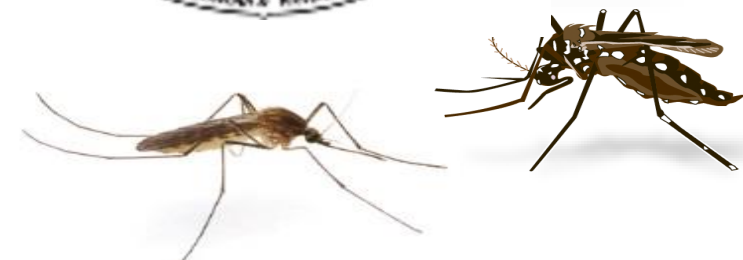


Controlling Malaria Epidemics during a Conflict: Evaluation of Permanet 3.0 for Malaria Prevention in an Internally Displaced People's Camp, Bentiu, Unity State, South Sudan.

Olivia Wetherill, Harriet Pasquale, Caroline Bower, Fatima Ahmed, Melinda Hadi, Sarah Wharton,
Valentina Buj, Elizabeth Ivanovich, Richard Allan



**REDUCING DEATHS AND SUFFERING FROM
TROPICAL DISEASES**



Rationale



Photo by: MENTOR, Bentiu PoC, South Sudan, August 2017

- Malaria morbidity and mortality continues to rise in areas affected by conflict and population displacement despite continued mass distribution of LLINs
- South Sudan is classified as one of the most unstable countries in the world with over 1.9 million people displaced, most of whom are living in overcrowded camps
- The sudden growth in proximity and density of populations with varying levels of malaria immunity pose a major risk for malaria epidemics to these already vulnerable populations
- Famine, declared in South Sudan, also impairs immune function and lessens efficacy of malaria treatment resulting in malaria mortality particularly for those under 5 years
- There is therefore an urgent need for novel tools to combat malaria epidemics for such vulnerable populations



REDUCING DEATHS AND SUFFERING FROM TROPICAL DISEASES



Insecticide Resistance & Permanet 3.0

- The global increase in vectors resistant to the pyrethroid classes of insecticide poses a major setback to current vector control interventions
- One of the most common resistance mutations is overexpression of P450 enzyme which allows pyrethroid toxin to be metabolised and excreted more efficiently
- The addition of piperonyl butoxide (PBO) to pyrethroid LLINs can counter this resistance by acting as a synergist to inhibit P450 thereby prolonging presence of pyrethroid within the mosquito for a toxic effect
- Permanet 3.0 by Vestergaard is a mosaic PBO LLIN containing PBO+deltamethrin on a polyethylene roof and deltamethrin only on polyester walls
- Village studies in Nigeria, Ghana and Togo showed higher efficacy on entomological parameters such as mortality and bloodfeeding relative to pyrethroid-only LLINs



**REDUCING DEATHS AND SUFFERING FROM
TROPICAL DISEASES**

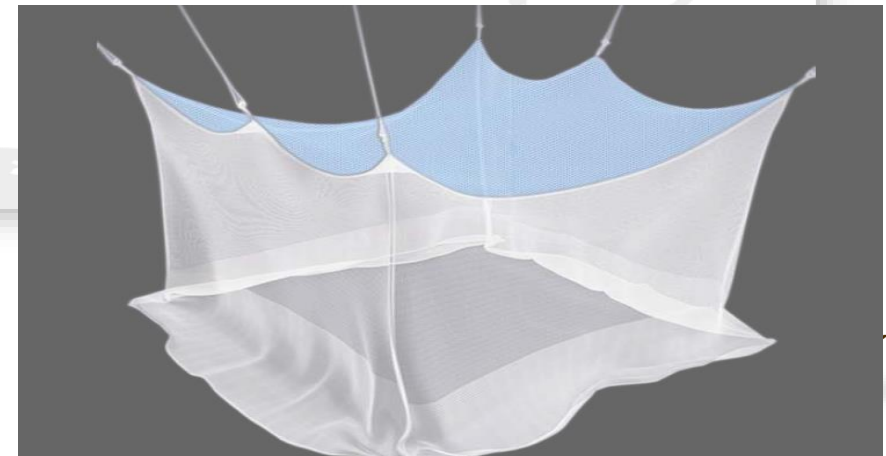
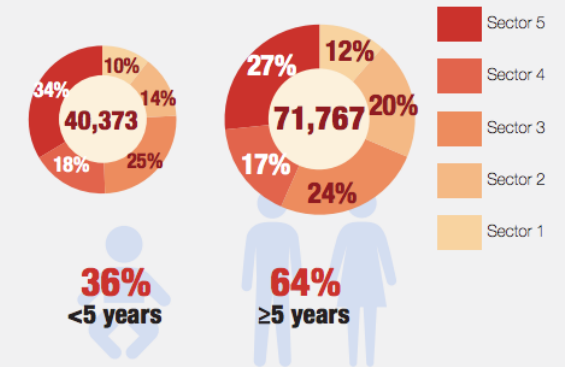


Image: Vestergaard, Permanet 3.0 Technical Basis for Deployment

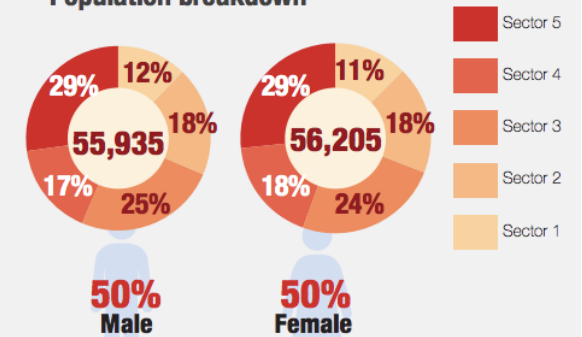
Setting: Bentiu Protection of Civilian Camp

- Located in Rubkona county, Unity State, Bentiu PoC formed quickly in 2013 as civilians sought refuge from the surrounding increase in violence and attacks
- Ethnic group of Bentiu PoC is Nuer although fighting can break out sporadically within the camp between county clans
- In 2017, over 160,000 were registered although population counts reported fluctuating population of approx. 120,000
- A significant proportion of the camp were children under 5 at 36% (over 40,000)
- The infrastructure is made of regularised sectors, blocks, rows and finally plot numbers
- One plot can have up to three structures with numerous families demonstrating the cramped living conditions and overcrowding
- Each sector has 1 health facility with 1 MSF hospital for inpatients

Population <5 and ≥5 years



Male and Female Population breakdown



Graphics: IOM CCCM Population Count, October 2017



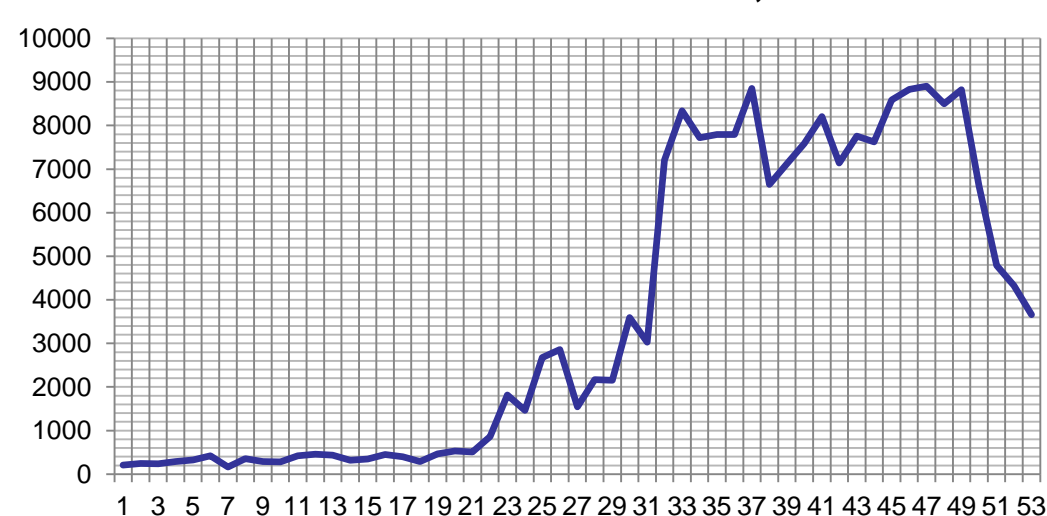
REDUCING DEATHS AND SUFFERING FROM TROPICAL DISEASES



Bentiu PoC Environment and Malaria Endemicity

- The camp is located in an inhabitable lowland and with limited resources nearby resulting in extreme flooding during the rainy season (April – November)
- A drainage system has now been engineered that pumps out excess water via central reservoir although the soil consistency does not soak water effectively leading to rapid formation of large breeding sites particularly in the channels around each block

Bentiu PoC Malaria Caseload, 2015



- In 2015, despite high coverage of pyrethroid-based LLINs and IRS campaign using ICON (pyrethroid) insecticide, the camp suffered a major malaria epidemic with a significant number of deaths
- Despite clear operational data indicating vector resistance to pyrethroids, mass distribution of pyrethroid LLINs was still implemented the following year in 2016.



REDUCING DEATHS AND SUFFERING FROM TROPICAL DISEASES



Insecticide Resistance in Bentiu PoC

- In January 2018, locally reared mosquitoes were tested for susceptibility to insecticides using WHO standard method
- High resistance to both permethrin (type 1) and deltamethrin (type 2) class of pyrethroids was confirmed
- Further testing to identify type of resistance was not performed however, 88% of mosquito samples from entomological assessment, were classified as *An. Gambiae* or *An. Colluzzii*.
- Therefore both metabolic and target site resistance were identified as the most likely causes
- Based on these findings, the mass distribution of pyrethroid-only LLINs were only providing a physical barrier as protection rather than intended “mass killing” effect





Insecticide Class	24 hour mortality
Pyrethroid (type 1)	3.75 % 
Pyrethroid (type 2)	10 % 
Carbamate	93.75 % 
Organophosphate	98.75 % 



Photo by: MENTOR, Bentiu PoC, South Sudan, January 2018



**REDUCING DEATHS AND SUFFERING FROM
TROPICAL DISEASES**



Aims and Objectives

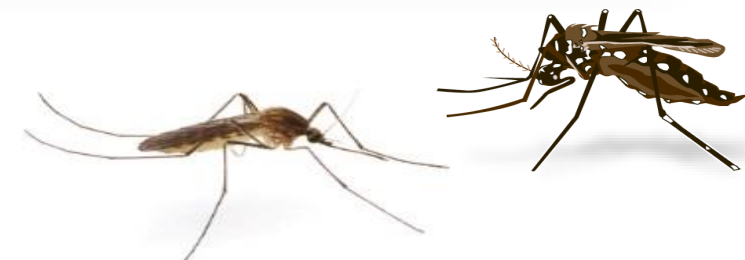
- **Aim:** Conduct a full-scale evaluation of PBO LLINs in preventing malaria morbidity and mortality among internally displaced population
- **Objectives:**
 - 1. Epidemiological Monitoring
 - 2. Durability Survey
 - 3. Entomological Assessment
- **Research questions:**
 - What is the impact of PBO LLINs on malaria vector populations and malaria transmission?
 - What are the malaria incidence rates for IDPs living with PBO LLINs compared to those living with standard LLINs / IRS?
 - How well does the PBO LLIN perform in terms of user acceptance and durability?



Photo by: MENTOR Initiative, Bentiu PoC South Sudan, February 2018



REDUCING DEATHS AND SUFFERING FROM TROPICAL DISEASES



Study Design

- The study used a prospective observational design in two phases
- Due to potential complications around dispersal capability of vectors, cluster or individual randomisation was not possible
- Sector 1 (exposed) and Sector 5 (unexposed) were monitored with Sectors 2-4 acting as the buffer zone with a minimum distance of 1km between the two study arms and 180m between Sector 1 and 2
- Sector 1 had 72% PN 3.0 LLIN coverage immediately after distribution
- Sector 5 had 75.8% coverage of pyrethroid-only LLINs however coverage of LLINs classified as “protective” (less materials degradation) was only 33%



REDUCING DEATHS AND SUFFERING FROM TROPICAL DISEASES



Ethical Considerations and Consent

- Ethical clearance was provided by South Sudan Ethical Reviews Board with agreement from Ministry of Health
- Agreement was also reached with camp management and all health partners before implementation
- Signed consent for epidemiological monitoring was obtained from all Block and Sector Leaders
- Heads of households signed (or in cases of illiteracy used fingerprints) for interviews and mosquito collections



**REDUCING DEATHS AND SUFFERING FROM
TROPICAL DISEASES**



First Phase: PN 3.0 Distribution

- 14,800 Permanet 3.0 LLINs were delivered to residents of Sector 1 and the part of Sector 2 via mass distribution in July-August
- Major fighting broke out across the camp during distribution of the first 8,500 PBO LLINs in early July. Activities resumed in August when the remaining 5,700 and 300 were distributed to Sector 1 and Sector 2 respectively.
- 100% PBO LLIN were distributed to households in Sector 1. A hang-up campaign calculated PN3.0 LLIN coverage at 72% 1 month post distribution in Sector 1
 - Biometric registration had never been updated since PoC formed, and many households registered for the camp were no longer living there whilst some non-registered residents were now living in Sector 1 therefore had not received an LLIN
- Distribution of standard (permethrin) LLINs to the remaining sectors was planned for July-August. However Global Fund / PSI experienced complications and were unable to deliver the LLINs.
- A survey in sector 5 identified LLIN coverage as 75.8% however coverage of LLINs classified as protective was only 33%



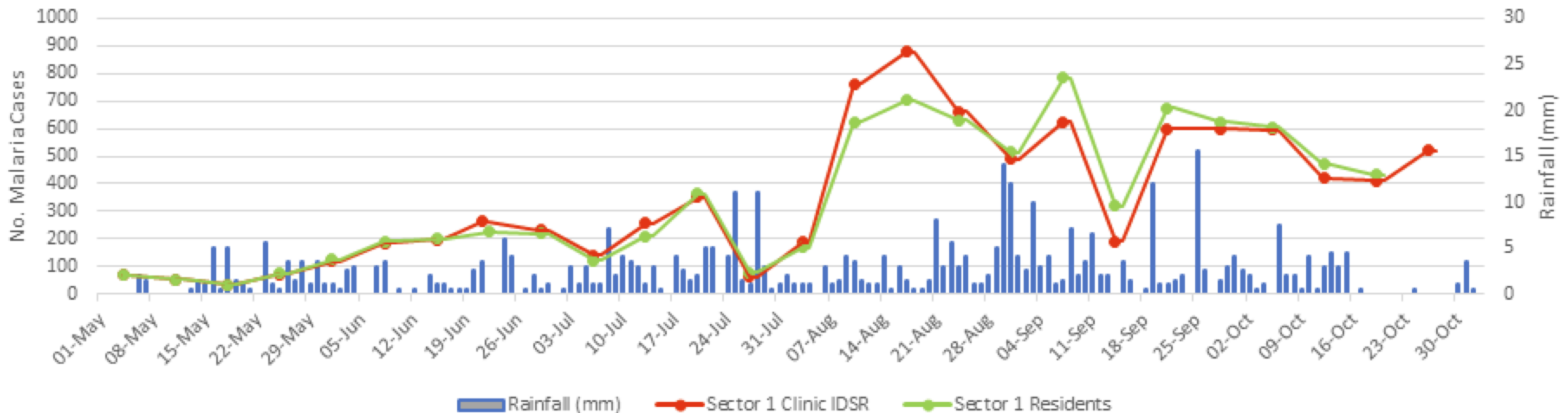
**REDUCING DEATHS AND SUFFERING FROM
TROPICAL DISEASES**



Second Phase: IRS Campaign

- In week 36, malaria cases were reaching peak levels with little to no protection afforded in control and buffer zone by 2-year old standard LLINs
- MENTOR therefore implemented IRS campaign with Ficam insecticide (bendiocarb class of insecticide) in Sectors 2-5 to mitigate the epidemic
- In Sector 5 control arm, coverage of IRS reached 94%
- Sector 1 was closely monitored throughout the rainy season and it was decided that the PN 3.0 LLINs were providing adequate protection therefore no IRS campaign was implemented for exposed group

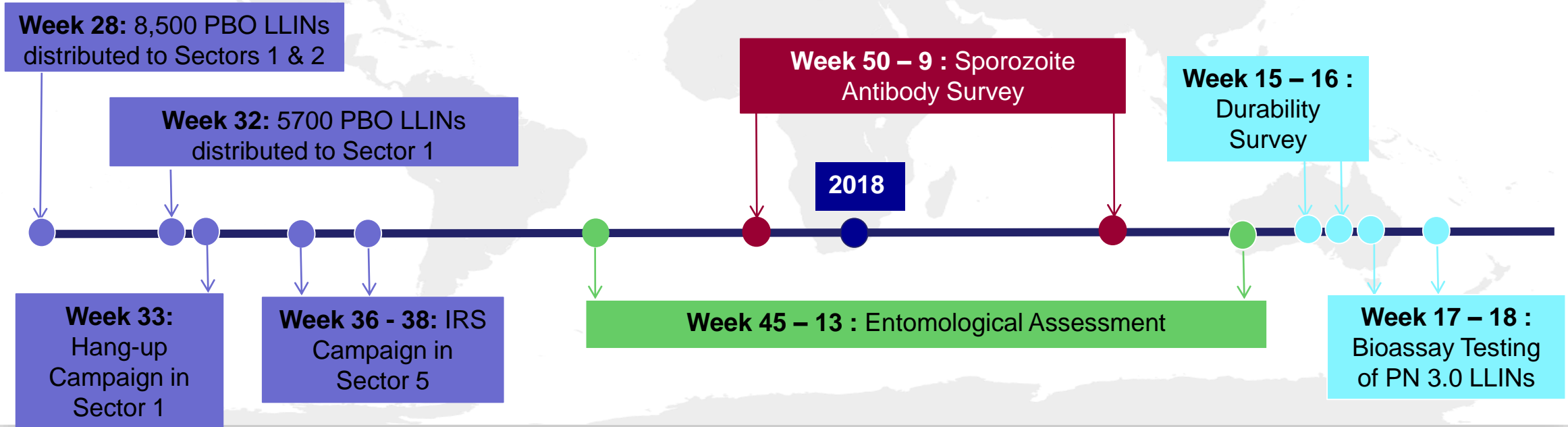
Malaria Cases & Rainfall: Bentiu PoC



Methodology and Timeline

The study employed three approaches to investigate distinct aspects of assessing LLIN effectiveness:

1. Epidemiological Monitoring
2. Durability Survey
3. Entomological Assessment



REDUCING DEATHS AND SUFFERING FROM TROPICAL DISEASES



1. Epidemiological Monitoring Methodology

- **Malaria Incidence:** Patients testing positive for RDT with residence in either Sector 1 or 5 was collected passively from all health facilities within the camp
- **Antibody Response Survey:**
 - Blood spot samples were collected from malaria RDT+ patients aged between 4 months – 13 years.
 - The level of anti-salivary IgG antibody in each blood sample is measured against extracted proteins from *Anopheles* salivary glands
 - IgG response correlates to biting pressure; a darker well indicates more infective bites

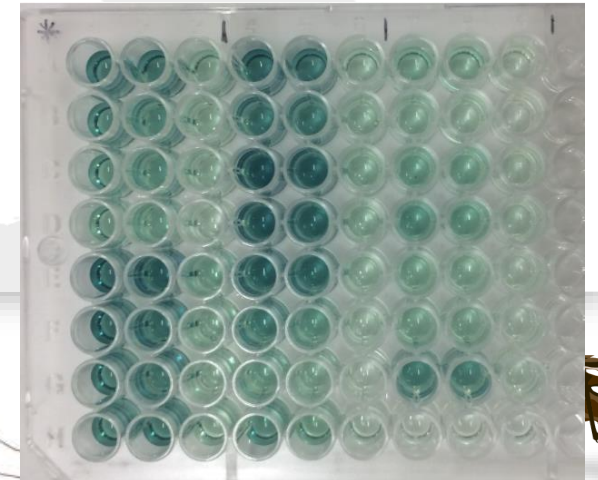
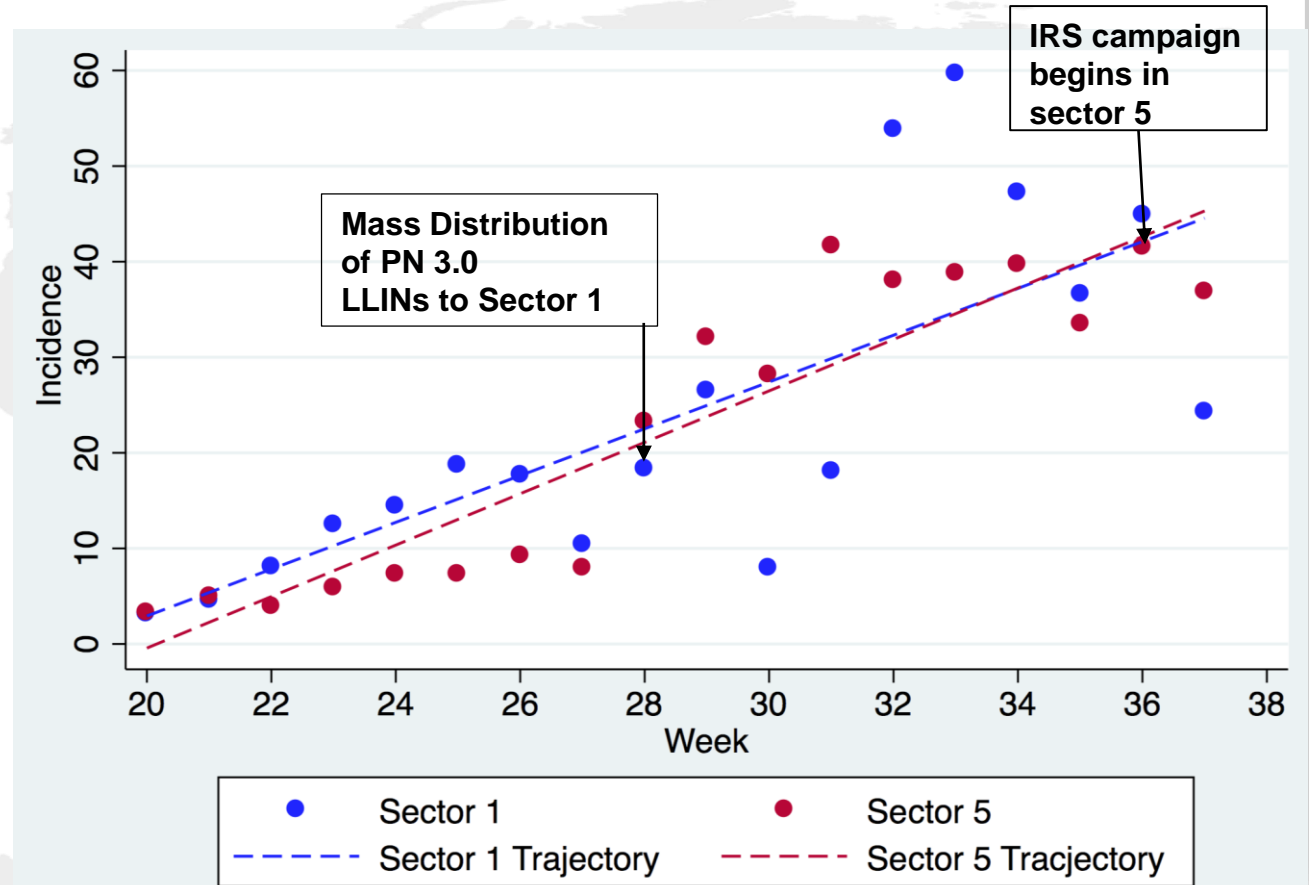


Photo by: Montpellier University

Preliminary Results: Before Peak of Epidemic

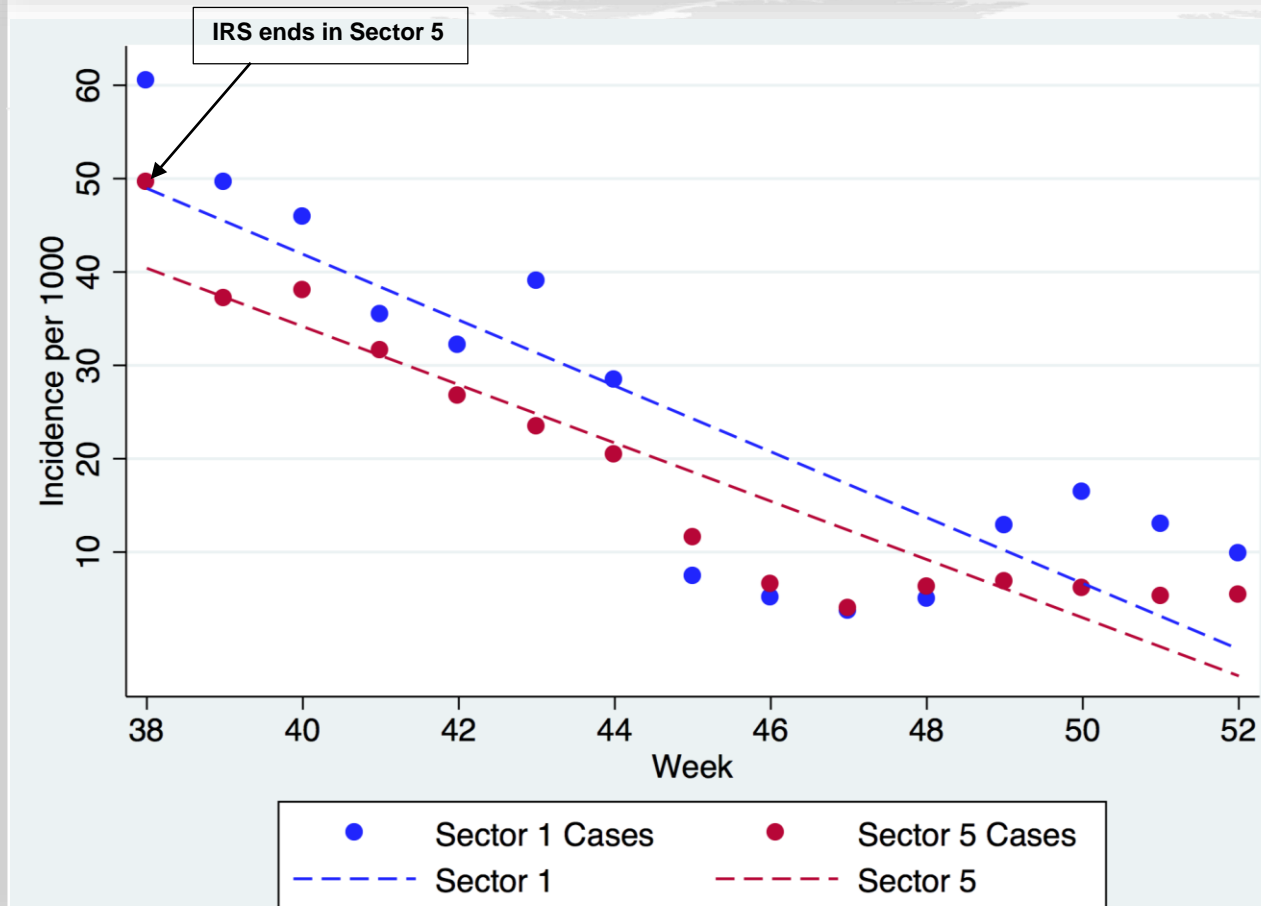
- Results show an overall slower increase in malaria cases leading up to the peak of the epidemic in Sector 1 suggesting the PBO LLINs offered better protection to the exposed group than those sleeping under old pyrethroid-based LLINs.
- Despite an initial increase of malaria cases after distribution, it appears the residents did not fully start to use PN 3.0 LLINs until after the hang-up campaign (week 34) where malaria rates fall



REDUCING DEATHS AND SUFFERING FROM TROPICAL DISEASES



Preliminary Results: After Peak of Epidemic



- After the peak of the epidemic and post IRS campaign in Sector 5 (plus the buffer zone), a slightly sharper rate of decline in malaria cases was seen in Sector 1 in comparison to Sector 5.
- PBO LLINs have proven themselves to be just as or even slightly more protective than IRS + standard LLINs at reducing a malaria epidemic



REDUCING DEATHS AND SUFFERING FROM TROPICAL DISEASES

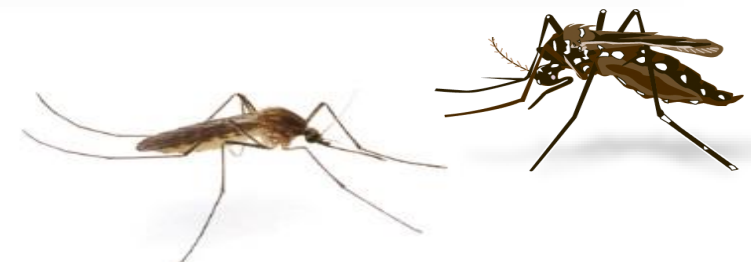


Challenges and Limitations in Malaria Incidence Data

- The preliminary results are based on gathered data from all healthcare facilities and MSF hospital. Due to concerns from partners on patient confidentiality and subsequent limited access to clinical records, individual patient data could not be collected. Results therefore could not be disaggregated by age or gender.
- Results are highly dependent on RDT supply to clinics which were frequently in short supply throughout the transmission season.
- Population entering and moving within the camp is mobile and no proof of address is needed during consultations. A significant number of people entered the camp every day to use health services however, for fear of being turned away, reported address as within the camp.
- During insecurity and workers strikes, clinics would shut overwhelming MSF or other sector health services. In times, methodical collection of patient information such as address was often missed by overworked health staff.

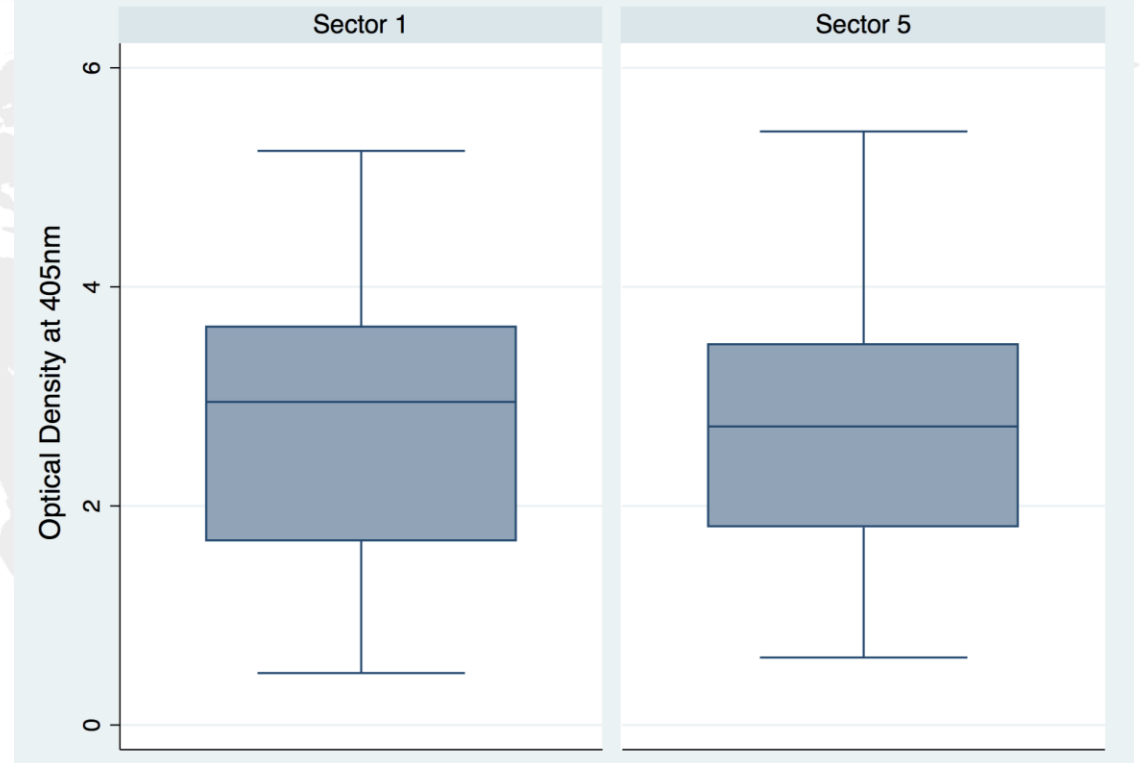


**REDUCING DEATHS AND SUFFERING FROM
TROPICAL DISEASES**



Antibody Response Survey

- 1,000 blood samples were sent to Montpellier university to test IgG antibody response
- Overall, there was no significant difference found in IgG responses between Sectors PN 3.0 LLINs and old standard LLINs + IRS ($p=0.58$, Mann-Whitney U test) implying a similar rate of infective bites between the two Sectors



REDUCING DEATHS AND SUFFERING FROM TROPICAL DISEASES



2. Durability Survey Methodology

Both extrinsic and intrinsic factors that may affect LLIN durability were assessed:

Element	Methodology
Net survivorship	<ul style="list-style-type: none">• In-depth questionnaire for 160 randomly selected shelters
Fabric integrity	<ul style="list-style-type: none">• Inspection of all LLINs using WHO standard hole index calculation
Insecticidal activity	<ul style="list-style-type: none">• WHO standard bioassay cones• Laboratory testing of chemical residual insecticidal concentration

- As control LLINs were not distributed in Sector 5, insecticidal activity was assessed by comparing old vs. unused Permanet 3.0 LLINs
- Cone bioassays were also performed using locally reared mosquitoes as opposed to standard WHO fully susceptible mosquitoes due to limitations on import laws and lack of resources for transporting them to field sites



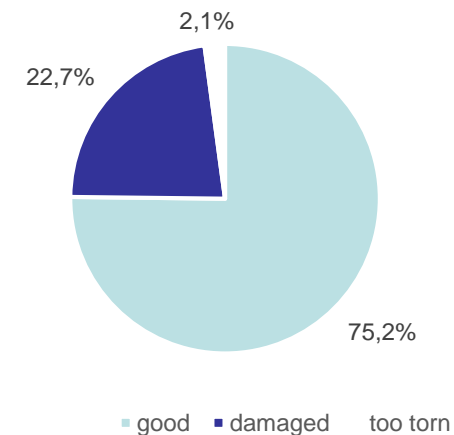
REDUCING DEATHS AND SUFFERING FROM TROPICAL DISEASES



Durability Survey 9 months post distribution

- Only 35% of the distributed PN 3.0 LLINs remained in Sector 1 with a coverage of 46.9%; most common reason for loss being LLIN had been given away or used in another location
 - Population is highly mobile and frequently move in / out of the camp
 - Many who received PN 3.0 LLINs are registered but no longer live in the camp
 - Lack of LLIN distribution to remaining sectors and communities outside the camp increased sharing of PN 3.0 LLINs with families from other sectors
- Usage of PN 3.0 LLINs the previous night in Sector 1 was significantly higher (98.6%) than usage of old LLINs in Sector 5 (90.4%)
- For 75.2% of surveyed PN 3.0 LLINs, the physical integrity was still classified as “good” (Proportionate hole index = 0-64)
- Assuming hole formation is constant, PHI increases 9.43 per month, a lower rate than in similar studies on PBO

Classification of PN 3.0 LLIN Physical Condition



REDUCING DEATHS AND SUFFERING FROM TROPICAL DISEASES

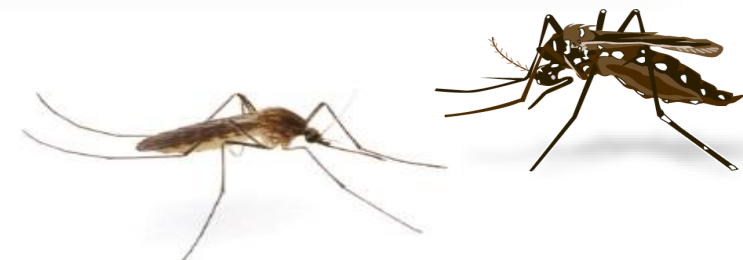


Insecticidal Activity: Bioassay Methodology

- 20 randomly selected PBO LLINs from the durability survey were tested for efficacy in killing local mosquito population
- Mosquito larvae were collected from the PoC and reared in field laboratory
- Comparisons were made between 20 new (unused) and 20 old (approx. 9 months in use) PN 3.0 LLINs
- Roof (containing PBO), upper wall and lower wall were all tested separately
- Female Anopheles mosquitoes were exposed to each piece for 3 minutes then transferred to netted cups with sugar solution
- 1 hour knockdown and 24 hour mortality were then measured
- Samples from the same LLINs were sent to Vestergaard for testing chemical content concentration for upper walls, lower walls and roof

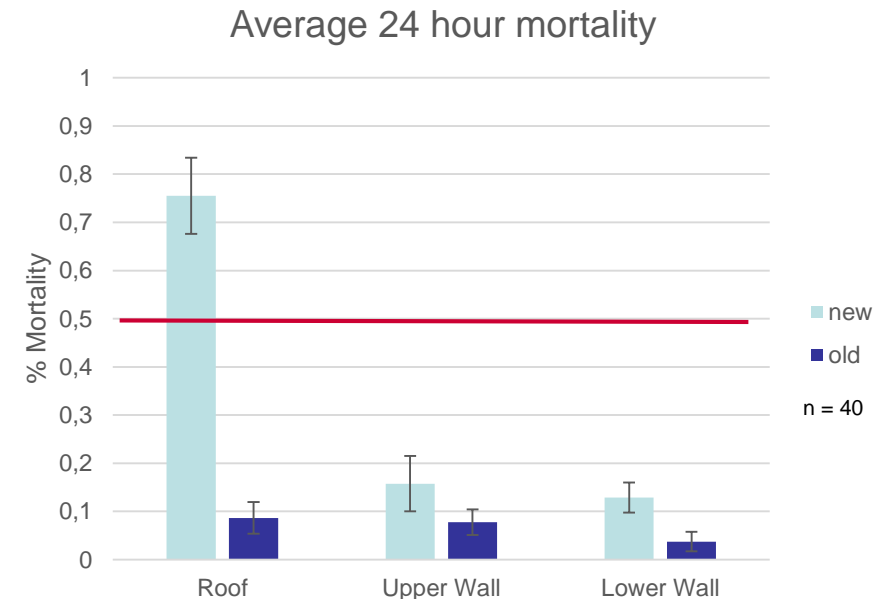
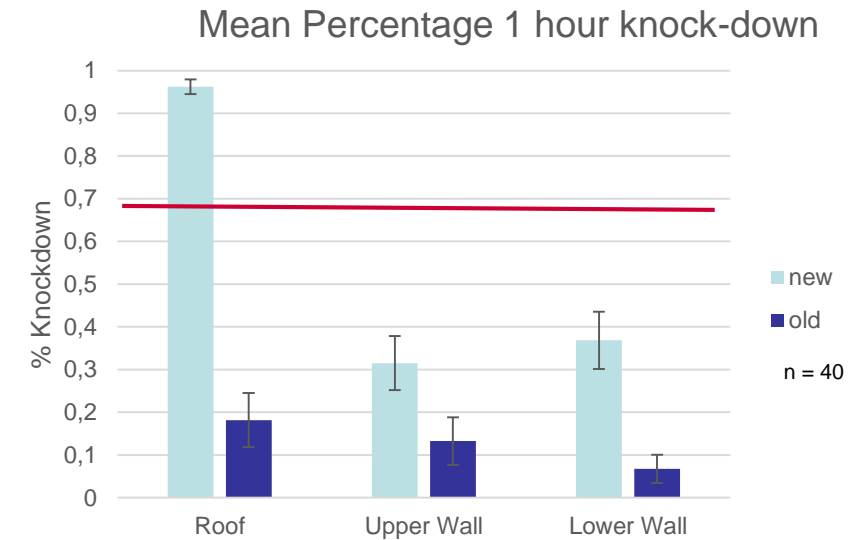


**REDUCING DEATHS AND SUFFERING FROM
TROPICAL DISEASES**



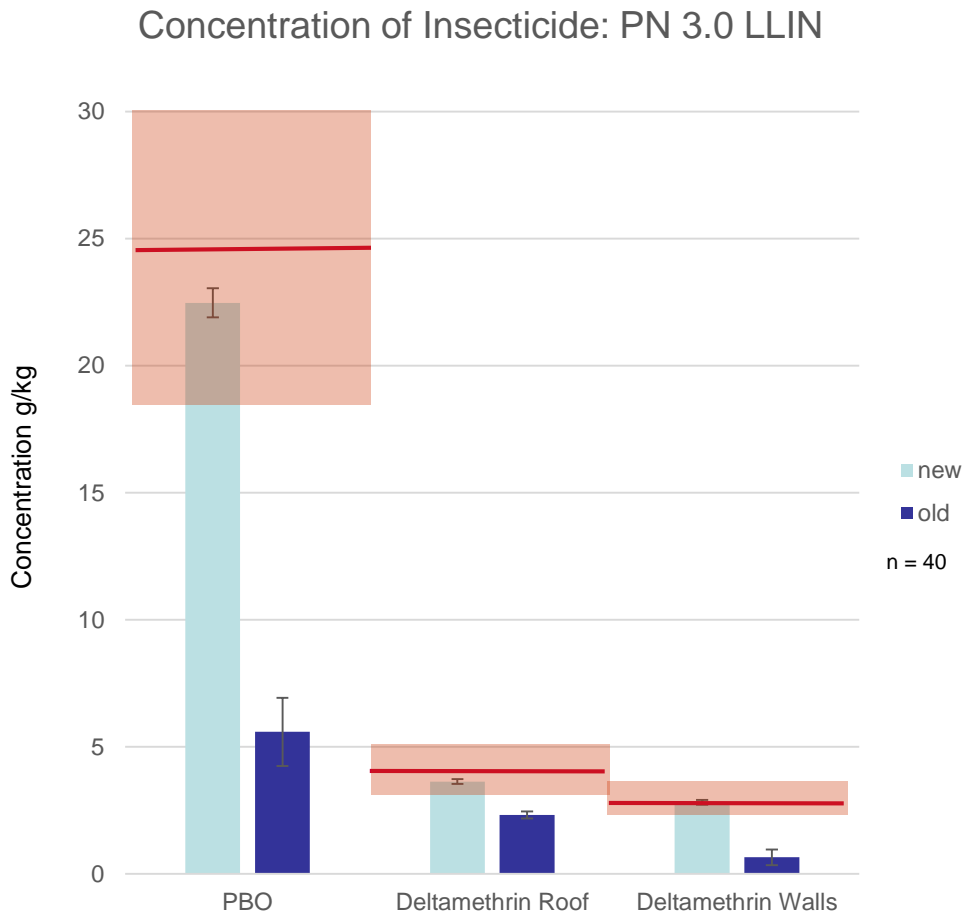
Cone Bioassay Results 9 months Post Distribution

- For LLINs to be classified as bioeffective, knockdown and mortality threshold (for locally reared mosquitoes) is considered to be 70% and 50% respectively (Allosogbe et al. 2017) – red lines
 - Roof of new (unused) PN 3.0 LLINs is therefore considered effective however the walls are not
- Significant differences were found for 1 hour knockdown between new and old ($p < 0.001$, binomial t-test)
- New PN 3.0 LLINs show significantly high mean percentage knockdown and mortality for the roof containing PBO with slightly lower values for the walls as they contain pyrethroid (deltamethrin) only
- Knockdown and mortality of old PN 3.0 LLIN roof pieces has dropped to levels similar to the walls implying there is no longer any residual PBO bioefficacy after only 9 months in use



REDUCING DEATHS AND SUFFERING FROM TROPICAL DISEASES

Chemical Content Analysis 9 months Post Distribution



- After 9 months in use, PN 3.0 LLINs lost 75.2% PBO concentration
- Minimum manufacturing concentrations (red box) stand at 25 ± 0.25 g/kg for PBO on roof, 4 ± 0.25 g/kg for deltamethrin in the roof and 2.8 ± 0.25 g/kg in the walls
- All LLINs 9 months post-use did not reach the minimum manufacturing concentration requirements in the walls or roof
- Multiple linear regression analysis found reductions in PBO concentrations in the roof are the key determinant of reduced efficacy for both knockdown and mortality. This is supported by a higher correlation coefficient between knockdown or mortality when PBO concentration is higher than with deltamethrin
- This contrasts to other research showing 60% PBO retention in PN 3.0 LLIN roofs after 20 washes (Corbel et al. 2014)



REDUCING DEATHS AND SUFFERING FROM TROPICAL DISEASES

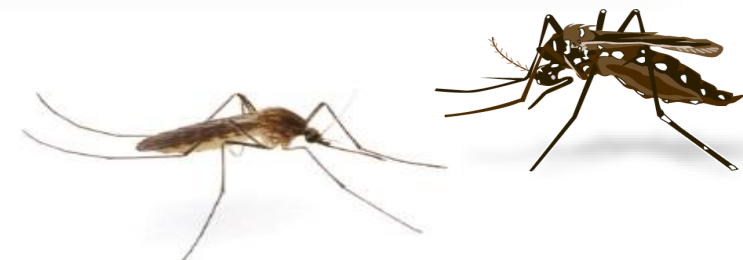


Insecticidal Activity Discussion and Conclusion

- Whilst the roof of new PN 3.0 LLINs shows a high success rate in knock-down and mortality, the walls of new LLINs have reduced efficacy mainly due to known resistance of local mosquito populations to the pyrethroids.
- Because PBO in new PN 3.0 LLINs is still effective, this confirms the most common type of resistance to pyrethroids in Bentiu PoC is P450 overexpression (metabolic)
- The reduced mortality efficacy of old PBO LLINs indicates that the PBO chemical has lost most if not all residual activity and supported by results from chemical content analysis which shows a significant reduction in PBO concentration in the roof to below manufacturer's requirements
- Household surveys found 63.5% of households in Sector 1 dried their PN 3.0 LLINs in the sun with 84.5% having washed in the last month demonstrating that, despite high IEC campaign coverage, habits of households to keep LLINs clean can dramatically impact the bioefficacy of the LLINs
- It is clear that the harsh conditions and handling of LLINs in the camp after only 9 months in use diminish retention of PBO thereby debilitating its synergistic effect on pyrethroid-resistant mosquitoes



**REDUCING DEATHS AND SUFFERING FROM
TROPICAL DISEASES**



3. Entomological Assessment

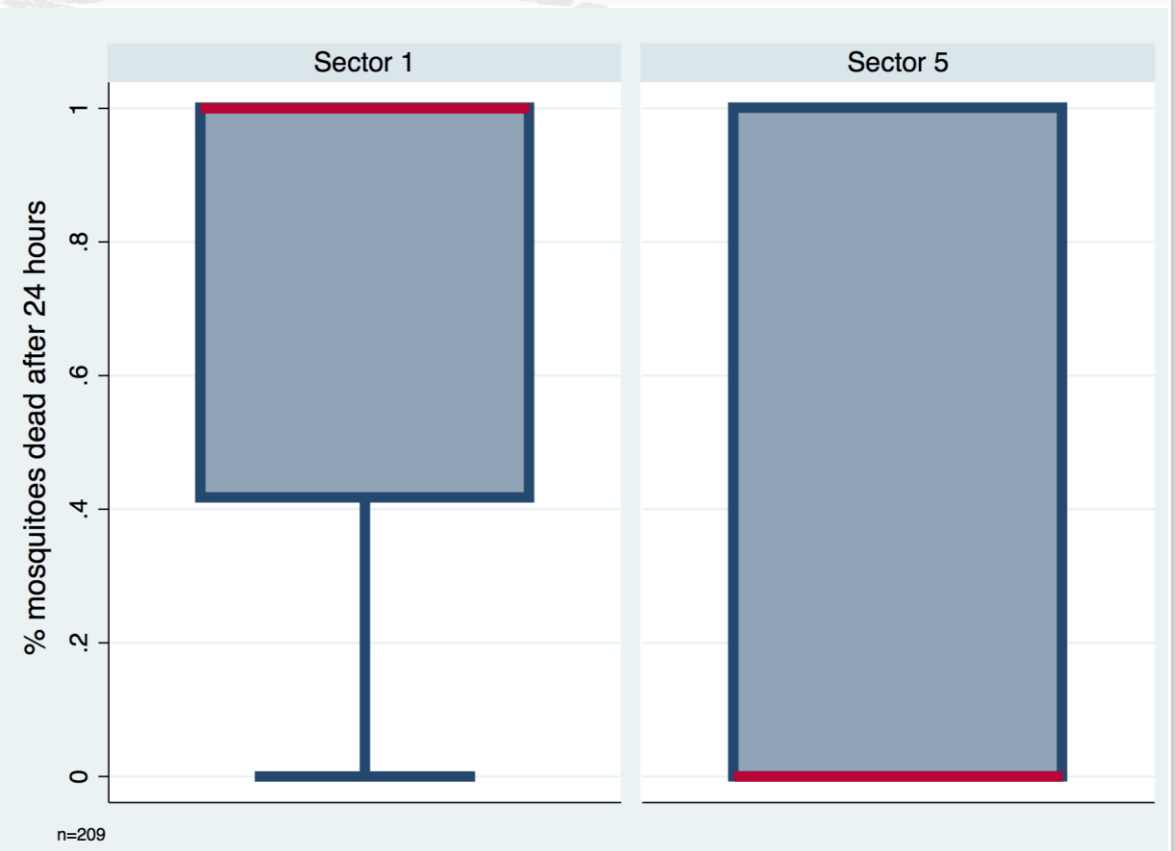
- Collection of mosquitoes was conducted monthly from randomly selected shelters in sectors 1 and 5 to test difference between PN 3.0 LLINs vs old pyrethroid LLINs + IRS
- A sub-sample of 200 mosquitoes were then to LSTM for species identification and blood meal analysis

Type of Collection	Data Obtained
Light Traps	Abundance Species composition Outdoor mosquitoes Delayed mortality
Exit Traps	Exiting Mosquitoes Delayed mortality
Morning hand collections	Delayed mortality

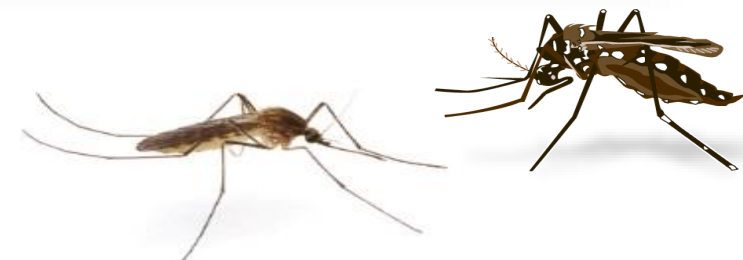


Entomological Results: Delayed Mortality

- Delayed mortality: a greater proportion of mosquitoes were dead at 24h in Sector 1 (71.8%) compared to Sector 5 (36.8%) with significant difference between the two sectors ($p < 0.005$, Mann-Whitney U test)
- This implies there is a higher mortality effects from PBO in Sector 1 as compared to Ficam insecticide in Sector 5



**REDUCING DEATHS AND SUFFERING FROM
TROPICAL DISEASES**

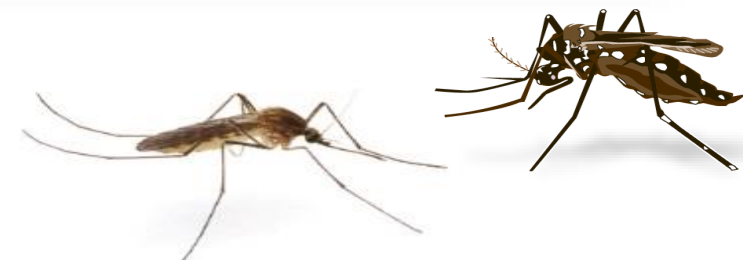


Conclusions on PN 3.0 LLIN effectiveness

- PN 3.0 LLINs may be more efficacious in decreasing rate of fall after malaria epidemic
 - Epidemiological data can be analysed in two phases: 1. start of season to peak, 2. peak to end of epidemic. Data from second phase shows us a rate of incidence decline is marginally greater in PN 3.0 LLIN sector than standard LLINs + IRS sector
- Residual bioefficacy of PBO is not long-lasting in the harsh environmental conditions of the camp
 - Only 24% of PBO was retained in the roof after 9 months in use
 - 88% reduction in mortality of local mosquitoes when comparing 9 months in use LLINs to unused LLINs
- However, the physical durability of the PN 3.0 LLINs is promising with a slower rate of PHI formation as compared to LLINs in other studies
- With highly mobile populations and displacement, the protection afforded by >80% coverage of LLINs is significantly compromised due to low retention of distributed materials even under tightly managed field study conditions
 - Only 35.7% of originally distributed LLINs were found in homes after 9 months although usage of those LLINs was high at 98.6%
 - The fluidity of the population means majority of distributed LLINs were shared with other family members or taken outside of the camp back to home communities
- Identification of resistance to pyrethroids in local mosquitoes proves standard pyrethroid LLINs are not an effective vector control tool in South Sudan



**REDUCING DEATHS AND SUFFERING FROM
TROPICAL DISEASES**



Operational Research Challenges in these Settings

- Randomised community cluster trials are impossible in conflict-affected displaced settings and therefore study design must be adjusted for the context
- Unpredictability of the setting and difficulties in procurements and logistics require flexibility in adjusting for the study
 - Major fighting suspended activities across the camp resulting in LLIN distribution being implemented in 2 stages
 - Sectors 2 – 5 had to receive emergency IRS due to issues from Global Fund / PSI in procuring standard Olyset LLINs
 - Procurement of materials for research heavily relied on delivery by UN cargo flights which were intermittent during rainy season and prioritized drug delivery
- Results from this study have shown importance of context-appropriate ways to more accurately determine how vector control tools can perform in different contexts
 - Chemical concentration is significantly lower than WHO standard 20 wash tests
- There is an urgent need to find novel vector control tools as evidenced by the increase in malaria death rates across South Sudan since 2009 despite a massive scale-up in LLIN distribution and usage



**REDUCING DEATHS AND SUFFERING FROM
TROPICAL DISEASES**

