Insecticide Resistance and the Future of Malaria Control in Zambia

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Abstract

Background: In line with the Global trend to improve malaria control efforts a major campaign of insecticide treated net distribution was initiated in 1999 and indoor residual spraying with DDT or pyrethroids was reintroduced in 2000 in Zambia. In 2006, these efforts were strengthened by the President’s Malaria Initiative. This manuscript reports on the monitoring and evaluation of these activities and the potential impact of emerging insecticide resistance on disease transmission.

Methods: Mosquitoes were captured daily through a series of 108 window exit traps located at 18 sentinel sites. Specimens were identified to species and analyzed for sporozoites. Adult Anopheles mosquitoes were collected resting indoors and larva collected in breeding sites were reared to F1 and F0 generations in the lab and tested for insecticide resistance following the standard WHO susceptibility assay protocol. Annual cross sectional household parasite surveys were carried out to monitor the impact of the control programme on prevalence of Plasmodium falciparum in children aged 1 to 14 years.

Results: A total of 619 Anopheles gambiae s.l. and 228 Anopheles funestus s.l. were captured from window exit traps throughout the period, of which 203 were An. gambiae malaria vectors and 14 An. funestus s.s. In 2010 resistance to DDT and the pyrethroids deltamethrin, lambda-cyhalothrin and permethrin was detected in both An. gambiae s.s. and An. funestus s.s. No sporozoites were detected in either species. Prevalence of P. falciparum in the sentinel sites remained below 10% throughout the study period.

Conclusion: Both An. gambiae s.s. and An. funestus s.s. were controlled effectively with the ITN and IRS programme in Zambia, maintaining a reduced disease transmission and burden. However, the discovery of DDT and pyrethroid resistance in the country threatens the sustainability of the vector control programme.

Introduction

Malaria vector control activities are substantially increasing in many malaria endemic countries [1], with some countries considering elimination [2]. Indoor Residual Spraying (IRS) and Insecticide Treated Nets (ITNs) form the backbone of these activities and both have been proven as excellent vector control strategies [3]. However, the number of insecticides recommended for both methods is severely restricted, hence monitoring and management of insecticide resistance within the control programme is essential if control is to be maintained [4].

Zambia first initiated IRS with DDT in the 1950s, at the same time malaria became a notifiable disease [3]. IRS coverage was reduced by 30% by 1973 due to economic constraints and environmental concerns about the use of DDT. The IRS programme stopped completely in the mid 1980s. With reduced vector control and the development of parasite resistance to antimalarial drugs [6], malaria cases increased from 121.5 per 1000 in 1976 to 394 cases per 1000 in 2002[5].

In 2000, Konkola Copper Mines, a private company, reintroduced IRS with pyrethroids and DDT in two districts in Zambia [7] The success of these IRS programmes led the National Malaria Control Programme (NMCP) to again implement an IRS programme alongside the distribution of Long Lasting Insecticide Treated Nets (LLINs) [8]. As these malaria vector control interventions are being scaled up their impact has been monitored [9].

In areas of hyper and holoendemic malaria, vector control is critical in reducing human malaria transmission and the related morbidity and mortality. Evidence-based deployment and optimal
assessment of vector control interventions is needed to formulate operational control policies. This requires the collation and assessment of a number of different data systems. To facilitate data handling the Zambian NMCP evaluated the Malaria Decision Support System (MDSS) developed by the Innovative Vector Control Consortium (IVCC) [10].

**Materials and Methods**

**Ethics clearance**

Ethical clearance for this study was obtained from the University of Zambia Biomedical Ethical Committee (Assurance No. FWA00000338, IRB00001131 of IOR G0000774 reference code 002-07-07). Written informed consent was obtained from all participants in this study.

**Study sites and interventions**

Zambia is situated in the Southern African region with a population of approximately 13 million, 45% of whom are below the age of fifteen [11]. Data were collected from 18 sentinel sites, distributed amongst nine districts within a 350 km radius of the capital, Lusaka (Figure 1). Sites were selected to assist in monitoring the expanding vector control programme. At each site annual household surveys were carried out to measure *Plasmodium falciparum* prevalence in children aged 1 to 14. Relative mosquito abundance and infectivity were measured as a proxy for transmission and insecticide resistance status was monitored. Indoor residual spraying was carried out in October each year from 2003 to 2010 with DDT at 2 g/m² (Avima, South Africa) in 5 sites only (Chimoto, Kabulongo, Kafue Estates, Mufweshya and Mukobeko). PermaNet® (Verstargaard) and Olyset® (Sumitomo), LLINs were distributed to all areas (Figure 1). Countrywide mass distribution of ITNs commenced in 2005, prior to this ITN distribution was through the antenatal clinics and commercial outlets. Over 5 million ITNs, enough to cover 96% of Zambia population, had been distributed by 2008 [8,9]. All vector control was carried out by the NMCP as part of their ongoing operational control activities.

**Plasmodium falciparum prevalence surveys**

Prevalence surveys were conducted at the end of the malaria transmission season in April/May in 2008, 2009 and 2010. Households were selected from strata formed by dividing sentinel sites into quadrants. In each quadrant a starting house was selected and all children aged 1 to 14 tested for *P. falciparum* infection using ICT™ malaria combo rapid diagnostic tests (R&R, Cape Town, South Africa). The investigator then proceeded to the next nearest house and tested all children until a total of 35 children per quadrant had been tested. Children testing positive for *P. falciparum* were offered treatment with Coartem® (artemether-lumefantrine) according to the National Malaria Control Programme (NMCP) guidelines. Any complicated malaria case was referred to the

![Figure 1. Map showing the spatial distribution of sentinel sites in Zambia.](doi:10.1371/journal.pone.0024336.g001)
nearest health centre. The sentinel site specific sample size was calculated to provide evidence at the 5% significance level of an absolute reduction in *P. falciparum* prevalence of 20%. Prevalence of *P. falciparum* infection and 95% confidence intervals (CI) for each sentinel site were estimated taking account of clustering by sentinel site using the statistical software package STATA (StataCorp LP. Stata Statistical Software: Release 10. College Station, TX, USA).

Sentinel sites were considered as the primary sampling unit. Logistic regression, allowing for complex survey designs, was performed to estimate the mean effect of the vector control intervention on prevalence compared to 2008 data.

**Mosquito Species Abundance and Infectivity**

Window exit traps were fitted to six houses in each sentinel site with the homeowners consent in April 2008 and operated until May 2010. Houses were selected to cover the whole of a sentinel site. Each house was constructed in traditional style and in good repair with a window in the bedroom for the exit trap to be fitted. The Chunga sentinel site was not used for exit window trap collections, as houses were constructed from concrete blocks with corrugated metal roofs. Mosquito collections were made daily by homeowners trained to empty the contents of the window trap into pre-labelled specimen jars containing isopropanol. Checklists were completed specifying nights for which traps were not operating.

**Table 1.** Prevalence of infection with *Plasmodium falciparum* in children 1 to - 14 years of age, by sentinel site, observed during household surveys in 2008, 2009 and 2010 in Zambia.

| Sentinel site | April/May 2008 | April/May 2009 | April/May 2010 | P(08-09) | P(09-10) |
|---------------|---------------|---------------|---------------|----------|----------|
| IRT sites     |               |               |               |          |          |
| Chimoto       | 3.2 (93)      | 0.7 (141)     | 3.4 (145)     | 0.206    | 0.182    |
| Kabulongo     | 11.4 (158)    | 0 (84)        | 4.6 (130)     | 0.0007   | 0.02*    |
| Mukobeko      | 7.157 (157)   | 6.7 (134)     | 6.2 (130)     | 0.933    | 0.89     |
| Kafue estate  | 2.3 (128)     | 0 (116)       | 3.7 (137)     | 0.129    | 0.054    |
| Mufweshya     | 4.3 (69)      | 0 (73)        | 1.8 (113)     | 0.038    | 0.18     |
| INN sites     |               |               |               |          |          |
| Chiawa        | 2 (148)       | 3.7 (134)     | 5.1 (136)     | 0.476    | 0.637    |
| Chibombo      | 21.2 (146)    | 9.3 (161)     | 3 (132)       | 0.0311   | 0.027    |
| Chikankata    | 1.1 (93)      | 0.7 (147)     | 0.7 (136)     | 0.765    | 1        |
| Chipepo       | 11 (73)       | 4.1 (123)     | 5 (120)       | 0.076    | 0.766    |
| Chisamba      | 0.5 (109)     | 0.7 (139)     | 2 (150)       | 0.874    | 0.429    |
| Chohana       | 8.9 (79)      | 3.2 (124)     | 1 (97)        | 0.101    | 0.283    |
| Chunga        | 9.6 (81)      | 4.2 (95)      | 1.9 (104)     | 0.83     | 0.352    |
| Mwanachingwala| 12.2 (66)     | 0.152         | 1.5 (131)     | 0.273    | 0.221    |
| Mulungushi    | 15.2 (46)     | 4.6 (131)     | 8.1 (123)     | 0.0172   | 0.326    |
| Munenga       | 1.5 (134)     | 0 (138)       | 0 (134)       | 0.22     | 0        |
| Myooye        | 0 (117)       | 0 (140)       | 3 (133)       | 0.083    |          |
| Rufunsia      | 23.1 (104)    | 40.7 (135)    | 58.2 (141)    | 0.0275   | 0.078    |
| All           | 6.9 (1823)    | 4.9 (2167)    | 6.8 (2192)    | 0.036    | 0.031    |
| IRS           | 6.3 (605)     | 1.8 (548)     | 4.0 (655)     | 0.10     | 0.22     |
| ITN           | 7.2 (1218)    | 5.9 (1619)    | 7.9 (1537)    | 0.60     | 0.11     |

*PI = Prevalence of infection,% 95% CI = 95% Confidence interval Bold = Change since 2008 was statistically significant.

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**Mosquito species identification**

Anopheline mosquitoes were identified morphologically as *Anopheles gambiae* Giles complex, *An. funestus* Giles group or *An. nili* Theobald group [12,13]. Sibling species were identified and *An. gambiae s.s* were identified as M or S molecular form using PCR [14–18].

**Malaria transmission**

DNA was extracted from the head and thorax of mosquitoes and tested for the presence of *P. falciparum* sporozoites [19]. Numbers of mosquitoes per trap per night were calculated for each vector species. Using the species specific estimated sporozoite prevalence the number of infectious mosquitoes per species per trap per night was calculated. The relative transmission index, defined as the ratio of infective numbers of mosquitoes per trap per night, relative to the baseline year of 2008, could not be computed because none of the specimens collected contained sporozoites [20].

**Insecticide Resistance**

Mosquitoes were collected during the rainy seasons of 2009 and 2010 from 17 localities. *Anopheles* mosquitoes were collected between 06.00 and 08.00 either by larval collections from breeding sites, or as blood fed adult females resting inside homes. Collected mosquitoes were transported to the laboratory, and reared to adults or transferred to individual oviposition tubes and tubes
allowed to lay eggs that were reared to adults at 26±2°C and 70–80% relative humidity.

Insecticide susceptibility assays were carried out on a random sample of 1–3 day old, sugar fed F1 and F0 adults following the procedure described by the World Health Organization (WHO 1998). Insecticides tested included bendiocarb (0.01%), DDT (4%), deltamethrin (0.05%), lambda-cyhalothrin (0.05%), malathion (5%), permethrin (0.75%), propoxur (0.1%).

Both Leu-Phe (west) and Leu-Ser (east) kdr mutations were investigated [21,22] using the taqman PCR [23].

Results

Malaria Prevalence in Children 1 to 14 years old

A total of 1,823 children aged between 1 to 14 years were tested for P. falciparum parasitaemia in all sentinel sites except Manueli and Nyamankalo in April/May 2008. Follow-up surveys were carried out in all sites in 2009 and 2010 with 2,176 children and 2,192 children respectively. The combined prevalence of infection with P. falciparum across all sites was 6.9% (95% CI = 3.9–12.1) in 2008, 4.9% in 2009 (95% CI 1.7–13.4) and 6.8% in 2010 (CI = 2.2–18.9) (Table 1).

No significant change in prevalence was observed in all ITN sites between years 2008, 7.2 (95% CI = 3.3–15.2), 2009, 5.9 (CI 95% = 1.9–17.3) to 2010, 7.9 (95% CI = 2–26.3). In IRS sites there was a significant overall decline in prevalence of infection between 2008 and 2009 from 6.3 (95% CI = 3.4–11.5) to 1.8 (95% CI = 0.4–8.4) (OR = 0.28, 95% CI = 0.05–1.44, P = 0.036). However, prevalence increased again in 2010 to 4.0 (95% CI = 2.47–5.47) (OR = 2.22, 95% CI = 0.57–8.65, P=0.031) (Table 1).

Mosquito Species Abundance, sporozoite rate and transmission

A total of 619 An. gambiae s.l. and 228 An. funestus s.l. were collected and morphologically identified from 108 window exit traps operating continuously from April 2008 to May 2010. Five hundred and twenty five An. gambiae s.l. were subsequently identified to species level. There were four An. gambiae s.s, 199 An. arabiensis and 322 An. quadriannulatus. Two hundred and four An. funestus s.l were identified to species, these were 14 An. funestus s.s, 98 An. parensis, 20 An. rivulorum, 18 An. leesoni, 16 An. vaneeldeni, 14 were identified as the recently described An. funestus-like, 1 An. nili s.s and 23 unidentified. Culicine mosquitoes were recorded in the absence of Anopheles to demonstrate that the traps were being operated correctly.

At 217, the numbers of actual vectors, An. gambiae s.s, An. arabiensis and An. funestus s.s. trapped was extremely low. All of these were tested for sporozoites and all were negative. Due to zero sporozoites a transmission index could not be calculated (Table 2).

Insecticide susceptibility

A total of 1,742 An. gambiae s.s. and 796 An. funestus s.s., from 17 localities, 11 of which were sentinel sites were assayed for insecticide susceptibility using the WHO protocol [24]. Prior to 2009, no resistance had been detected to any insecticide in An. gambiae s.l or An. funestus in Zambia (Table 3 and Table 4). Between 2009 and 2010 resistance to pyrethroids (deltamethrin,

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### Table 2. Vector Abundance, Infectivity by period of time and intervention type.

| Year          | All sites | All ITN sites | All IRS sites |
|---------------|-----------|---------------|---------------|
|               | 04/08-4/09 | 05/09-5/10 | 04/08-4/09 | 05/09-5/10 | 04/08-4/09 | 05/09-5/10 |
| **An. gambiae s.l.** |           |               |               |               |               |               |
| No. caught      | 409       | 210           | 395           | 195           | 14           | 15           |
| No. analyzed for species id | 360       | 167           | 354           | 157           | 6            | 10           |
| An. arabiensis propn (%) | 24.0      | 32.9          | 23.8          | 34.4          | 28.6         | 13.3         |
| An. gambiae s.s propn (%) | 0.49      | 0.95          | 0.51          | 1.03          | 0.00         | 0.00         |
| **An. gambiae s.s** |           |               |               |               |               |               |
| No. Estimated   | 2         | 2             | 2             | 2             | 0            | 0            |
| No per trap per 100 nights | 0.03      | 0.03          | 0.03          | 0.03          | 0.00         | 0.00         |
| No. Sporozoite positive | 0(n = 2)  | 0(n = 2)      | 0(n = 2)      | 0(n = 2)      | 0(n = 0)     | 0(n = 0)     |
| **An. arabiensis** |           |               |               |               |               |               |
| No. Estimated   | 113       | 86            | 104           | 83            | 9            | 3            |
| No per trap per 100 nights | 1.59      | 1.21          | 1.46          | 1.17          | 0.13         | 0.04         |
| No. Sporozoite positive | 0(n = 125) | 0(n = 98)   | 0(n = 104)    | 0(n = 92)     | 0(n = 9)     | 0(n = 6)     |
| **An. funestus s.l.** |           |               |               |               |               |               |
| No. caught      | 105       | 123           | 94            | 113           | 11           | 10           |
| No. analyzed for species id | 99        | 105           | 91            | 95            | 8            | 10           |
| An. funestus s.s | 8         | 5             | 8             | 4             | 0            | 1            |
| An. funestus s.s propn (%) | 7.62      | 4.07          | 8.51          | 3.54          | 0.00         | 10.00        |
| **An. funestus s.s** |           |               |               |               |               |               |
| No. Estimated   | 8         | 6             | 8             | 5             | 0            | 1            |
| No per trap per 100 nights | 0.12      | 0.08          | 0.12          | 0.07          | 0.00         | 0.01         |
| No. Sporozoite positive | 0(n = 8)  | 0(n = 9)      | 0(n = 8)      | 0(n = 6)      | 0(n = 0)     | 0(n = 1)     |

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lambda-cyhalothrin and permethrin) and DDT was detected in both An. gambiae s.s. and An. funestus s.s. No resistance was detected to the carbamate (bendiocarb) or the organophosphate (malathion) in either species. Limited numbers of mosquitoes were found at all sites, which correlates with the species abundance calculated from the window trap collections.

Prior to interventions mosquitoes from tested from 8 locations in Zambia were fully susceptible to the WHO discriminating dosage of deltamethrin, lambda-cyhalothrin, DDT, propoxur and malathion in 1999. Three of these locations, Chipulukusu, Mukobeko and Mushili had IRS introduced in 2003. In 2010, significant (P < 0.001) resistance was detected in An. gambiae s.s. in Chipulukusu to deltamethrin (13.5%), permethrin (61%) and DDT (43%) and Mushili to DDT (55%). In Mukobeko no resistance was detected in An. gambiae s.s. to deltamethrin but a slight increase in resistance was detected in An. funestus s.s. (96% P<0.1). In Chibombo where only ITNs have been distributed resistance to deltamethrin was detected in An. funestus s.s. (88.9% P<0.001) in 2010 (Table 3 & 4 and Figure 2).

In 2010 new sites were surveyed for resistance. In the IRS sites An. funestus s.s. was susceptible to deltamethrin and DDT in Kabulongo, Katete and Mufweshya, but had low level resistance to deltamethrin (95.6%) and DDT (98%) in Kafue. Resistance to DDT was (3.8%) and deltamethrin (95.2%) was detected in An. gambiae s.s. from Kizingezinge.

Similarly in 2010 nine new sites that had only received ITNs as interventions were surveyed for insecticide resistance. In An. gambiae s.s. resistance to deltamethrin ranged from 41.8–100% in these sites with resistance to lambda-cyhalothrin being detected in Nyamankalo (83.3%) and DDT in Myooye (69%). Similarly a range of resistance was detected to deltamethrin in An. funestus s.s. (66.6–100%), permethrin resistance was also detected in Nanga Farms (90.9%) and to DDT in Myooye (94%) and Nyamankalo (88%) (Table 3 &4).

### Table 3. WHO susceptibility test results on 1–3 dayold An. gambiae s.s of 17 localities in Zambia.

| Location | 1999 | 2009-2010 |
|----------|------|-----------|
|          | deltamethrin (0.05%) | permethrin (0.75%) | λ-cyhalothrin (0.05%) | DDT (4%) | Malathion (5%) | Bendiocarb (0.01%) |
| ITN only sites | | | | | | |
| Chibombo* | 100 | - | - | - | - | 11 | 100 |
| Chingola* | 15 | 100 | - | - | - | 5 | 100 |
| Chipulukusu | 46 | 100b | 121 | 100b | | |
| Livingstone10 | 17 | 100 | - | - | - | 13 | 100 |
| Lusaka10 | 7 | 100 | - | - | - | 6 | 100 |
| Mukobeko10 | 19 | 100a | - | - | - | 11 | 100 |
| Mushili | - | - | - | 73 | 100b | | |
| Samfya10 | 8 | 100 | - | - | - | 5 | 100 |

% = percentage mortality.
a = p<0.1,
b = p<0.001,
* = 1999 Unpublished baseline data collected by TDRC,
# = 1999 Unpublished data collected by NMCP.
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Insecticide Resistance Impact on Malaria Control

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Molecular forms and \textit{kdr} mutations

In four localities with pyrethroid and DDT resistant \textit{An. gambiae} and one (Chipepo) with high pyrethroid resistance, PCR assays, to check for the presence of east (leu-ser) (Ranson et al. 2000) and west (leu-phe) (Martinez-Torres et al. 1998) \textit{kdr} mutations were carried out on a random sample. All 159 \textit{An. gambiae s.s} were identified as the molecular S-form and all were negative for east \textit{kdr}. In four of the sites, Chipepo, Chipulkusu, Kizhingezhinge and Mushili all samples carried the west \textit{kdr} mutation. In Myooye, only one sample was homozygous for west \textit{kdr} (Table 5).

Discussion

The Roll Back Malaria, United Nations Millennium Development Goals and World Health Assembly universal access and coverage targets for malaria prevention and treatment have been established to stimulate the reduction in disease transmission. To meet these targets malaria control interventions are now being scaled up [25,26]. In order to obtain and maintain these goals there is a need for continuous surveillance, monitoring and evaluation of malaria control programmes to make informed decisions and guide control efforts.

In Zambia, the initial re-deployment of vector control interventions, ITN (1999) and IRS (2000), was based on minimal empirical evidence. Subsequent monitoring and evaluation has allowed more informed decisions to be made on targeting these interventions. Zambia has achieved high coverage of IRS (20% of households) and ITNs (60% plus of households) as of 2008 [9], and now needs to sustain these efforts.

Prevalence of parasites in children has been frequently used as a surrogate measure for malaria transmission intensity [27]. Previous studies conducted in Zambia, prior to or early into the re-introduction of interventions, as population based surveys, or from hospital based routine surveillance show widely heterogeneous results [7,28,29]. In this study, the overall average \textit{P. falciparum} prevalence in children between the ages of 1 and 14

\begin{table}
\centering
\caption{WHO susceptibility test results on 1-3-d-old \textit{An. funestus s.s} of 17 localities in Zambia.}
\begin{tabular}{|l|c|c|c|c|c|c|c|c|c|}
\hline
\textbf{Location} & \textbf{1999} & & & & & & & & \\
 & & \textbf{deltamethrin (0.05\%)} & \textbf{\textit{\lambda}-cyhalothrin (0.05\%)} & \textbf{DDT (4\%)} & \textbf{Propoxur (0.01\%)} & \textbf{Malathion (5\%)} & & \\
 & \textbf{n} & \textbf{%} & \textbf{n} & \textbf{%} & \textbf{n} & \textbf{%} & \textbf{n} & \textbf{%} & \\
\hline
\textbf{ITN only sites} & & & & & & & & & \\
Chibombo* & 72 & 100 & - & - & - & - & 19 & 100 & - & - \\
Chingola* & - & - & - & - & - & - & 3 & 100 & 16 & 100 \\
Livingstone & 5 & 100 & 7 & 100 & 6 & 100 & - & - & - & - \\
Mukobeko# & 25 & 100 & 15 & 100 & 25 & 100 & - & - & - & - \\
Mushili* & 4 & 100 & - & - & - & - & - & - & 4 & 100 \\
\hline
\textbf{2009-2010} & & & & & & & & & \\
\textbf{Location} & \textbf{deltamethrin (0.05\%)} & \textbf{permethrin (0.75\%)} & \textbf{\textit{\lambda}-cyhalothrin (0.05\%)} & \textbf{DDT (4\%)} & \textbf{Bendiocarb (0.01\%)} & & & & \\
& \textbf{n} & \textbf{%} & \textbf{n} & \textbf{%} & \textbf{n} & \textbf{%} & \textbf{n} & \textbf{%} & \textbf{n} & \textbf{%} & \\
\hline
\textbf{ITN only sites} & & & & & & & & & \\
Chibombo* & 9 & 88.9 & - & - & - & - & - & - & - & - \\
Chipepo & 4 & 100 & - & - & - & - & - & - & - & - \\
Manuvi & 11 & 72.7 & - & - & - & - & - & - & - & - \\
Mulungushi & - & - & - & - & 4 & 100 & - & - & 7 & 100 \\
Mwanachingwala & 22 & 81.8 & - & - & - & - & - & - & - & - \\
Myooye & 27 & 96.2 & - & - & - & - & 62 & 94 & - & - \\
Nanga Farms & 30 & 100 & 11 & 90.9 & - & - & 10 & 100 & - & - \\
Nyamankalo & 87 & 80.5 & - & - & - & - & 33 & 88 & - & - \\
Rufunsa & 66 & 66.6 & - & - & - & - & - & - & - & - \\
\textbf{IRS sites} & & & & & & & & & \\
Kabulongo & 15 & 80 & - & - & - & - & 14 & 100 & - & - \\
Kafue & 23 & 95.6 & - & - & - & - & 90 & 98 & - & - \\
Katete & - & - & - & - & - & - & 5 & 100 & - & - \\
Mufweshya & 18 & 100 & - & - & - & - & 21 & 100 & - & - \\
Mukobeko# & 26 & 96 & - & - & - & - & - & - & - & - \\
\hline
\end{tabular}
\end{table}

\% = percentage mortality.
\* = p>0.1
\* = p<0.001
\(* = 1999 Unpublished baseline data collected by TDRC,
\# = 1999 Unpublished data collected by NMCP.

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years was below 10% implying low transmission. This low prevalence has been achieved in part due to effective vector control interventions. Between 2003 and 2008 approximately 5.9 million ITNs (since 2007 all have been LLINs) have been distributed and IRS expanded from 5 to 36 districts, increasing the number of structures sprayed from less than 100,000 to over 1 million [9]. At the same time Zambia expanded clinical control of malaria through improved diagnostics (microscopy and RDTs), introducing ACTs into all clinics and expanding the network of community health workers.

Species density and infectivity measures are important indicators of malaria transmission [20,30]. The endophilic nature of An. funestus s.s. and An. gambiae s.s makes these species susceptible to both IRS and ITNs, which reduce abundance and sporozoite rates [20,31]. Anopheles arabiensis on the other hand is generally exophilic and may be the cause of underlying transmission in Zambia, a topic that warrants further investigation. In this study, both IRS and ITNs are associated with a lower abundance of An. gambiae s.s., and An. funestus s.s. than An. arabiensis. However, IRS is associated with lower abundance than ITNs, with no An. gambiae s.s collected from window traps in IRS areas. This may account for the greater impact of IRS on prevalence. Similar results of IRS having a more prompt and powerful impact than ITNs on species abundance has been observed [32,33]. The apparent elimination of An. gambiae s.s in IRS areas and suppression of An. funestus s.s. and An. arabiensis to minimal levels, coupled with the absence of vector infectivity in both IRS and ITNs settings is striking. This effect of reducing abundance and infectivity of malaria vectors results in community wide protection [34,35].

In contrast to the current study, where no infected mosquitoes were collected, previous studies before and early into vector control in Zambia found P. falciparum sporozoites in all three major vectors (An. gambiae s.s., An. arabiensis and An. funestus s.s.) [36–38], although this may in part be a function of the different collection methods employed. The identification of An. nili and An. funestus-like species in Zambia, as well as the presence of An. rivulorum increases our knowledge of the range of these species. Further work to assess their transmission potential in Zambia is necessary.

Indoor interventions have been shown to significantly reduce transmission in neighboring Tanzania. However, a residual transmission of disease remained through outdoor biting of An. gambiae s.l. [39]. This may account in part for the transmission that is still observed here, which will not have been detected in the methods used. This requires further investigation if the control programme is to reduce prevalence further.

The evidence of pyrethroid resistance spreading in Africa is mounting [20,40,41] and in some cases has resulted in policy changes in vector control interventions [20,41,42]. High levels of

Table 5. Detection of Leu-Phe (West) kdr in survivors populations of An. gambiae s.s. tested for DDT and pyrethroid resistance.

| Location         | Homozygous Leu-Phe mutation | Heterozygous Leu-Phe mutation | Homozygous wild type |
|------------------|------------------------------|------------------------------|---------------------|
| ITN only sites   |                              |                              |                     |
| Chipepo          | 21                           | 0                            | 0                   |
| Myooye           | 1                            | 0                            | 10                  |
| IRS sites        |                              |                              |                     |
| Chipulukusu      | 45                           | 1                            | 0                   |
| Kizhingezhinge   | 20                           | 0                            | 0                   |
| Mushili          | 58                           | 3                            | 0                   |

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pyrethroid and DDT resistance were detected for the first time in both the An. gambiae s.s and An. funestus s.s in Zambia during this study, and these findings were subsequently confirmed by other groups. There was significant variation in the frequency of resistance detected between IRS and ITN localities, with higher levels of resistance (P < 0.0001) being detected in IRS areas compared to ITNs areas.

Pyrethroid-DDT cross resistance from a common kdr mechanism, has been reported in An. gambiae s.s in Africa [21,40,43]. Samples of An. gambiae s.s that were pyrethroid and DDT resistant were tested for east and west kdr mutations. The west kdr mutation was found in all sites tested. This is the most southerly documented detection of this mutation. It is not yet clear whether this resistance has arisen de novo in Zambia or whether it has spread from other locations in Africa, but analysis of introns within the kdr gene would clarify the origin of the resistance. Anopheles funestus was also resistant to pyrethroids and DDT at a high frequency. To date there have been no reports of sodium channel mutations in An. funestus. The DDT and pyrethroid resistance detected here could arise from two separate metabolic resistance mechanisms, GST and P450 [44] respectively, or may be the first instance of kdr-type resistance in this species documented. Sequencing of the relevant areas of the sodium channel gene in resistant specimens of An. funestus is needed to clarify this. However it is notable that the pyrethroid resistant An. funestus found in Mozambique is not accompanied by resistance to DDT [41,45]. The detection of multiple resistance in both major vectors of malaria in Zambia may have grave implications for the malaria control programme. It may compromise the efficacy of interventions and potentially lead to the failure of IRS and ITNs based vector control. While this data serves to highlight the threats more information is required on all the underlying mechanisms of insecticide resistance so that a suitable insecticide resistance management plan may be put in place to sustain vector control.

Monitoring malaria cases in Kwa-Zulu Natal, South Africa, picked up the failure of pyrethroids in the IRS programme in the 1990s due to P450 mediated pyrethroid-resistance selection in An. funestus, resulting in IRS being successfully reintroduced [46], The resulting decline in malaria was reinforced by a change in drug treatment policy [47]. In Bioko Island, Equatorial Guinea, IRS with pyrethroids had a significant impact on the abundance of An. funestus but not on An. gambiae. However, a reduced sporozoite level in An. gambiae was observed that will have lowered transmission. With the detection of high frequencies of kdr in An. gambiae on the island a policy change to carbamate was made which appeared to significantly reduce the abundance of An. gambiae and maintained the low levels of An. funestus [20].

In 2000 the percentage of children aged 1–5 years positive for parasites fell from 25% to <15% in Zambia due to increased malaria control efforts (Chizema-Kawesha, 2010). The monitoring, evaluation and surveillance carried out over a three year period demonstrates the success of Zambia’s control programme in reducing the burden of malaria further as signified by reduced prevalence of malarial infection to 6.8%. However, the recent detection of high insecticide resistance to pyrethroids and DDT in An. gambiae and An. funestus along with kdr detection in An. gambiae threatens the sustainability of this programme. While the lack of sporozoite positive mosquitoes in the window traps is consistent with reduced levels of transmission the rebound in prevalence of P. falciparum in IRS areas may be due to the increased levels of insecticide resistance and the beginning of control programme failure.

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Author Contributions

Conceived and designed the experiments: MC IK EC-K. Performed the experiments: MC IK EC-K AMR VM. Analyzed the data: MC IK EC-K AMR VR FNP MK VM. Contributed reagents/materials/analysis tools: MC SC. Wrote the paper: EC-K IK JH MC. Managed the project in Zambia: EC KSB. Completed field collection: DM EC MC MK.

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