 49/154) to ‘unserviceable’ condition (36%, 80/221).

None of the other factors were associated with malaria infection once adjusted for the other factors in the model. This included sex, wealth, livestock, household head education, IRS spraying 18 months earlier, household owning at least one LLIN, and usage of LLIN of different conditions based on hole index categories.

Mosquito fauna and risk factors for vector density
A total of 29,401 and 2,668 mosquitoes were collected in 770 indoor light trap collections and in 120 outdoor tent trap collections. Anophelines comprised 64% of the indoor collections and 57% of the outdoor collections. Mean vector density per collection night was 24.5 indoors and 12.7 outdoors. Of the Anophelines collected 4.6% were identified as An.funestus s.l. and the remaining were An.gambiae s.l. Among the 969 An.gambiae s.l. identified to species by PCR 93.5% were An.gambiae s.s., 4.5% were An.arabiensis, and 2.2% did not amplify. There was no evidence of difference in ratio of An.gambiae s.s. to An. arabiensis between indoor and outdoor collections ($\chi^2$ 3.5, p = 0.18). Of the 289 An.funestus identified by PCR 81.7% were An.funestus s.s., 15.6% An.leesoni, 1.0% An.parensis and 1.7% did not amplify. A subsample of 4,311 specimens was tested for CSP. The sporozoite rate was 4.7%. Both An.funestus and An.gambiae s.s were found positive for CSP. The entomological inoculation rate was 0.34 (95%CI: 0.20–0.49) infectious bites per person-night during the period of collection, and this rate varied from 0 to 2.7 between village clusters. Resistance testing using WHO susceptibility tests in 2015 indicated pyrethroid resistance frequency of 8.8% for An. gambiae and
54.5% for An. funestus and a resistance intensity of 38 for An. gambiae s.l. and 34 for An. funestus [15].

Altitude of households selected during the entomology survey ranged from 1,133 to 1,651 m, with a mean of 1,303 m. It rained during 43.4% of collections and the wind was moderate or strong during 43.6% of collections. 34.7% of households did not have animals. For those that owned animals, 39% kept them indoor. 50% of families had poorly constructed houses and 24% had improved houses. 57.9% of houses had open eaves. The median number of rooms was 5 per house and median household size was 5 persons. Only 19.4% of families cooked inside the house. Houses had been sprayed 3 times since the first IRS campaign in 2007. An average of 65.7% of households were using at least one LLIN.

The univariate analyses showed evidence for negative associations between vector density and improved housing, number of rooms, households using at least one LLIN and number of times the house had been sprayed. Cooking inside the house was associated with higher vector densities. Altitudes above 1242 meters was associated with a decreased risk of indoor vectors, whereas light wind or no wind during collection was associated with increased risk.

House structure had an IRR of 0.75 (95% CI 0.60–0.90), indicating a 25% decreased risk of vector exposure in improved housing. Using at least one LLIN in the household and several times sprayed was associated with reduced vector density. There was no evidence of association with rain during collection night, open eaves or animals present inside the house.

The multivariate model retained five risk factors as significant: altitude, wind during collection, presence of livestock, house construction and LLIN usage. Number of rooms, showed co-linearity with house construction and, was therefore removed from the model. There was strong association with vector density, altitude and wind. Vector density was negatively associated with increasing altitude, being 65% less at the higher altitudes and with nights with moderate or strong winds. Improved housing structure was associated with reduced vector density whereas presence of livestock was associated with higher vector density. Net usage showed an association with density after adjusting for other factors. The variables that showed no association with vector density in the multivariate
model were: history of IRS, cooking indoors and presence of open eaves.

Discussion

This study investigated the effects of LLIN distributed during and after the universal coverage campaign of 2011 in an area of intense pyrethroid resistance in northwest Tanzania. The aim was to explore the effects of net deterioration and other risk factors for malaria infection and vector density. The assessment was carried out 3.5 years after the campaign by which time many of the original LLIN had developed holes or been replaced by newer ones. Malaria prevalence was over 60% in the study area in 2014, while the estimated number of infective bites per day was 0.34. These rates were much higher than the rates observed in the same area in 2011 shortly after the campaign [17]. An increase in malaria infection was also observed in other regions. After the initial decrease in malaria infection to 9% (ranging 1–25%), malaria prevalence increased to 14% (ranging from 1–41%) across seven regions of the country by 2014 [3]. Various factors may have contributed to the rebound of malaria in the study area: these include reduced LLIN effectiveness caused by increasing insecticide resistance, deterioration of the LLINs’ physical condition, and reduced LLIN coverage and usage.

LLIN ownership had decreased from 90.8% after the universal coverage campaign to 69% in 2014 [16]. LLIN usage had also decreased from 55.7–27.7%. While good household access to LLIN and individual LLIN use were both associated with reduced risk of malaria infection in the risk factor analysis, the reduction in LLIN coverage and usage after the campaign would have contributed to the observed increase in malaria prevalence in 2014. The reported reasons for not using LLINs included: LLINs becoming torn over time and the poor access to new LLINs, which is consistent with findings from other studies [27]. Even with good access to LLIN malaria infection prevalence was still 59%, indicating loss of effectiveness due to other reasons.

About 53% of the nets which were still in use had holes and around 35% of the nets were badly torn (pHI > 643). Previous studies have indicated that personal and community effectiveness of LLINs declines over time due to factors such as net coverage, aging net damage and insecticide resistance [13, 14]. Protection against highly resistant mosquitoes is reduced when LLIN develop holes because such mosquitoes are more able to penetrate the net via the holes, bite and blood feed [13, 14]. In the
present study LLIN use and LLIN access were both associated with malaria protection. However, the level of protection was not associate with hole index category. It is possible the sample size was underpowered; only 581 (15%) of the 3861 children tested for malaria had their nets assessed for hole index. However, even a small degree of holing might be sufficient to for a LLIN to lose its capacity to protect when pyrethroid resistance reaches high frequency and intensity [13, 14]. There was significantly greater protection from malaria of LLIN less than two years of age; such nets would have higher concentrations of pyrethroid and were potentially more repellent to insecticide resistant mosquitoes. It would appear that once nets are more than two years of age they lose some capacity to protect against mosquitoes that are highly resistant. This observation should be kept in perspective; the actual difference in infection prevalence was between 53% in users of LLIN < 2 years old and 64% in LLIN > 2 years old. Standard LLIN were simply no longer adequately protective against this highly resistant vector population.

Other main risk factors for malaria infection prevalence in children 6 months to 14 years included living in area of lower altitude, in poorly constructed houses and with open eaves, being 5 to 14 years old, and coming from a lower socio-economic category. The same risk factors are also associated with higher vector density. These findings are consistent with other studies [28]. Some studies have reported an increased parasitaemia in children living at low altitudes compared to those who lived at high altitudes [29]. High altitudes are associated with lower temperatures, which have an impact on growth of mosquito larvae and therefore mosquito abundance [30]. Lower temperature increases the interval between biting/feeding cycles and also retards the rate of malaria parasite development within the mosquitoes.

The observed increased risk of malaria infection in people living in poor housing is consistent with previous studies [31, 32]. Living in improved housing and in houses with closed eaves was also associated with reduced vector density. A recent systematic review and meta-analysis indicated that improved housing is protective against malaria infection by 50% [32]. House designs with open eaves and unscreened windows have been associated with increased risk for malaria infection, as a gap in the eaves is an entry point for malaria vectors [33].
Increased risk of malaria infection in older children (the 5–14 year age group) compared to younger children seen in this study is a trend which has been observed in other studies conducted in Tanzania [34]. Older children have higher exposure to mosquito bites as they have more active outdoors at night and more exposed to mosquito bites compared to younger age groups. In addition, due to the acquired anti-malarial immunity in older children, most have persistent asymptomatic malaria infections that are less likely to be treated, unlike younger children [35]. Lower LLIN usage has been also observed in this older age groups [36].

Household that own livestock had higher vector density, but this did not translate in increased malaria infection prevalence when adjusted for other co-variates. This suggests that livestock may attract Anopheles but did not lead to an increase in human biting rate. The impact that livestock have on malaria and vector density is complex. Some studies indicate that livestock provides zooprophylactic protection [37], while other studies showed that livestock increases risk [38]. It depends on how the livestock are deployed within the household compound relative to humans and the anthophilic/zoophilic plasticity of the vector species. Further study of vector composition between household with or without livestock would be required to better understand the difference in the present study.

**Conclusion**

Three years and half after the universal coverage campaign LLIN usage had decreased considerably despite some limited distribution of new LLIN in the interim. Children living in households with full access to LLIN per sleeping place were at lower risk of malaria; however, malaria prevalence in this group remained high. Standard pyrethroid-only LLIN were no longer sufficiently protective even when in good condition and relatively new. More efforts should be made to maintain high coverage of effective LLIN between campaigns. Other malaria risk factors identified were consistent with studies in other areas or countries. National malaria control programs should consider targeting interventions to high risk groups such as children up to 15 years of age through school net distribution. House improvements were identified as another factor for consideration.

**Declarations**
Ethical approval and consent to participants

The trial was approved by the ethics review committees of the Kilimanjaro Christian Medical College, the National Institute for Medical Research Tanzania and the London School of Hygiene and Tropical Medicine. Written informed consent was obtained from all participants or the parents/guardians if under 15 years old.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Financial Support

This study was funded under the Joint Global Health Trials Scheme by DFID, Medical Research Council and, Wellcome Trust (Grant Ref: MR/L004437/).

Availability of Data and Material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors’ contribution

NP, FWM, IK, MR, JFM designed the study. JFM, NP EL, JDC, AW, AM implemented the study. JFM and NP analyzed the data. JFM, NP, IK, MR interpreted the data. JFM and NP wrote the first draft of the manuscript. MR and IK critically revised the manuscript for important content. EL, JDC, AW, AM, FWM, WK, IK, and MR in particular revised the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements

The authors express their sincere thanks to all colleagues and staff from Kilimanjaro Christian Medical University College in Muleba and Moshi, and from the National Institute of Medical Research Mwanza involved in the project for their hard work. We acknowledge the assistance provided by staff of the Muleba District Office, the village and hamlet leaders. We thank, NMCP, USAID/PMI for their support. This study was funded under the Joint Global Health Trials Scheme by DFID, Medical Research Council and, Wellcome Trust (Grant Ref: MR/L004437/).

References

1. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, Battle K, Moyes CL, Henry A, Eckhoff PA, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature. 2015;526:207-11.
2. WHO. World Malaria Report 2018. 2018.
3.
NBS. National Bureau of Statistics, Demographic and Health Survey and Malaria Indicator Survey 2015-2016. Dar Es Salaam. Tanzania: National Bureau of Statistics; 2016.

4. Bonner K, Mwita A, McElroy PD, Omari S, Mzava A, Lengeler C, Kaspar N, Nathan R, Nggeba J, Mtung’e R, Brown N. Design, implementation and evaluation of a national campaign to distribute nine million free LLINs to children under five years of age in Tanzania. Malar J. 2011;10:73.

5. Renggli S, Mandike R, Kramer K, Patrick F, Brown NJ, McElroy PD, Rimisho W, Msengwa A, Mnzava A, Nathan R, et al. Design, implementation and evaluation of a national campaign to deliver 18 million free long-lasting insecticidal nets to uncovered sleeping spaces in Tanzania. Malar J. 2013;12:85.

6. Levitz L, Janko M, Mwandagalirwa K, Thwai KL, Likwela JL, Tshefu AK, Emch M, Meshnick SR. Effect of individual and community-level bed net usage on malaria prevalence among under-fives in the Democratic Republic of Congo. Malar J. 2018;17:39.

7. Koenker HM, Yukich JO, Mkindi A, Mandike R, Brown N, Kilian A, Lengeler C. Analysing and recommending options for maintaining universal coverage with long-lasting insecticidal nets: the case of Tanzania in 2011. Malar J. 2013;12:150.

8. Kramer K, Mandike R, Nathan R, Mohamed A, Lynch M, Brown N, Mnzava A, Rimisho W, Lengeler C. Effectiveness and equity of the Tanzania National Voucher Scheme for mosquito nets over 10 years of implementation. Malar J. 2017;16:255.

9. Acosta A, Obi E, Ato Selby R, Ugot I, Lynch M, Maire M, Belay K, Okechukwu A, Inyang U, Kafuko J, et al. Design, Implementation, and Evaluation of a School Insecticide-Treated Net Distribution Program in Cross River State, Nigeria. Glob Health Sci Pract. 2018;6:272-87.

10. Gnanguenon V, Azondekon R, Oke-Agbo F, Beach R, Akogbeto M. Durability assessment results suggest a serviceable life of two, rather than three, years for the current long-lasting insecticidal (mosquito) net (LLIN) intervention in Benin. BMC Infect Dis. 2014;14:69.

11. Ranson H, N’Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol. 2011;27:91–8.

12. Kabula B, Tungu P, Malima R, Rowland M, Minja J, Wililo R, Ramsan M, McElroy PD, Kafuko J, Kulkarni M, et al. Distribution and spread of pyrethroid and DDT resistance among the Anopheles gambiae complex in Tanzania. Med Vet Entomol. 2014;28:244–52.

13. Ochomo EO, Bayoh NM, Walker ED, Abongo BO, Ombok MO, Ouma C, Githeko AK, Vulule J, Yan G, Gimnig JE. The efficacy of long-lasting nets with declining physical integrity may be compromised in areas with high levels of pyrethroid resistance. Malar J. 2013;12:368.

14. Asidi A, N’Guessan R, Akogbeto M, Curtis C, Rowland M. Loss of household protection from use of insecticide-treated nets against pyrethroid-resistant mosquitoes, benin. Emerg Infect Dis. 2012;18:1101–6.

15. Protopopoff N, Mosha JF, Lukole E, Charlwood JD, Wright A, Mwalimu CD, Manjurano A, Mosha FW,
Kisinza W, Kleinschmidt I, Rowland M. Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two factorial design trial. Lancet. 2018;391:1577-88.

16. West PA, Protopopoff N, Rowland MW, Kirby MJ, Oxborough RM, Mosha FW, Malima R, Kleinschmidt I. Evaluation of a national universal coverage campaign of long-lasting insecticidal nets in a rural district in north-west Tanzania. Malar J. 2012;11:273.

17. West PA, Protopopoff N, Wright A, Kivaju Z, Tigererwa R, Mosha FW, Kisinza W, Rowland M, Kleinschmidt I: Enhanced protection against malaria by indoor residual spraying in addition to insecticide treated nets: is it dependent on transmission intensity or net usage? PLoS One 2015, 10:e0115661.

18. Protopopoff N, Matowo J, Malima R, Kavishe R, Kaaya R, Wright A, West PA, Kleinschmidt I, Kisinza W, Mosha FW, Rowland M. High level of resistance in the mosquito Anopheles gambiae to pyrethroid insecticides and reduced susceptibility to bendiocarb in north-western Tanzania. Malar J. 2013;12:149.

19. Protopopoff N, Wright A, West PA, Tigererwa R, Mosha FW, Kisinza W, Kleinschmidt I, Rowland M. Combination of Insecticide Treated Nets and Indoor Residual Spraying in Northern Tanzania Provides Additional Reduction in Vector Population Density and Malaria Transmission Rates Compared to Insecticide Treated Nets Alone: A Randomised Control Trial. PLoS One. 2015;10:e0142671.

20. WHO. Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions. 2011.

21. WHO. Vector Control Technical Export Group report to MPAC September 2013: Estimating functional survival of long-lasting insecticidal nets from field data. World Health Organisation; 2013.

22. Charlwood JD, Rowland M, Protopopoff N, Le Clair C. The Furvela tent-trap Mk 1.1 for the collection of outdoor biting mosquitoes. Peerj. 2017;5:e3848.

23. Gillies MTC. M.: A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region). Johannesburg South Africa: South African Institute for Medical Research; 1987.

24. Wirtz RA, Zavala F, Charoenvit Y, Campbell GH, Burkot TR, Schneider I, Esser KM, Beaudoin RL, Andre RG. Comparative testing of monoclonal antibodies against Plasmodium falciparum sporozoites for ELISA development. Bull World Health Organ. 1987;65:39-45.

25. Bass C, Williamson MS, Field LM. Development of a multiplex real-time PCR assay for identification of members of the Anopheles gambiae species complex. Acta Trop. 2008;107:50-3.

26. Koekemoer LL, Kamau L, Hunt RH, Coetzee M. A cocktail polymerase chain reaction assay to identify members of the Anopheles funestus (Diptera: Culicidae) group. Am J Trop Med Hyg. 2002;66:804-11.

27. Koenker H, Kilian A, Zegers de Beyl C, Onyefunafoa EO, Selby RA, Abeku T, Fotheringham M, Lynch M. What happens to lost nets: a multi-country analysis of reasons for LLIN attrition using 14 household surveys in four countries. Malar J. 2014;13:464.
28. Essendi WM, Vardo-Zalik AM, Lo E, Machani MG, Zhou G, Githeko AK, Yan G, Afrane YA. Epidemiological risk factors for clinical malaria infection in the highlands of Western Kenya. Malar J. 2019;18:211.

29. Mmbando BP, Vestergaard LS, Kitua AY, Lemnge MM, Theander TG, Lusingu JP. A progressive declining in the burden of malaria in north-eastern Tanzania. Malar J. 2010;9:216.

30. Bødker RAJ, Shayo D, Kisinza W, Msangeni HA, Pedersen EM, Lindsay SW. Relationship between altitude and intensity of malaria transmission in the Usambara Mountains, Tanzania. J Med Entomol 2003.

31. Lwetoijera DW, Kiware SS, Mageni ZD, Dongus S, Harris C, Devine GJ, Majambere S. A need for better housing to further reduce indoor malaria transmission in areas with high bed net coverage. Parasit Vectors. 2013;6:57.

32. Tusting LS, Ippolito MM, Willey BA, Kleinschmidt I, Dorsey G, Gosling RD, Lindsay SW. The evidence for improving housing to reduce malaria: a systematic review and meta-analysis. Malar J. 2015;14:209.

33. Lindsay SW, Jawara M, Paine K, Pinder M, Walraven GEL, Emerson PM. Changes in house design reduce exposure to malaria mosquitoes. Tropical medicine international health: TM IH. 2003;8:512–7.

34. Winskill P, Rowland M, Mtove G, Malima RC, Kirby MJ. Malaria risk factors in north-east Tanzania. Malar J. 2011;10:98.

35. Nankabirwa J, Brooker SJ, Clarke SE, Fernando D, Gitonga CW, Schellenberg D, Greenwood B. Malaria in school-age children in Africa: an increasingly important challenge. Trop Med Int Health. 2014;19:1294–309.

36. Noor AM, Gething PW, Alegana VA, Patil AP, Hay SI, Muchiri E, Juma E, Snow RW. The risks of malaria infection in Kenya in 2009. BMC Infect Dis. 2009;9:180.

37. Mayagaya VSNG, Lyimo IN, Kihonda J, Mtambala H, Ngonyani H, Russell TL, Ferguson HM: The impact of livestock on the abundance, resting behaviour and sporozoite rate of malaria vectors in southern Tanzania. Malar J 2015, 21;14:17.

38. Kitau J, Oxborough RM, Tungu PK, Matowo J, Malima RC, Magesa SM, Bruce J, Mosha FW, Rowland MW. Species shifts in the Anopheles gambiae complex: do LLINs successfully control Anopheles arabiensis? PLoS One. 2012;7:e31481.

Figures
Figure 1

Study profile for Household, individual selection and entomological monitoring

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.
Letter_to_editor_1st_April.docx