

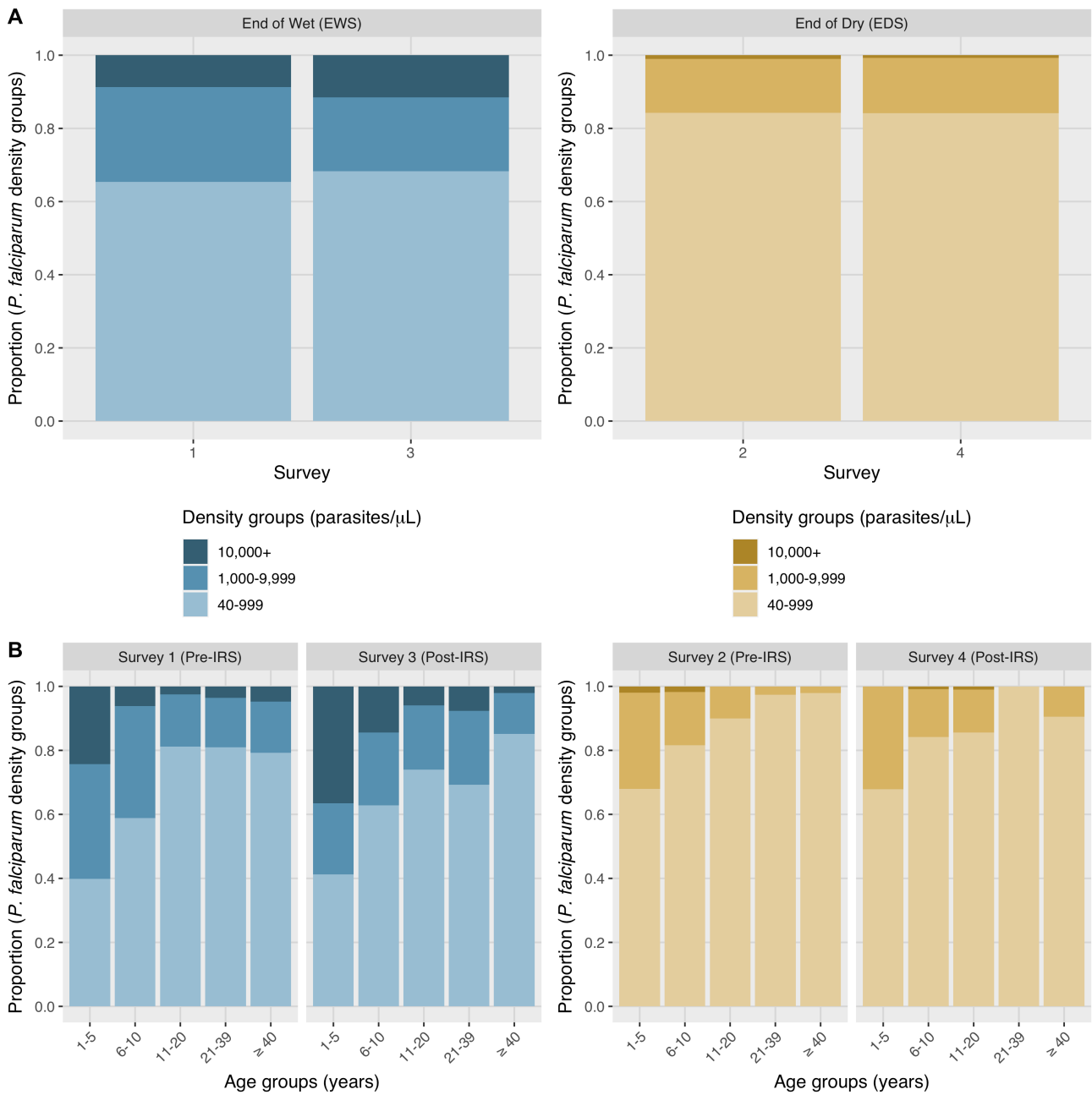
## Supporting Information (Figures and Tables)

# Indoor residual spraying with a non-pyrethroid insecticide reduces the reservoir of *Plasmodium falciparum* in a high-transmission area in northern Ghana

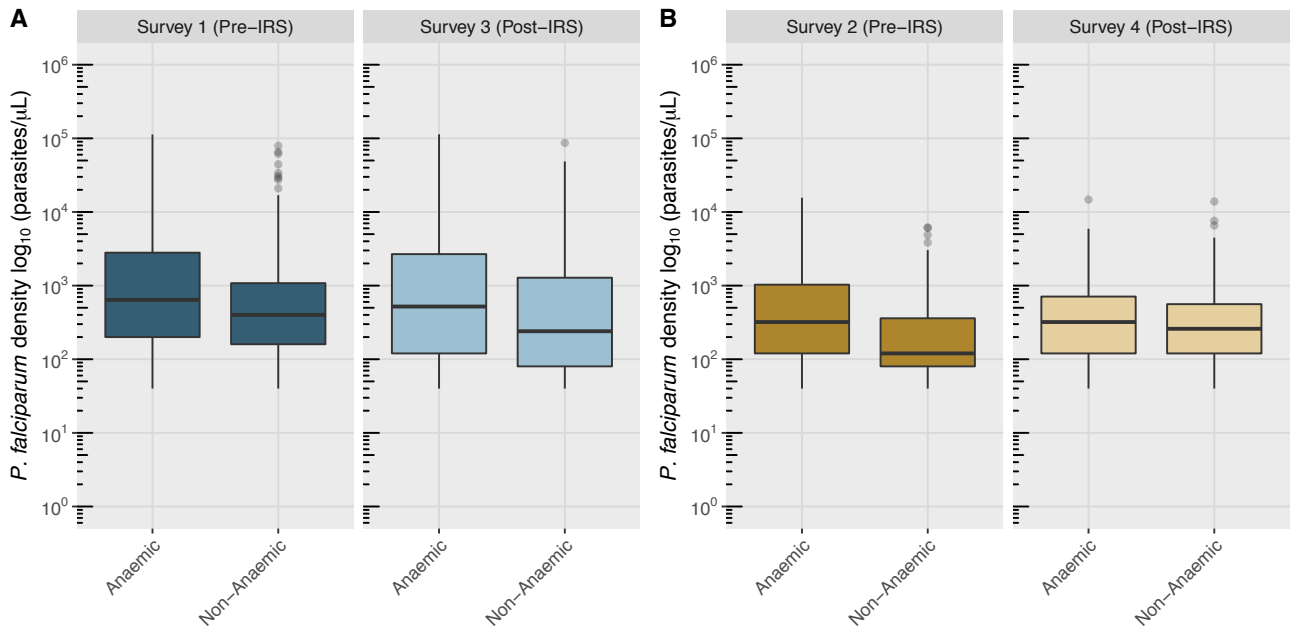
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<sup>¶</sup>KET and ARO contributed equally to this work as first authors.

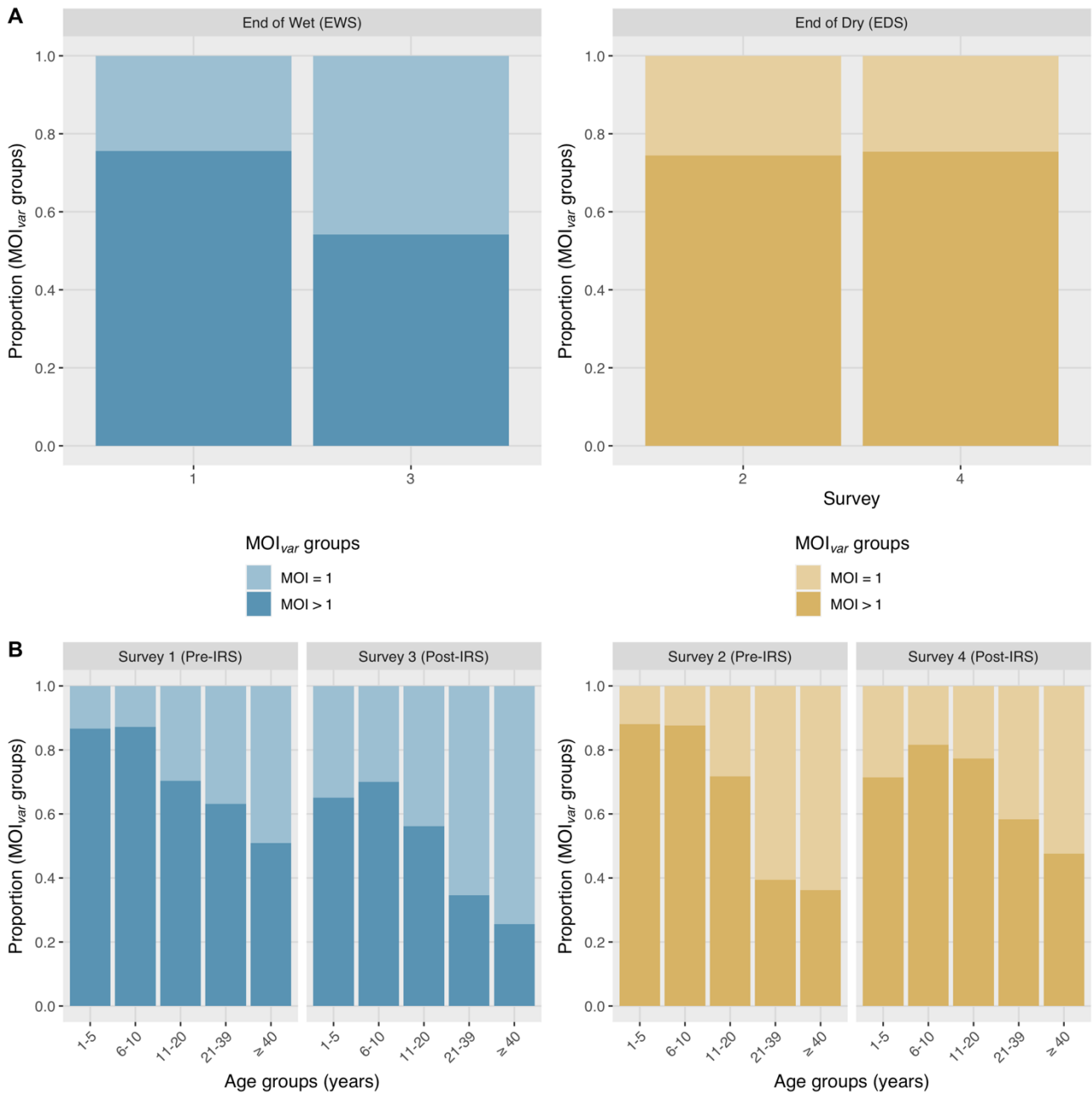
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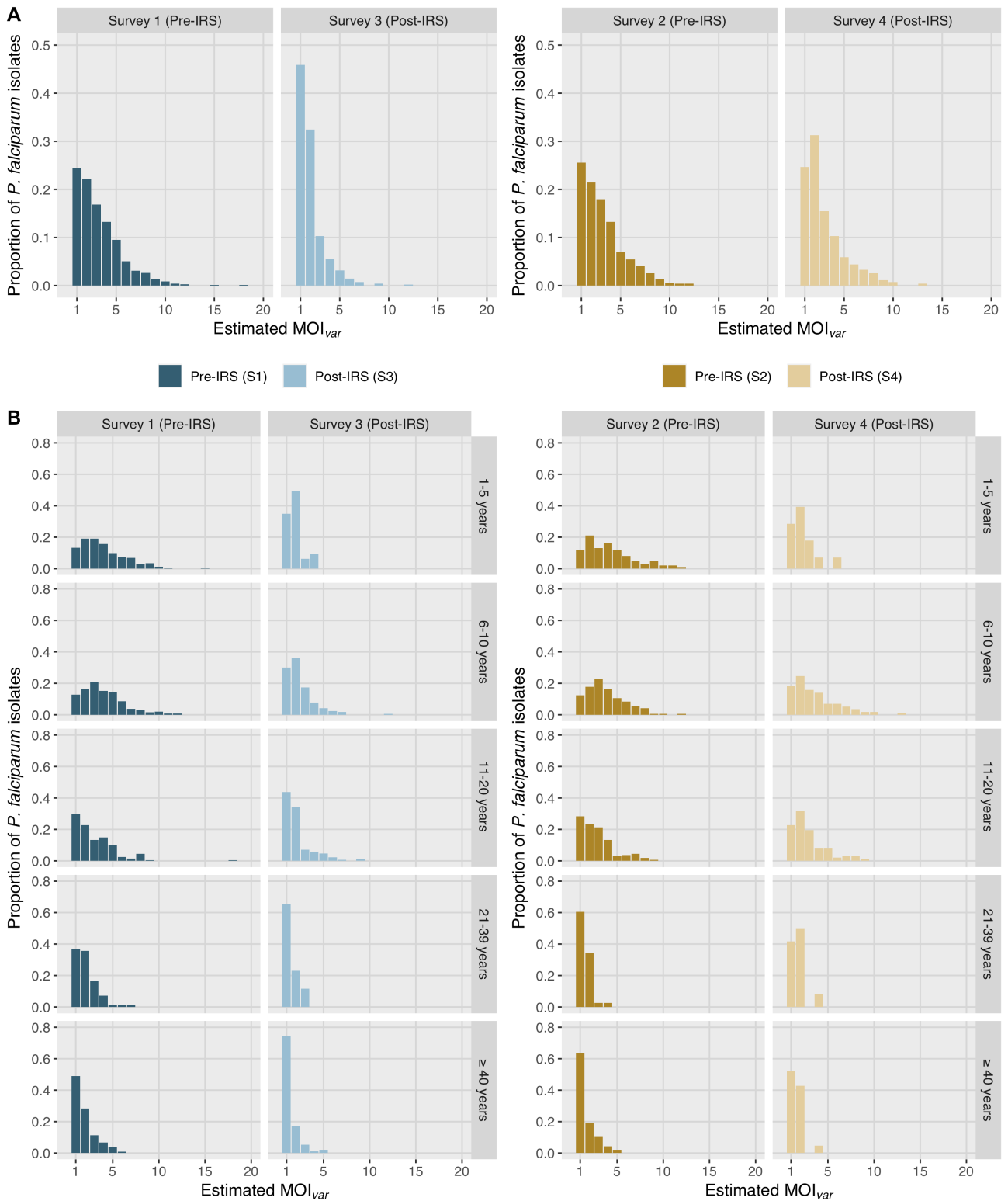
**S1 Fig.** The proportion microscopic *P. falciparum* infections categorized into low (40-999 parasites/ $\mu$ L), moderate (1,000-9,999 parasites/ $\mu$ L), and high ( $\geq$  10,000 parasites/ $\mu$ L) parasite densities groups pre- to post-IRS at the end of the wet (blue) and dry (gold) seasons. Proportion of microscopic *P. falciparum* infections categorized pre- to post-IRS at the **A.** end of the wet and dry season surveys and **B.** across each age group (years) at the end of the wet and dry season surveys.



**S2 Fig. Density of microscopic *P. falciparum* infections grouped based on anaemia status pre- to post-IRS at the end of the wet (blue) and dry (gold) seasons.** Box and whisker plots of the log-transformed microscopic *P. falciparum* infection densities (parasites/ $\mu\text{L}$ ) grouped based on anaemia status pre- to post-IRS **A.** at the end of the wet season surveys and **B.** at the end of the dry season surveys. The boxes represent the inter-quartile ranges (IQR) and the horizontal lines represent the median log<sub>10</sub>-transformed *P. falciparum* infection densities (parasites/ $\mu\text{L}$ ). The whiskers are used to depict the largest and smallest log<sub>10</sub>-transformed infection densities and the grey dots outside the whiskers are used to denote outliers. The parasite densities were log<sub>10</sub>-transformed to remove skewness. Anaemia status was defined according to the WHO guidelines for age and gender (S1 Table). (Median *P. falciparum* density (value/ $\mu\text{L}$ ), Inter Quartile Range [IQR] in anaemic vs. non-anaemic: Survey 1 (640 [200 – 2,800] vs. 400 [160 – 1,080], respectively), Survey 2 (320 [120 – 1,030] vs. 120 [80 – 360], respectively), Survey 3 (520 [120 – 2,690] vs. 240 [80 – 1,280], respectively), Survey 4 (320 [120 – 710] vs. 250 [120 – 560], respectively).



**S3 Fig.** The proportion of microscopic *P. falciparum* infections categorized as single-genome (estimated  $MOI_{var} = 1$ ) or multi-genome (estimated  $MOI_{var} > 1$ ) pre- to post-IRS at the end of the wet (blue) and dry (gold) seasons. Proportion of microscopic *P. falciparum* infections categorized pre- to post-IRS at the **A.** end of the wet and dry season surveys and **B.** across each age group (years) at the end of the wet and dry season surveys.



**S4 Fig. Relative  $MOI_{var}$  frequency distributions pre- to post-IRS at the end of the wet (blue) and dry (gold) seasons.** On the horizontal axis are the discrete estimated  $MOI_{var}$  categories (i.e., range  $MOI_{var}$  1-20) for each microscopic *P. falciparum* infection. The vertical axis depicts the relative proportion of infections found in each of these  $MOI_{var}$  categories at the **A.** end of the wet and dry season surveys and **B.** across each age group (years) at the end of the wet and dry season surveys. Please S4 Table for the number of *P. falciparum* infections during each survey.

S1 Table. Demographic characteristics of the study population during each survey.

Demographic characteristics <sup>a</sup>	Pre-IRS		Post-IRS	
	Survey 1 End of wet season (October 2012)	Survey 2 End of dry season (May/June 2013)	Survey 3 End of wet season (October 2015)	Survey 4 End of dry season (May/June 2016)
<b>Age groups<sup>b</sup></b>				
All	1923	1902	2022	2091
1-5 years	356 (18.5)	351 (18.5)	405 (20.0)	358 (17.1)
6-10 years	395 (20.5)	404 (21.2)	409 (20.2)	425 (20.3)
11-20 years	413 (21.5)	406 (21.3)	467 (23.1)	514 (24.6)
21-39 years	326 (17.0)	315 (16.6)	297 (14.7)	331 (15.8)
≥ 40 years	433 (22.5)	426 (22.4)	444 (22.0)	463 (22.2)
<b>Sex<sup>c</sup></b>				
Female	1031 (53.6)	1055 (55.5)	1093 (54.1)	1124 (53.8)
Male	892 (46.4)	847 (44.5)	929 (45.9)	967 (46.2)
<b>Catchment area<sup>d</sup></b>				
Vea/Gowrie	919 (47.8)	925 (46.8)	1000 (49.5)	1026 (49.1)
Soe	1004 (52.2)	977 (51.4)	1022 (50.5)	1065 (50.9)
<b>LLIN usage (previous night)</b>				
No	210 (10.9)	286 (15.0)	191 (9.4)	274 (13.1)
Yes	1713 (89.1)	1616 (85.0)	1831 (90.6)	1817 (86.9)
<b>Antimalarial treatment (previous 2-weeks)<sup>e</sup></b>				
No treatment	1127 (58.6)	1748 (91.9)	1645(81.4)	1922 (91.9)
Treatment	796 (41.4)	154 (8.1)	298 (14.7)	157 (7.6)
Don't know	0 (0)	0 (0)	79 (3.9)	12 (0.6)
<b>Anaemia Status<sup>f</sup></b>				
Anaemic	896 (46.7)	605 (32.2)	829 (41.1)	602 (28.8)
Non-anaemic	1,022 (53.3)	1,275 (67.8)	1,188 (58.9)	1,487 (71.2)

<sup>a</sup> Data reflect the number (% (n/N)) of subjects.

<sup>b</sup> During each survey a similar proportion of participants were surveyed in all the age groups.

<sup>c</sup> During each survey a similar proportion of female and male participants were surveyed in each age category, except for the adult age groups (>20 years) where significantly more females than males were surveyed ( $p$ -value  $\leq 0.038$ ).

<sup>d</sup> During each survey a similar proportion of participants were surveyed in each catchment area.

<sup>e</sup> Indicates those participants who reported they were sick, sought treatment, and were provided with an antimalarial treatment in the previous two-weeks.

<sup>f</sup> Anaemia status was defined according to the WHO guidelines for age and gender. Participants in each Survey were excluded from the anaemia status category if their haemoglobin was not measured on the day the survey was conducted: Survey 1 (N = 5), Survey 2 (N = 22), Survey 3 (N = 5), and Survey 4 (N = 2).

S2 Table. Parasitological parameters and DBL $\alpha$  type sequencing data for the microscopic *P. falciparum* infections during each survey.

Parasitological parameters	Pre-IRS		Post-IRS	
	Survey 1 End of wet season (October 2012)	Survey 2 End of dry season (May/June 2013)	Survey 3 End of wet season (October 2015)	Survey 4 End of dry season (May/June 2016)
<b>Number of participants</b> <sup>a</sup>	1923 (100)	1902 (100)	2022 (100)	2091 (100)
<b>Microscopic <i>P. falciparum</i> prevalence</b> <sup>b</sup>	808 (42.0)	513 (27.0)	545 (27.0)	272 (13.0)
<b>Age groups</b>				
1-5 years	173 (48.6)	100 (28.5)	63 (15.6)	28 (7.8)
6-10 years	243 (61.5)	169 (41.8)	167 (40.8)	114 (26.5)
11-20 years	202 (48.9)	159 (39.2)	169 (36.2)	97 (18.9)
>20 years	190 (25.0)	85 (11.5)	146 (19.7)	33 (4.2)
<b>Microscopic <i>P. falciparum</i> isolates with DBL<math>\alpha</math> type sequencing data</b> <sup>c</sup>	685 (84.8)	440 (85.8)	413 (75.8)	238 (87.5)
<b>Age groups</b>				
1-5 years	158 (91.3)	94 (94.0)	51 (81.0)	25 (89.3)
6-10 years	217 (89.3)	156 (92.3)	146 (87.4)	103 (90.4)
11-20 years	167 (82.7)	136 (85.5)	129 (76.3)	85 (87.6)
>20 years	143 (75.3)	54 (63.5)	87 (59.6)	25 (75.8)

<sup>a</sup> Number of participants surveyed that were analysed by microscopy.

<sup>b</sup> Data reflect the number (% (n/N)) of participants sampled that were microscopically positive for *P. falciparum* (including mixed *P. falciparum* infections) relative to the number of participants surveyed in the total population or by the age groups presented.

<sup>c</sup> Data reflect the number (% (n/N)) of microscopic *P. falciparum* isolates that had DBL $\alpha$  type sequencing data relative the number of participants surveyed that were microscopically positive for *P. falciparum* (including mixed *P. falciparum* infections) in the total population or by the age groups presented. *P. falciparum* isolates that had low DNA quality and/or sequencing quality (i.e., < 20 DBL $\alpha$  types, S3 Table) were removed when the sequencing dataset was cleaned (please see the Methods for additional details).

S3 Table. DBL $\alpha$  type sequencing results for all isolates that were positive for a *P. falciparum* infection by microscopy.

Survey	<i>P. falciparum</i> positive isolates sequenced	<i>P. falciparum</i> isolates with DBL $\alpha$ type sequencing data (i.e., $\geq 20$ DBL $\alpha$ types) <sup>a</sup>	<i>P. falciparum</i> isolates with no DBL $\alpha$ type sequencing data (i.e., 0 DBL $\alpha$ types) <sup>b</sup>	<i>P. falciparum</i> isolates with limited DBL $\alpha$ type sequencing data (i.e., 1 to $< 20$ DBL $\alpha$ types) <sup>c</sup>
1	808	685 (84.8)	66 (8.2)	57 (7.0)
2	513	440 (85.8)	45 (8.8)	28 (5.4)
3	545	413 (75.8)	35 (6.4)	97 (17.8)
4	272	238 (87.5)	13 (4.8)	21 (7.7)
TOTAL	2,138	1,776 (83.1)	159 (7.4)	203 (9.5)

<sup>a</sup> Data reflect the number (% (n/N)) of *P. falciparum* isolates that had DBL $\alpha$  type sequencing data relative to the number of participants sampled that had microscopically positive for *P. falciparum* (including mixed *P. falciparum* infections).

<sup>b</sup> Data reflect the number (% (n/N)) of microscopic *P. falciparum* isolates that had no DBL $\alpha$  type sequencing data relative to the number of participants sampled that were microscopically positive for *P. falciparum* (including mixed *P. falciparum* infections).

<sup>c</sup> Data reflect the number (% (n/N)) of microscopic *P. falciparum* isolates that had between DBL $\alpha$  type sequencing data (i.e., 1 to  $< 20$  DBL $\alpha$  types) relative the number of participants sampled that were microscopically positive for *P. falciparum* (including mixed *P. falciparum* infections).



S4 Table. Parasitological parameters of the *P. falciparum* infections during each survey.

Parasitological parameters	Pre-IRS		Post-IRS	
	Survey 1 End of wet season (October 2012)	Survey 2 End of dry season (May/June 2013)	Survey 3 End of wet season (October 2015)	Survey 4 End of dry season (May/June 2016)
<b>Number of participants<sup>a</sup></b>	1923 (100)	1902 (100)	2022 (100)	2091 (100)
<b>Microscopic <i>P. falciparum</i> prevalence<sup>b</sup></b>				
<b>Age groups</b>				
All	<b>808 (42.0)</b>	<b>513 (27.0)</b>	<b>545 (27.0)</b>	<b>272 (13.0)</b>
1-5 years	173 (48.6)	100 (28.5)	63 (15.6)	28 (7.8)
6-10 years	243 (61.5)	169 (41.8)	167 (40.8)	114 (26.5)
11-20 years	202 (48.9)	159 (39.2)	169 (36.2)	97 (18.9)
21-39 years	84 (25.8)	38 (12.1)	52 (17.5)	12 (3.6)
≥ 40 years	106 (24.5)	47 (11.0)	94 (21.2)	21 (4.5)
<b>Sex</b>				
Female	379 (36.8)	239 (22.6)	269 (24.6)	123 (10.9)
Male	429 (48.1)	274 (32.3)	276 (29.7)	149 (15.4)
<b>Catchment area</b>				
Vea/Gowrie	356 (38.7)	255 (27.6)	261 (26.1)	97 (9.5)
Soe	452 (45.0)	258 (26.4)	284 (27.8)	175 (16.4)
<b>Microscopic <i>P. falciparum</i> density<sup>c</sup></b>				
<b>Age groups</b>				
All	<b>520 [160-1640]</b>	<b>160 [80-560]</b>	<b>320 [120-1800]</b>	<b>280 [120-640]</b>
1-5 years	1640 [400-9840]	440 [120-1270]	1840 [240-19,940]	400 [80-1650]
6-10 years	760 [240-1840]	240 [120-680]	520 [200-2720]	380 [160-720]
11-20 years	320 [160-760]	120 [80-400]	280 [120-1000]	240 [160-480]
21-39 years	200 [120-720]	120 [40-160]	200 [80-1730]	120 [80-200]
≥ 40 years	200 [120-670]	80 [40-140]	120 [40-320]	120 [80-160]
<b>Sex</b>				
Female	520 [160-1620]	160 [80-460]	320 [80-1960]	280 [120-480]
Male	480 [160-1640]	200 [120-600]	320 [120-1300]	320 [120-720]
<b>Catchment area</b>				
Vea/Gowrie	360 [200-1200]	160 [80-520]	240 [80-1280]	280 [120-560]
Soe	680 [200-2170]	200 [80-600]	400 [120-2210]	280 [120-640]
<b>Submicroscopic <i>P. falciparum</i> prevalence<sup>d,e</sup></b>	1115 (100)		1473 (100)	
<b>Age groups</b>				
All	<b>612 (54.9)</b>		<b>295 (20.0)</b>	
1-5 years	82 (44.8)		36 (11.1)	
6-10 years	90 (59.2)		56 (23.1)	
11-20 years	145 (68.7)		93 (31.4)	
21-39 years	128 (52.9)		51 (20.9)	
≥ 40 years	167 (51.1)		57 (16.3)	
<b>Sex</b>				
Female	340 (52.1)		149 (18.1)	
Male	272 (58.7)		146 (22.5)	
<b>Catchment area</b>				
Vea/Gowrie	258 (45.1)		118 (16.0)	
Soe	354 (64.1)		177 (24.0)	

<sup>a</sup> Number of participants surveyed that were analysed by microscopy.

<sup>b</sup> Data reflect the number (% (n/N)) of participants that were microscopically positive for *P. falciparum* (including mixed *P. falciparum* infections).

<sup>c</sup> Median parasite density for microscopically positive *P. falciparum* (including mixed *P. falciparum* infections) (value/μL, Inter Quartile Range [IQR]) samples.

<sup>d</sup> Data reflect the number (% (n/N)) of participants sampled in Survey 1 and Survey 3 that were PCR positive (i.e., submicroscopic) for *P. falciparum* (including mixed *P. falciparum* infections).

<sup>e</sup> For the submicroscopic *P. falciparum* infections in Survey 3, there were 1,473 participants included in the analysis: exclusions were participants where a dried blood spot was not available for PCR (N=4).

S5 Table. Absolute decrease in the probability of having a microscopic *P. falciparum* infection post-IRS in Bongo at the end of the wet and dry seasons. Results are expressed in terms of Attributable Risk (AR) and Attributable Risk percentage (AR%).

Outcome	Demographic characteristics	Pre-IRS to Post-IRS End of wet season Survey 1 to Survey 3			Pre-IRS to Post-IRS End of dry season Survey 2 to Survey 4		
		AR (95% CI)	AR% (95% CI)	p-value	AR (95% CI)	AR% (95% CI)	p-value
Positive for a microscopic <i>P. falciparum</i> infection	All	0.151 (0.121, 0.180)	36.9 (29.9, 41.3)	< 0.001	0.140 (0.115, 0.164)	51.8 (44.9, 57.8)	< 0.001
	<b>Age Groups</b>						
	1-5 years	0.330 (0.268, 0.393)	68.0 (58.9, 75.1)	< 0.001	0.207 (0.152, 0.262)	72.6 (59.4, 81.5)	< 0.001
	6-10 years	0.207 (0.139, 0.275)	33.6 (23.6, 42.3)	< 0.001	0.150 (0.086-0.214)	35.9 (22.1, 47.2)	< 0.001
	11-20 years	0.127 (0.062, 0.192)	26.0 (13.6, 36.7)	< 0.001	0.203 (0.145, 0.261)	51.8 (40.2, 61.2)	< 0.001
	21-39 years	0.083 (0.018, 0.147)	32.1 (7.5, 50.1)	0.013	0.084 (0.043, 0.126)	70.0 (43.6, 84.0)	0.013
	≥ 40 years	0.033 (-0.022, 0.089)	13.5 (-10.4, 32.3)	0.243	0.065 (0.030, 0.100)	58.9 (32.4, 75.0)	0.243
	<b>Sex</b>						
	Female	0.122 (0.083, 0.161)	33.1 (23.6, 41.3)	< 0.001	0.117 (0.086, 0.148)	51.7 (41.0, 60.5)	< 0.001
	Male	0.184 (0.140, 0.228)	38.2 (30.3, 45.2)	< 0.001	0.169 (0.131, 0.208)	52.4 (43.2, 60.1)	< 0.001
<b>Catchment area</b>							
Vea/Gowrie	0.126 (0.085, 0.168)	32.6 (23.1, 41.0)	< 0.001	0.181 (0.147, 0.215)	65.7 (57.4, 72.4)	< 0.001	
Soe	0.172 (0.131, 0.214)	38.3 (30.4, 45.3)	< 0.001	0.100 (0.064, 0.135)	37.8 (26.2, 47.6)	< 0.001	

AR (Attributable Risk = (Pre-IRS Risk) - (Post-IRS Risk))

AR% (Attributable Risk Percentage = ((Pre-IRS Risk) - (Post-IRS Risk)/ (Pre-IRS Risk)) \* 100%)

CI=confidence interval

S6 Table. Association between the IRS and microscopic *P. falciparum* infection prevalence at the end of the wet seasons.

Factor	Microscopic <i>P. falciparum</i> infection <sup>a</sup>			
	Unadjusted		Adjusted <sup>b</sup>	
	OR (95% CI)	p-value	aOR (95% CI)	p-value
<b>IRS/Survey</b>				
Pre-IRS (Survey 1, October 2012)	1.00	-	1.00	-
Post-IRS (Survey 3, October 2015)	0.51 (0.45-0.58)	< 0.001	0.47 (0.40-0.54)	< 0.001
<b>Age groups</b>				
1-5 years	1.00	-	1.00	-
6-10 years	2.31 (1.88-2.85)	< 0.001	2.27 (1.84-2.79)	< 0.001
11-20 years	1.58 (1.29-1.94)	< 0.001	1.52 (1.23-1.89)	< 0.001
21-39 years	0.61 (0.48-0.79)	< 0.001	0.57 (0.44-0.74)	< 0.001
≥ 40 years	0.66 (0.52-0.82)	< 0.001	0.63 (0.50-0.80)	< 0.001
<b>Sex</b>				
Female	1.00	-	1.00	-
Male	1.44 (1.25-1.66)	< 0.001	1.27 (1.10-1.47)	0.001
<b>Catchment area</b>				
Vea/Gowrie	1.00	-	1.00	-
Soe	1.22 (1.06-1.41)	0.005	1.24 (1.08-1.44)	0.003
<b>LLIN usage (previous night)</b>				
No	1.00	-	1.00	-
Yes	0.76 (0.61-0.95)	0.015	0.79 (0.62-1.01)	0.058
<b>Antimalarial treatment (previous 2-weeks)</b>				
No treatment	1.00	-	1.00	-
Treatment	1.11 (0.96-1.28)	0.174	0.86 (0.73-1.01)	0.060

OR=odds ratio; aOR=adjusted odds ratio; CI=confidence interval, to deal with the repeated measures the cluster sandwich variance estimator was used

<sup>a</sup> Participants were excluded from the model if their antimalarial treatment in the previous two weeks was not known: Survey 3 (N = 79).

<sup>b</sup> Age group, sex, catchment area, LLIN usage the previous night, and antimalarial treatment in the previous two weeks are adjusted for in the multivariable logistic regression model.

S7 Table. Association between the IRS and microscopic *P. falciparum* infection prevalence at the end of the dry seasons.

Factor	Microscopic <i>P. falciparum</i> infection <sup>a</sup>			
	Unadjusted		Adjusted <sup>b</sup>	
	OR (95% CI)	<i>p</i> -value	aOR (95% CI)	<i>p</i> -value
<b>IRS/Survey</b>				
Pre-IRS (Survey 2, May/June 2013)	1.00	-	1.00	-
Post-IRS (Survey 4, May/June 2016)	0.41 (0.35-0.47)	< 0.001	0.36 (0.31-0.43)	< 0.001
<b>Age groups</b>				
1-5 years	1.00	-	1.00	-
6-10 years	2.34 (1.85-2.96)	< 0.001	2.42 (1.91-3.08)	< 0.001
11-20 years	1.75 (1.37-2.23)	< 0.001	1.86 (1.44-2.39)	< 0.001
21-39 years	0.38 (0.27-0.54)	< 0.001	0.37 (0.26-0.53)	< 0.001
≥ 40 years	0.38 (0.27-0.52)	< 0.001	0.38 (0.28-0.53)	< 0.001
<b>Sex</b>				
Female	1.00	-	1.00	-
Male	1.53 (1.29-1.80)	< 0.001	1.32 (1.11-1.58)	0.002
<b>Catchment area</b>				
Vea/Gowrie	1.00	-	1.00	-
Soe	1.23 (1.04-1.45)	0.013	1.33 (1.12-1.59)	0.001
<b>LLIN usage (previous night)</b>				
No	1.00	-	1.00	-
Yes	0.88 (0.71-1.10)	0.264	1.03 (0.81-1.30)	0.825
<b>Antimalarial treatment (previous 2 weeks)</b>				
No treatment	1.00	-	1.00	-
Treatment	0.56 (0.40-0.79)	0.001	0.53 (0.37-0.76)	< 0.001

OR=odds ratio; aOR=adjusted odds ratio; CI=confidence interval, to deal with the repeated measures the cluster sandwich variance estimator was used

<sup>a</sup> Participants were excluded from the model if their antimalarial treatment in the previous two weeks was not known: Survey 4 (N = 12)

<sup>b</sup> Age group, sex, catchment area, LLIN usage the previous night, and antimalarial treatment in the previous two weeks are adjusted for in the multivariable logistic regression model.

**S8 Table. Stratum-specific estimates for the association between the IRS and microscopic *P. falciparum* prevalence at the end of the wet seasons.** The reference for all comparisons was Survey 1 (pre-IRS, October 2012).

Factor	Microscopic <i>P. falciparum</i> infection <sup>a</sup>		
	Pre-IRS Survey 1 (October 2012) aOR <sup>b</sup>	Post-IRS Survey 3 (October 2015) aOR (95% CI) <sup>b</sup>	<i>p</i> -value
<b>Age groups</b>			
1-5 years	1.00	0.19 (0.13-0.27)	< 0.001
6-10 years	1.00	0.41 (0.31-0.56)	< 0.001
11-20 years	1.00	0.56 (0.42-0.73)	< 0.001
21-39 years	1.00	0.63 (0.43-0.94)	0.023
≥ 40 years	1.00	0.83 (0.59-1.18)	0.311

aOR=adjusted odds ratio; CI=confidence interval, to deal with the repeated measures the cluster sandwich variance estimator was used

<sup>a</sup> Participants were excluded from the model if their antimalarial treatment in the previous two weeks was not known: Survey 3 (N = 79).

<sup>b</sup> Age group, sex, catchment area, LLIN usage the previous night, and antimalarial treatment in the previous two weeks are adjusted for in the multivariable logistic regression model.

S9 Table. Stratum-specific estimates for the association between the IRS and microscopic *P. falciparum* infection prevalence at the end of the dry seasons. The reference for all comparisons was Survey 2 (pre-IRS, May/June 2013).

Factor	Microscopic <i>P. falciparum</i> infection <sup>a</sup>		
	Pre-IRS Survey 2 (May/June 2013) aOR <sup>b</sup>	Post-IRS Survey 4 (May/June 2016) aOR (95% CI) <sup>b</sup>	p-value
<b>Age groups</b>			
1-5 years	1.00	0.21 (0.13-0.33)	< 0.001
6-10 years	1.00	0.50 (0.38-0.68)	< 0.001
11-20 years	1.00	0.34 (0.25-0.46)	< 0.001
21-39 years	1.00	0.25 (0.12-0.52)	< 0.001
≥ 40 years	1.00	0.39 (0.23-0.65)	< 0.001
<b>Catchment area</b>			
Vea/Gowrie	1.00	0.24 (0.19-0.31)	< 0.001
Soe	1.00	0.49 (0.39-0.61)	< 0.001

aOR=adjusted odds ratio; CI=confidence interval, to deal with the repeated measures the cluster sandwich variance estimator was used

<sup>a</sup> Participants were excluded from the model if their antimalarial treatment in the previous two weeks was not known: Survey 4 (N = 12).

<sup>b</sup> Age group, sex, catchment area, LLIN usage the previous night, and antimalarial treatment in the previous two weeks are adjusted for in the multivariable logistic regression model.

S10 Table. Association between the IRS and *P. falciparum* infection (i.e., microscopic or submicroscopic) prevalence at the end of the wet seasons.

Factor	<i>P. falciparum</i> infection (i.e., microscopic or submicroscopic) <sup>a</sup>			
	Unadjusted		Adjusted <sup>b</sup>	
	OR (95% CI)	<i>p</i> -value	aOR (95% CI)	<i>p</i> -value
<b>IRS/Survey</b>				
Pre-IRS (Survey 1, October 2012)	1.00	-	1.00	-
Post-IRS (Survey 3, October 2015)	0.25 (0.22-0.29)	< 0.001	0.22 (0.19-0.26)	< 0.001
<b>Age groups</b>				
1-5 years	1.00	-	1.00	-
6-10 years	2.49 (2.01-3.09)	< 0.001	2.69 (2.15-3.35)	< 0.001
11-20 years	2.49 (2.02-3.06)	< 0.001	2.79 (2.22-3.50)	< 0.001
21-39 years	1.14 (0.92-1.42)	0.245	1.08 (0.85-1.37)	0.546
≥ 40 years	1.07 (0.87-1.30)	0.529	1.05 (0.84-1.31)	0.692
<b>Sex</b>				
Female	1.00	-	1.00	-
Male	1.43 (1.25-1.63)	< 0.001	1.37 (1.18-1.59)	< 0.001
<b>Catchment area</b>				
Vea/Gowrie	1.00	-	1.00	-
Soe	1.60 (1.40-1.83)	< 0.001	1.71 (1.47-1.98)	< 0.001
<b>LLIN usage (previous night)</b>				
No	1.00	-	1.00	-
Yes	0.72 (0.58-0.90)	0.004	0.82 (0.64-1.05)	0.110
<b>Antimalarial treatment (previous 2 weeks)</b>				
No treatment	1.00	-	1.00	-
Treatment	1.35 (1.17-1.56)	< 0.001	0.88 (0.74-1.04)	0.141

OR=odds ratio; aOR=adjusted odds ratio; CI=confidence interval, to deal with the repeated measures the cluster sandwich variance estimator was used

<sup>a</sup> Participants were excluded from the model if their (i) antimalarial treatment in the previous two weeks was not known: Survey 3 (N = 79) and/or (ii) the participant dried blood spot was not available for PCR (N=4). Age group, sex, catchment area, LLIN usage the previous night, antimalarial treatment in the previous two weeks.

<sup>b</sup> Age group, sex, catchment area, LLIN usage the previous night, and antimalarial treatment in the previous two weeks are adjusted for in the multivariable logistic regression model.

**S11 Table. Absolute decrease in the probability of having a *P. falciparum* infection (i.e., microscopic or submicroscopic) post-IRS in Bongo at the end of the wet season.** Results are expressed in terms of Attributable Risk (AR) and Attributable Risk percentage (AR%).

Outcome	Demographic characteristics	Pre-IRS to Post-IRS End of wet season Survey 1 to Survey 3		
		AR (95% CI)	AR% (95% CI)	p-value
Positive for a <i>P. falciparum</i> infection (i.e., microscopic or submicroscopic)	<b>All</b>	0.322 (0.293, 0.351)	43.6 (40.3, 46.8)	< 0.001
	<b>Age Groups</b>			
	1-5 years	0.466 (0.403, 0.529)	65.1 (58.1, 70.9)	< 0.001
	6-10 years	0.298 (0.238, 0.358)	35.3 (28.7, 41.4)	< 0.001
	11-20 years	0.277 (0.220, 0.334)	32.9 (26.6, 38.7)	< 0.001
	21-39 years	0.302 (0.227, 0.377)	46.5 (36.3, 55.1)	< 0.001
	≥ 40 years	0.290 (0.227, 0.354)	46.1 (37.4, 53.5)	< 0.001
	<b>Sex</b>			
	Female	0.315 (0.274, 0.355)	45.1 (40.2, 49.6)	< 0.001
	Male	0.330 (0.288, 0.372)	42.0 (37.3, 46.4)	< 0.001
	<b>Catchment area</b>			
	Vea/Gowrie	0.288 (0.245)	43.1 (37.7, 48.1)	< 0.001
Soe	0.351 (0.312, 0.391)	43.8 (39.4, 48.1)	< 0.001	

AR (Attributable Risk = (Pre-IRS Risk) - (Post-IRS Risk))

AR% (Attributable Risk Percentage = ((Pre-IRS Risk) - (Post-IRS Risk) / (Pre-IRS Risk)) \* 100%)

CI=confidence interval



**S12 Table. Stratum-specific estimates for the association between the IRS and *P. falciparum* (i.e., microscopic or submicroscopic) infection prevalence at the end of the wet seasons.** The reference for all comparisons was Survey 1 (pre-IRS, October 2012).

Factor	<i>P. falciparum</i> infection (i.e., microscopic or submicroscopic) <sup>a</sup>		
	Pre-IRS Survey 1 (October 2012) aOR <sup>b</sup>	Post-IRS Survey 3 (October 2015) aOR (95% CI) <sup>b</sup>	<i>p</i> -value
<b>Age groups</b>			
1-5 years	1.00	0.14 (0.10-0.19)	< 0.001
6-10 years	1.00	0.19 (0.13-0.27)	< 0.001
11-20 years	1.00	0.22 (0.16-0.31)	< 0.001
21-39 years	1.00	0.28 (0.20-0.39)	< 0.001
≥ 40 years	1.00	0.30 (0.22-0.41)	< 0.001
<b>Catchment area</b>			
Vea/Gowrie	1.00	0.28 (0.23-0.34)	< 0.001
Soe	1.00	0.16 (0.13-0.20)	< 0.001

aOR=adjusted odds ratio; CI=confidence interval, to deal with the repeated measures the cluster sandwich variance estimator was used

<sup>a</sup> Participants were excluded from the model if their (i) antimalarial treatment in the previous two weeks was not known: Survey 3 (N = 79) and/or (ii) the participant dried blood spot was not available for PCR (N=4).

<sup>b</sup> Age group, sex, catchment area, LLIN usage the previous night, and antimalarial treatment in the previous two weeks are adjusted for in the multivariable logistic regression model.

S13 Table. The estimated number of *P. falciparum* genomes.

	Estimated number of <i>P. falciparum</i> genomes <sup>a</sup>			
	Pre-IRS		Post-IRS	
	Survey 1 End of wet season (October 2012)	Survey 2 End of dry season (May/June 2013)	Survey 3 End of wet season (October 2015)	Survey 4 End of dry season (May/June 2016)
<b>Survey</b>	<b>2,624</b>	<b>1,637</b>	<b>1,083</b>	<b>806</b>
<b>Age groups</b>				
1-5 years	683 (26.0)	423 (25.8)	120 (11.1)	65 (8.1)
6-10 years	961 (36.6)	634 (38.7)	401 (37.0)	408 (50.6)
11-20 years	603 (23.0)	448 (27.4)	355 (32.8)	279 (34.6)
21-39 years	175 (6.7)	56 (3.4)	76 (7.0)	21 (2.6)
≥ 40 years	202 (7.7)	76 (4.6)	131 (12.1)	33 (4.1)

<sup>a</sup>MOI<sub>div</sub> used to estimate the number of diverse *P. falciparum* genomes per isolates (see Methods for additional details). Data reflect the number (% (n/N)) of subjects.

## Supporting Information (Checklist)

# Indoor residual spraying with a non-pyrethroid insecticide reduces the reservoir of *Plasmodium falciparum* in a high-transmission area in northern Ghana

Kathryn E. Tiedje<sup>¶</sup>, Abraham R. Oduro<sup>¶</sup>, Oscar Bangre, Lucas Amenga-Etego, Samuel K Dadzie, Maxwell A Appawu, Kwadwo Frempong, Victor Asoala, Shazia Ruybal-Pésantez, Charles A. Narh, Samantha L. Deed, Dionne C. Argyropoulos, Anita Ghansah, Samuel A. Agyei, Sylvester Segbaya, Kwame Desewu, Ignatius Williams, Julie A. Simpson, Keziah Malm, Mercedes Pascual, Kwadwo A. Koram, and Karen P. Day\*

<sup>¶</sup> KET and ARO contributed equally to this work as first authors.

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# Inclusivity in global research

PLOS’ policy on inclusivity in global research aims to improve transparency in the reporting of research performed outside of researchers’ own country or community and ensures that PLOS publications reporting global research adhere to high standards for research ethics and authorship. Authors of relevant research articles may be asked to complete the questionnaire below, which outlines ethical, cultural, and scientific considerations specific to inclusivity in global research. This questionnaire may be requested when researchers have travelled to a different country to conduct research, if research uses samples collected in another country, research with Indigenous populations or their lands, or if research is on cultural artefacts. Researchers travelling to another country solely to use laboratory equipment will not normally be required to complete the questionnaire. However, the questionnaire can be requested at the journal’s discretion for any submission – if you have been requested to complete this questionnaire by the PLOS journal you submitted to, please do so.

Please complete the questionnaire below and include this as a Supporting Information file with your manuscript. Note that if your paper is accepted for publication, this checklist will be published with your article in the supporting information files. Please ensure that you reference the checklist in the main body of your manuscript. We suggest adding a subsection ‘Inclusivity in global research’ to your Methods section and adding the following sentence: “Additional information regarding the ethical, cultural, and scientific considerations specific to inclusivity in global research is included in the Supporting Information (SX Checklist)”

The questions have been designed to be applicable to a wide range of study types, and there are subsections for both human subjects research and non-human subjects research. If any of the questions are not relevant to your research please mark them as “N/A” as appropriate.

## Ethical considerations, permits and authorship

*This section is applicable to all research types.*

Provide details as to who granted permissions and/or consent for the study to take place in the Methods section of your manuscript. This should include the names of **all** ethics boards, governmental organizations, community leaders or other bodies that provided approval for the study. If individuals provided approval refer to these people by their role or title but do not list their name(s).

Reported on page number: Reported on page number: 16 of the Methods and Materials in the “Ethical approval” section.

If there were any deviations from the study protocol after approval was obtained please provide details of these changes in the Methods section of your manuscript.

Reported on page number: N/A

Did this study involve local collaborators that are residents of the country where the research was conducted or members of the community studied? If you do not have any authors from said communities, please provide an explanation for this below.

Yes, this study involved local collaborators that are residents of Ghana and/or members of the Bongo District community being studied. The manuscript was co-first authored by Abraham R. Oduro who is from Navrongo Health Research Centre (Navrongo, Upper East Region, Ghana) and is based in Ghana. In addition, other co-authors from Ghana were directly involved with this study: Oscar Bangre, Lucas Amenga-Etego, Samuel K Dadzie, Maxwell A Appawu, Kwadwo Frempong, Victor Asoala, Charles A. Narh, Anita Ghansah, Samuel A. Agyei, Sylvester Segbaya, Kwame Desewu, Ignatius Williams, Keziah Malm, and Kwadwo A. Koram.

Everyone listed as an author should meet PLOS' criteria for authorship and all individuals who meet these criteria should be included in the author byline, rather than the acknowledgements. Authorship criteria is based on the International Committee of Medical Journal Editors (ICMJE) Uniform Requirements for Manuscripts Submitted to Biomedical Journals - for further information please see here: <https://journals.plos.org/plosone/s/authorship>.

### **Human subjects research (e.g. health research, medical research, cross-cultural psychology)**

Did you obtain written informed consent from a representative of the local community or region before the research took place? How did you establish who speaks for the community? Details of written informed consent obtained from study participants should be reported separately in the Methods section of your manuscript.

Yes, prior to this research taking place, written informed consent was sought and obtained from the key stakeholders in Bongo District, specifically the Paramount Chief of the Bongo traditional area, the Bongo District Assembly, the Bongo District Health Directorate, and the Regional Health Directorate (Upper East Region). To engage with the local Bongo District community prior to the start of the study, community durbars were organized with the traditional rulers and community members. Community consent was granted to the investigators to proceed with the study. The study coordinator(s) from the Navrongo Health Research Center directed and presented at these key stakeholder meetings and local community durbars in Bongo District. They study coordinator(s) were specifically chosen as they were from the region, understand the local customs, and spoke the local language (i.e., Gurene).

How did members of the local community provide input on the aims of the research investigation, its methodology, and its anticipated outcome(s)?

The members of the local community provided a clear definition of the boundaries for agroecological zones (i.e., catchment areas Vea/Gowrie and Soe), community profiles/details, and population structure at the baseline. This information helped the research team to refocus some of the study objectives.

When engaging with the local community, how did you ensure that the informed consent documents and other materials could be understood by local stakeholders?

All informed consent documents (i.e., consent/assent forms, plain language states, oral consent procedures/text) were presented to the local stakeholders by the study coordinator(s) from the Navrongo Health Research Center. These meetings involved presentations and discussions on these documents, which were prepared and provided in the local language (i.e., Gurene) and English. During these meetings the local stakeholders also had a chance to speak directly to the study coordinator(s) if they required more information and/or had any questions. In addition, prior to the start of the study in Bongo District, sensitization meetings were organized to explain the purpose of the study to the local community and to provide an opportunity for community members to ask questions, provide comments, etc.

Will the findings of the research be made available in an understandable format to stakeholders in the community where the study was conducted (e.g. via a presentation, summary report, copies of publications, etc.)? Please provide details of how this will be achieved.

The findings of this research have been made available to the key stakeholders and local community (i.e., Paramount Chief of Bongo, his divisional chiefs, the Queen Mothers, and community members) using community-based debriefing presentations/meetings and radio broadcasts. In addition, presentations/meetings were also undertaken with the Bongo District Health Directorate and the Regional Health Directorate (Upper East Region). These presentations/meetings were prepared, coordinated, and delivered by the study coordinator(s) from the Navrongo Health Research Center in the local language (i.e., Gurene) and English, so that they were accessible to the local community. During these presentations/meetings, the study coordinator(s) were available to respond to questions and comments. Finally, summary reports and copies of publications related to this research have been made and will continue to be made available to key stakeholders and local community.

**Non-human subjects research using specimens/ animals collected as part of the study, or those housed in archival collections. Examples include archaeology, paleontology, botany and zoology.**

Did the permission you obtained from a local authority to perform the study include an agreement on access to outputs and benefit sharing? This may include procedures to enable fair distribution of the benefits and resources arising from the research performed. Please include any details of Prior Informed Consent and Benefit Sharing Agreements obtained. These may be required by field-specific regulations, for example the Convention on Biological Diversity (CBD) and the associated Nagoya Protocol.

Permission was obtained from the independent Ethics Bodies/Institutional Boards in Ghana who operate according to ICH/GCP principles and ensure there is equitable/fair distribution of resources and that the research will have benefits for the local community. The research team and the funding agencies are bound by ethical principles to ensure that the informed consent documents clearly defined the risks and benefits of being involved in this study.

If the material used in your study was imported, please A) provide the year it was imported and B) indicate whether permits were obtained to import/export the materials used, C) provide details of any permits obtained. If this information is not available, please indicate this.

- A) The biological specimens (i.e., dried blood spots) for this study were imported into Australia between 2014 and 2016.
- B) All necessary import permits were obtained to import of these biological specimens (i.e., dried blood spots) into Australia. No export permits were required for these shipments to be sent from Ghana.
- C) These three shipments were covered by two different import permits issued to the University Melbourne by the Australian government (AIQS-IP14004415 and AIQS-0000356592). A Material Transfer Agreement for the shipment of the biological samples from Navrongo Health Research Centre to University of Melbourne was signed prior to the shipments.

If you used archival specimens, please state how the material used in your study was acquired by the institute it is held in and provide details of any permits obtained for the original excavations/ sample collection. If this information is not available, please indicate this.

N/A

How was the potential cultural significance of the materials collected in your study to local communities considered in your research design? Were Indigenous peoples and/or local researchers and institutions involved with archaeological excavations / collection of specimens? If so, please provide a description of their involvement.

All biological specimens (i.e., dried blood spots) collected in this study were collected in Bongo District by local researchers from the Navrongo Health Research Center. In addition, literate local residents (Gurene and English) from Bongo District were trained as field workers and were directly involved in liaising with the local community and in the collection of study data onto the structured questionnaires.

If your manuscript includes photographs of human remains please indicate whether authors obtained permission from descendants or affiliated cultural communities to do so.

N/A



## Supporting Information (Appendix)

# Indoor residual spraying with a non-pyrethroid insecticide reduces the reservoir of *Plasmodium falciparum* in a high-transmission area in northern Ghana

Kathryn E. Tiedje<sup>¶</sup>, Abraham R. Oduro<sup>¶</sup>, Oscar Bangre, Lucas Amenga-Etego, Samuel K Dadzie, Maxwell A Appawu, Kwadwo Frempong, Victor Asoala, Shazia Ruybal-Pésantez, Charles A. Narh, Samantha L. Deed, Dionne C. Argyropoulos, Anita Ghansah, Samuel A. Agyei, Sylvester Segbaya, Kwame Desewu, Ignatius Williams, Julie A. Simpson, Keziah Malm, Mercedes Pascual, Kwadwo A. Koram, and Karen P. Day\*

<sup>¶</sup> KET and ARO contributed equally to this work as first authors.

\* Corresponding author: Karen P. Day; karen.day@unimelb.edu.au

## S1 Appendix. Structured questionnaire.

### SECTION 1: PARTICIPANT DATA FORM

1. Study ID 

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**STUDY\_ID**

2. Date surveyed (DD-MM-YYYY) 

--	--	--	--	--	--	--	--

**DATE**

3. Relationship of respondent to study participant 

1. Self	2. Mother	3. Father	4. Guardian
5. Other (specify)			

**RELATION**

4. Sex 

1. Male	2. Female
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**SEX**

5. Date of birth (DD-MM-YYYY) 

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**DOB**

6. Compound Name \_\_\_\_\_ **CPD\_NAM**

7. Compound ID 

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**CPD\_ID**

8. Name of village \_\_\_\_\_ **VILLAGE**

9. Name of Section \_\_\_\_\_ **SECTION**

10. Educational level of participant 

1. None	2. Prim	3. JSS/Middle	4. SSS/Voc	5. Above SSS
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**EDUC**

11. Occupation of participant 

1. House wife	2. Farmer	3. Trader	4. Student/pupil
5. Public/Civil servant	6. Unemployed		8. NA
7. Other (specify)			

**OCCUP**

**SECTION 2: MALARIA PREVENTION**

12. What are you (your HH) doing to prevent you (your ward/child) from getting malaria? (*circle all mentioned, multiple responses allowed*)

1. Bed nets	2. Repellents	3. Strong scented flowers	<b>MAL_P</b>
4. Clean environment	5. Insecticide spray	6. Nothing	
7. Other (specify)			

13. At any time in the past 12 months, has anyone come into your home to spray the interior walls against mosquitoes?

1. Yes	2. No	9. DK	<b>SPRAY</b>
--------	-------	-------	--------------

14. If YES, who sprayed your home?

1. Government Program	2. Private Company	3. NGO	8. NA	9. DK	<b>SPRAYHOME</b>
5. Other (specify)					

15. Does your household have any bed nets that can be used while sleeping?

1. Yes	2. No	<b>OWNNET</b>
--------	-------	---------------

16. If YES, how many mosquito nets does your household have?

Number:	8. NA	<b>NUMNET</b>
---------	-------	---------------

17. Has the bed net been treated with insecticide?

1. Yes	2. No	8. NA	9. DK	<b>TREATNET</b>
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18. When was the bed net acquired?

1. <6months	2. > 6months	3. >2 years	8. NA	9. DK	<b>TBNAQUIRE</b>
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19. How was the bed net acquired?

1. Free distribution	2. Purchased	3. ANC	8. NA	9. DK	<b>HBNAQUIRE</b>
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20. Did you (your ward/child) sleep under a bed net last night?

1. Yes	2. No	<b>SLEEPNET</b>
		<b>CODES1S2</b>

21. SECTION 1/SECTION 2 completed by

**SECTION 3: SYMPTOMATIC SCREENING**

22. The stud. Do you (your ward/child) have health insurance? 

1. Yes	2. No
--------	-------

**INSURANCE**

23. Is the health insurance still valid /working? 

1. Yes	2. No	8. NA
--------	-------	-------

**INSUR\_V**

24. Have you used the health insurance this year? 

1. Yes	2. No	8. NA
--------	-------	-------

**INSUR\_USE**

25. Have you (your child/ward) been sick within the past two weeks? 

1. Yes	2. No
--------	-------

**SICK**

**IF THE RESPONSE TO QUESTION 25 IS NO, SKIP DIRECTLY TO SECTION 4**

26. If you (your ward/child) were sick within the **PAST TWO WEEKS**, did you have any of the following symptoms?

a. Fever	1. Yes	2. No	8. NA	<b>FEVER</b>
b. Headache	1. Yes	2. No	8. NA	<b>HEADACHE</b>
c. Chills	1. Yes	2. No	8. NA	<b>CHILL</b>
d. Rigors	1. Yes	2. No	8. NA	<b>RIGORS</b>
e. Convulsion	1. Yes	2. No	8. NA	<b>CONVULSE</b>
f. Cough	1. Yes	2. No	8. NA	<b>COUGH</b>
g. Ear pain/discharge	1. Yes	2. No	8. NA	<b>EARPAIN</b>
h. Diarrhea	1. Yes	2. No	8. NA	<b>DIARRHOEA</b>
i. Other (Specify)				<b>OTHER</b>

27. Did you (your child/ward) seek treatment for the illness? 

1. Yes	2. No	8. NA
--------	-------	-------

**SEEKT**

**IF THE RESPONSE TO QUESTION 27 IS NO, SKIP DIRECTLY TO SECTION 4**

28. Where did you (your child/ward) go for treatment during this illness?  
*(circle all mentioned, multiple responses allowed)*

1. Home	2. Hospital	3. Health Centre/ Clinic	4. CHPs compound	<b>FACILITY</b>
5. Traditional healer/ Herbalist		6. Drug store /chemical seller	8. NA	

29. Was blood taken from your (your ward/child's) finger/heel for testing? 

1. Yes	2. No
--------	-------

**BLDTEST**

30. Were you (your ward/child) given a malaria treatment? 

1. Yes	2. No	9. DK
--------	-------	-------

**MAL\_T**

31. If YES to malaria treatment, what antimalarial treatment were you (your child/ward) given?

1. ASAQ	2. AL	3. DHP	4. SP	5. CQ
6. Quinine	7. AS	10. AQ	11. Other	
9. DK	8. NA			

**ANTIMAL**

32. SECTION 3 completed by

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**CODES3**

**SECTION 4: PHYSICAL EXAMINATION**

33. Auxiliary Temperature (°C):

		•	
--	--	---	--

**TEMP**

34. Weight (kg):

		•	
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**WEIGHT**

35. Systolic blood pressure (mmHg):

--	--	--

**SBP**

36. Diastolic blood pressure (mmHg):

--	--	--

**DBP**

37. SECTION 4 completed by

--	--

**CODES4**

**SECTION 5: SPECIMENS COLLECTED AT ENROLMENT**

	<b>SPECIMEN</b>			
38.	Filter paper blood blot	1. Yes	2. No	<b>BBLOT</b>
39.	Blood smear	1. Yes	2. No	<b>BSMEAR</b>

**LABORATORY RESULTS**

40. Haemoglobin concentration (g/dl)

		•	
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**Hgb**

41. Rapid Malaria Test (RDT)

1. Positive	2. Negative	3. Not done
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**RAMAT**

42. SECTION 5 completed by

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**CODES5**