

1 **No evidence for manipulation of *Anopheles gambiae*, *An. coluzzii* and *An. arabiensis***
2 **host preference by *Plasmodium falciparum***

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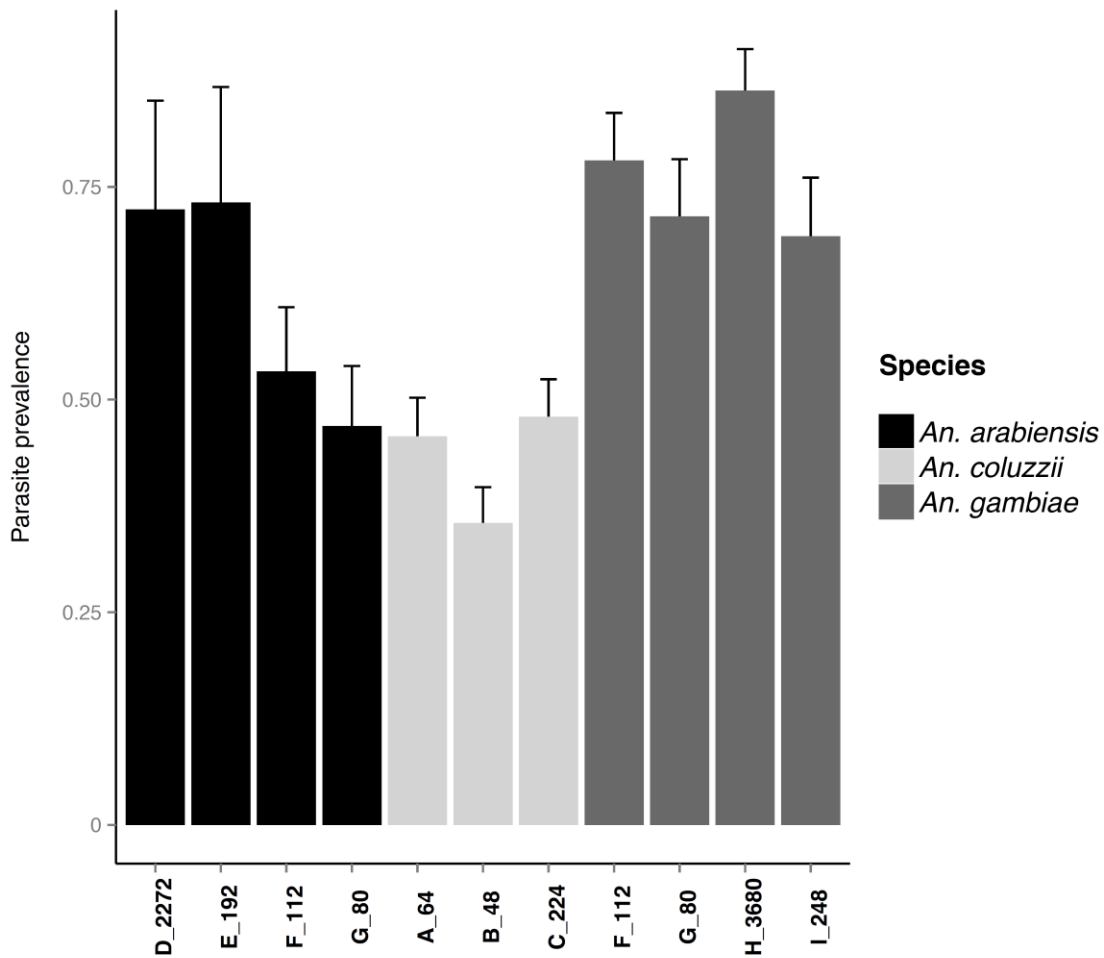
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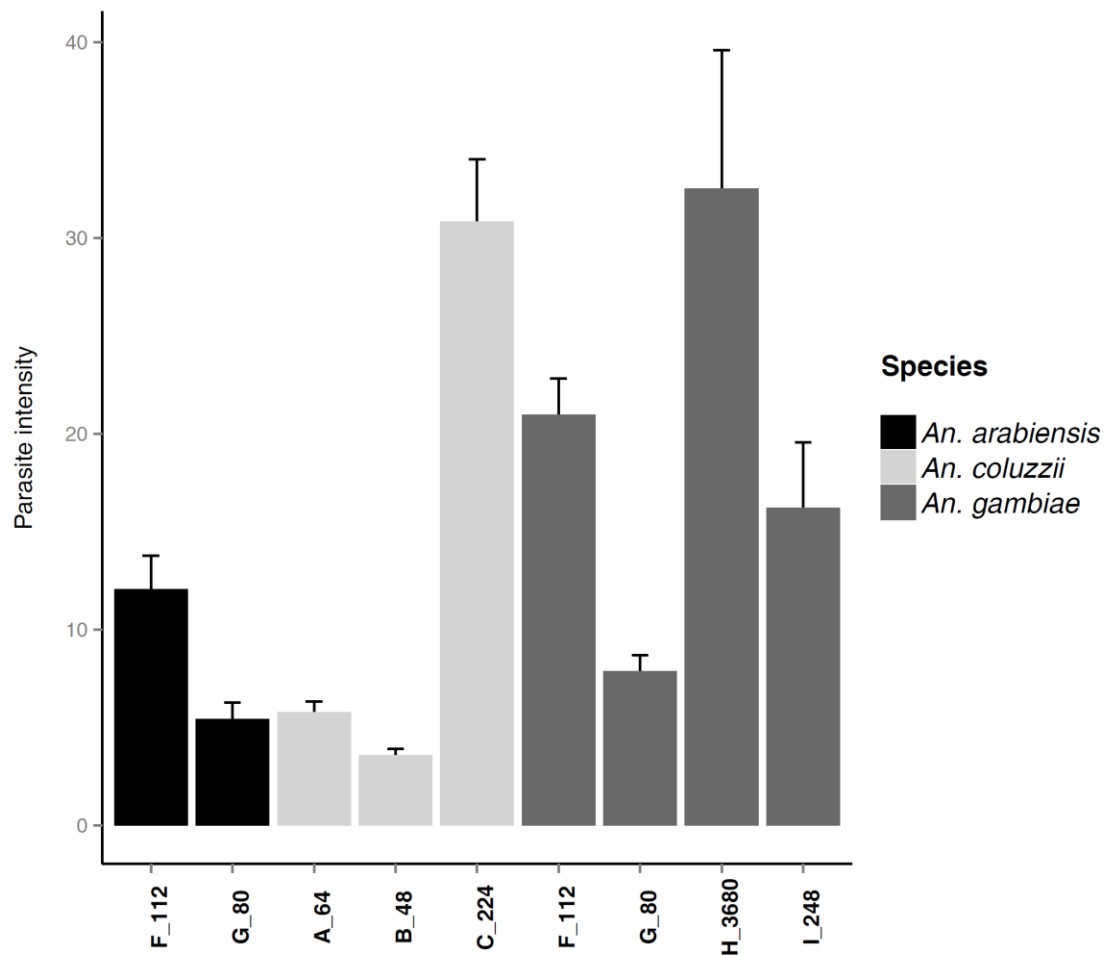
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Supplementary Figure S1. Parasite prevalence (proportion of infected females $\pm$ 95% confidence interval) at the oocyst stage of the three mosquito species. Letters below the bar indicate the gametocyte carrier and its gametocytemia.



Supplementary Figure S2. Parasite intensity (mean number of parasites in the midgut of infected females $\pm$ se) at the oocyst stage of the three mosquito species. Letters below the bar indicate the gametocyte carrier and its gametocytemia.



30 **Supplementary Table S1.** Details of carrier, gametocytemia, test period and total number of behavioral runs for each
 31 replicate
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Species	Experiment al replicate	Carrier	Gametocytes/ μl	Mean oocyst (Range)	Test period	Total runs
<i>Anopheles coluzzii</i>	1	A	64	5.79 (1 - 23)	Test period 1 (6, 7, 8 dpi) Test period 2 (13, 14, 15 dpi)	Test period 1: H-C: 18
	2	B	48	3.59 (1 – 17)	Test period 1 (6, 7, 8 dpi) Test period 2 (13, 14, 15 dpi)	H-O: 18
	3	C	224	30.85 (1 - 163)	Test period 1 (6, 7, 8 dpi), Test period 2 (13, 16, 17 dpi)	Test period2: H-C: 18 H-O: 18
<i>Anopheles arabiensis</i>	1	D	2272	NA	Test period 2 (17 dpi)	Test period 1:
	2	E	192	NA	Test period 2 (17 dpi)	H-C: 2
	3	F*	112	12.06 (1 - 65)	Test period 1 (5, 6, dpi)	H-O: 2
					Test period 2 (17, 18 dpi)	C-O: 2
		G*	80	5.42 (1 - 34)	Test period 1 (5, 6, dpi) Test period 2 (17, 18 dpi)	Test period2: H-C: 6 H-O: 6 C-O: 6
<i>Anopheles gambiae</i>	1	F*	112	20.99 (1 – 79)	Test period 1 (8, 9, dpi) Test period 2 (17, 18 dpi)	Test period 1: H-C: 8
					Test period 1 (8, 9, dpi) Test period 2 (17, 18 dpi)	H-O: 8 C-O: 8
		H	3680	32.53 (1 – 222)	Test period 1 (7, 8, dpi) Test period 2 (18, 19 dpi)	Test period2: H-C: 8
	2	I	248	16.21 (1 – 82)	Test period 1 (7, 8, dpi) Test period 2 (18, 19 dpi)	H-O: 8 C-O: 8

33 * indicates that the serum of the carrier was replaced by European serum
 34 dpi: day post infection

GLMM models for two groups of mosquitoes: infected (exposed-infected) and uninfected controls (unexposed).

Supplementary Table S2: Effect of infection treatment, combination and test period on *Anopheles coluzzii* activation rate

Variable	LRT	df	P
Infection treatment	0.50	1	0.48
Combination	3.21	1	0.07
Test period	0.08	1	0.78
Test period:combination	1.06	1	0.30
Infection treatment:combination	0.22	1	0.64
Infection treatment:test period	1.13	1	0.29

Supplementary Table S3: Effect of infection treatment, combination, test period on *Anopheles gambiae* activation rate

Variable	LRT	df	P
Infection treatment	0.22	1	0.64
Combination	3.26	2	0.20
Test period	3.94	1	0.047 *
Combination:test period	8.44	2	0.01*
Infection treatment:combination	2.21	2	0.33
Infection treatment:test period	0.23	1	0.63

Supplementary Table S4: Post hoc tests results of the significant effect of the interaction between combination and test period on *Anopheles gambiae* activation rate.

Pairwise	LRT	df	P
Human-calf vs calf-control	0.59	1	0.32
Human-calf vs human-control	7.79	1	0.02*
Calf-control vs human-control	3.71	1	0.11

Supplementary Table S5: Effect of infection treatment, combination on *Anopheles arabiensis* mosquito activation rate.

Variable	LRT	df	p
Test period 1 (oocyst stage)			
Infection treatment	0.58	1	0.45
Combination	10.34	2	0.005**
Infection treatment: combination	4.95	2	0.08
Test period 2 (sporozoite stage)			
Infection treatment	6.69	1	0.01**
Combination	6.61	2	0.04*
Infection treatment:combination	0.18	2	0.92

Supplementary Table S6: Post hoc test result for the significant effect of combination on *Anopheles arabiensis* activation rate during test period 1

Pairwise	LRT	df	P
Human-calf vs calf-control	1.79	1	0.18
Human-calf vs human-control	3.43	1	0.06
Calf-control vs human-control	9.96	1	0.002**

Supplementary Table S7: Post hoc test results for the significant effect of combination on *Anopheles arabiensis* activation rate during test period 2.

Pairwise	LRT	df	P
Human_calf vs calf control	0.01	1	0.90
Human_calf vs human control	4.97	1	0.03*
Calf_control vs human control	4.68	1	0.03*

Supplementary Table S8: Effect of infection treatment and test period on *Anopheles coluzzii* host choice

Variable	LRT	df	P
Human_calf combination			
Infection treatment	0.73	1	0.40
Test period	0.03	1	0.87
Infection treatment:test period	0.31	1	0.58
Human_control combination			
Infection treatment	1.24	1	0.27
Test period	0.02	1	0.89
Infection treatment:test period	0.62	1	0.43

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60 **Supplementary Table S9: Effect of infection treatment, test period on *Anopheles***

61 ***gambiae* host choice**

Variable	LRT	df	P
Human_calf combination			
Infection treatment	0.12	1	0.73
Test period	1.96	1	0.16
Infection treatment: test period	0.47	1	0.49
Human_control combination			
Infection treatment	2.92	1	0.09
Test period	1.66	1	0.20
Infection treatment: test period	0.82	1	0.37
Calf_control combination			
Infection treatment	0.75	1	0.75
Test period	0.40	1	0.53
Infection treatment:test period	4.67	1	0.03*

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63 **Supplementary Table S10: Effect of infection treatment on *Anopheles arabiensis* host**

64 **choice during test period 1.**

Variable	LRT	df	p
Human-calf combination			
Volunteer	0.69	1	0.41
Infection treatment	0.87	1	0.35
Human-control combination			

Infection treatment	0.16	1	0.69
Calf-control combination			
Infection treatment	1.01	1	0.31

Supplementary Table S11: Effect of infection treatment and volunteer on *Anopheles arabiensis* host choice during test period 2.

Variable	LRT	df	p
Human-calf combination			
Infection treatment	0.36	1	0.55
Human-control combination			
Infection treatment	1.39	1	0.24
Calf-control combination			
Infection treatment	0.06	1	0.81

GLMM models for all three groups of mosquitoes: exposed-infected, exposed-uninfected and unexposed.

Supplementary Table S12: Effect of infection status, combination and test period on *Anopheles coluzzii* mosquito activation rate.

Variable	LRT	df	P
Test period	0.01	1	0.91
Infection status	1.12	2	0.57
Combination	3.09	1	0.08
Infection status: combination	0.27	2	0.88
Infection status:test period	2.17	2	0.34
Test period:combination	1.81	1	0.18

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Supplementary Table S13: Effect of infection status and test period on *Anopheles coluzzii* mosquito host choice for the two types of combination.

Variable	LRT	df	P
Human-calf combination			
Infection status	1.56	2	0.46
Test period	0.54	1	0.46
Infection status:test period	0.35	2	0.84
Human-control combination			
Infection status	1.62	2	0.45
Test period	0.03	1	0.85
Infection status:test period	1.28	2	0.53

Supplementary Table S14: Effect of infection status, volunteer, test period on *Anopheles gambiae* mosquito activation rate.

Variable	LRT	df	P
Infection status	15.68	2	< 0.001***
Combination	15.66	2	<0.001***
Test period	3.96	1	0.046 *
Infection status:combination	10.87	4	0.03*
Test period:combination	6.78	2	0.03*
Infection status:test period	0.21	2	0.90

We did not run the model for the host choice due to insufficient data.

We found a significant effect of infection status effect on mosquito activation rate. The activation rate of exposed-uninfected females (12.06 ± 4.84 %, 21/174) was significantly lower than the activation rate of exposed-infected and uninfected control females (18.42 ± 3.18 %, 105/570 and 19.25 ± 2.99 %, 129/670, respectively; table S14a). This may

suggest an energy cost of resisting infection whereby females that invested in immunity and resisted the infection had less energy to invest in host-seeking. In addition, there was a significant infection status by combination interaction although post-hoc test for pairwise interactions showed non-significant differences (see table S14b & c).

Supplementary Table S14a: Post hoc tests results of the significant effect of the infection status on *Anopheles gambiae* activation rate.

Pairwise	LRT	df	P
Control – exposed infected	0.26	1	0.55
Control – exposed uninfected	9.59	1	0.006**
Exposed infected – Exposed uninfected	8.11	1	0.008**

Supplementary Table S14b: Post hoc tests results of the significant effect of the interaction between combination and infection status on *Anopheles gambiae* activation rate.

Pairwise interaction	LRT	df	P
Ctrl-Inf * CO-HC	0.96	1	1
Ctrl-Uninf * CO-HC	2.89	1	0.62
Inf-Uninf * CO-HC	1.21	1	1
Ctrl-Inf * CO-HO	0.26	1	1
Ctrl-Uninf * CO-HO	4.24	1	0.32
Inf-Uninf * CO-HO	4.93	1	0.24
Ctrl-Inf * HC-HO	2.12	1	0.87
Ctrl-Uninf * HC-HO	0.90	1	1
Inf-Uninf * HC-HO	2.01	1	0.87

Ctrl: Control; Inf: Exposed infected; Uninf: Exposed uninfected; HO: Human odour –

Outdoor air, CO: Calf odour– Outdoor air; HC : Human odour-Calf odour

Supplementary Table S14c: Post hoc tests results of the significant effect of the interaction between combination and infection status on *Anopheles gambiae* activation rate.

Pairwise	LRT	df	P
CO-HC * T1-T2	0.20	1	0.66
CO-HO * T1-T2	4.38	1	0.07
HC-HO * T1-T2	5.57	1	0.054

CO: Calf – Outdoor air; HC: Human odour – Calf odour; HO: Human odour – Outdoor air; T1: Testing period 1; T2: Testing period 2.

Supplementary Table S15: Effect of infection status, volunteer and combination on *Anopheles arabiensis* mosquito activation rate.

Variable	LRT	df	P
Test period 1 (oocyst stage)			
Infection status	0.55	2	0.76
Combination	14.92	2	<0.001***
Infection status:combination	5.46	4	0.24
Test period 2 (sporozoite stage)			
Infection status	9.45	2	0.009**
Combination	11.38	2	0.003**
Infection status:combination	1.59	4	0.81

We did not run the model for the host choice due to insufficient data.