

SUPPLEMENTAL INFORMATION

Over a 2-week period in June 2012 at the end of the dry season, blood samples (thick/thin blood films and dried blood spot) and malaria related questionnaires were collected after obtaining informed consent from 698 participants between the ages of 1–85 years during this cross-sectional survey (Supplemental Table 1). From this survey, 267 (38.3%) participants were microscopy positive for any *Plasmodium* spp. with most being infected with *P. falciparum* (97.4%, *N* = 260), a few infected with mixed *P. falciparum*/*P. malariae* infections (1.9%, *N* = 5), and 0.7% (*N* = 2) with *P. malariae* infections (Supplemental Table 1). All the participants with *P. malariae* infections (*N* = 2) by microscopy were confirmed to be harboring *P. falciparum* infections by *m*sp2 PCR and are included in the analyses as mixed *P. falciparum*/*P. malariae* infections. Supplemental Table 1 summarizes the parasitological

characteristics of the total cross-sectional study population by participants' age group, gender and catchment area. *P. falciparum* infections occurred across all age groups in both catchment areas with the highest prevalence of *P. falciparum* infection occurring among children between 6–10 years of age, with 58.1% (95% CI: 54.1–62.1%) of 6–10 year olds across both catchment areas having a *P. falciparum* infection (Supplemental Table 1). When male participants were compared with females there were no differences in the prevalence *P. falciparum* infection for the total study population and within/between the catchment areas. From this cross-sectional survey on the reservoir of *P. falciparum* infections in BD, 200 participants with confirmed *P. falciparum* infections (including mixed *P. falciparum*/*P. malariae* infections) were selected for the microsatellite analyses.

SUPPLEMENTAL TABLE 1
Demographics and parasitological characteristics of the study population collected at the end of the dry season in June 2012

Characteristic	Total	Veal/Gowrie	Soe
Age groups*			
All	698	354 (50.7)	344 (49.3)
1–5 years	133 (19.1)	76 (21.5)	57 (16.6)
6–10 years	155 (22.2)	79 (22.3)	76 (22.1)
11–20 years	140 (20.1)	73 (20.6)	67 (19.5)
21–39 years	112 (16.0)	49 (13.8)	63 (18.3)
≥ 40 years	158 (22.6)	77 (21.8)	81 (23.5)
Gender*			
Female	355 (50.9)	182 (51.4)	173 (50.3)
Male	343 (49.1)	172 (48.6)	171 (49.7)
Microscopic <i>Plasmodium</i> spp. prevalence (<i>N</i> = 267)			
<i>P. falciparum</i>	260 (97.4)	140 (96.6)	120 (98.4)
<i>P. falciparum</i> / <i>P. malariae</i>	5 (1.9)	4 (2.7)	1 (0.8)
<i>P. malariae</i>	2 (0.7)	1 (0.7)	1 (0.8)
<i>Plasmodium</i> spp. median density (<i>N</i> = 267)†			
All	280 [120–880]	280 [120–800]	280 [150–1,000]
1–5 years	960 [480–2,200]	900 [640–1,860]	960 [440–2,760]
6–10 years	240 [120–940]	200 [120–700]	400 [140–1,240]
11–20 years	240 [120–520]	240 [120–490]	240 [120–660]
21–39 years	180 [110–500]	240 [120–470]	160 [80–730]
≥ 40 years	120 [80–230]	100 [80–280]	160 [80–200]
Female	290 [120–890]	240 [120–800]	280 [120–880]
Male	320 [160–880]	280 [120–880]	360 [160–960]
<i>P. falciparum</i> prevalence (<i>N</i> = 267)‡			
All	267 (38.3)	145 (41.0)	122 (35.5)
1–5 years	51 (38.3)	28 (36.8)	23 (40.4)
6–10 years	90 (58.1)	45 (57.0)	45 (59.2)
11–20 years	72 (51.4)	42 (57.5)	30 (44.8)
21–39 years	22 (19.6)	10 (20.4)	12 (19.0)
≥ 40 years	32 (20.3)	20 (26.0)	12 (14.8)
Female	134 (37.7)	71 (39.0)	63 (36.4)
Male	133 (38.8)	74 (43.0)	59 (34.5)
<i>P. falciparum</i> median MOI (<i>N</i> = 267)§			
All	2 [1–3]	2 [1–3]	2 [1–3]
1–5 years	2 [1–3]	2 [1–3]	1.5 [1–3.25]
6–10 years	2 [1–3]	2 [1–3]	3 [2–4]
11–20 years	2 [1–3]	2 [2–3]	2 [1–3]
21–39 years	1 [1–2]	1 [1–3]	1 [1–2]
≥ 40 years	1 [1–2]	1 [1–2]	1 [1–2]

IQR = inter quartile range; MOI = multiplicity of infection; PCR = polymerase chain reaction.

* Data reflect No. (% (n/N)) of subjects.

† Median parasite density for microscopically positive *P. falciparum* (including mixed *P. falciparum*/*P. malariae* infections) (value/μL, IQR) samples.

‡ Data reflect No. (% (n/N)) of participants sampled that were positive for *P. falciparum* (including mixed *P. falciparum*/*P. malariae* infections) by microscopy and *m*sp2 PCR.

§ Data reflect the median MOI for the participants sampled that were positive for *P. falciparum* (including mixed *P. falciparum*/*P. malariae* infections) by microscopy and *m*sp2 PCR (median, [IQR]).

SUPPLEMENTAL TABLE 2

Allelic size range, number of alleles, expected heterozygosity, and microsatellite loci genotyping success

Locus	Chromosome	Range (bp)	A	H_e	Genotyping success (%)
TA1	6	151–202	17	0.89	83.0
TA60	13	66–99	10	0.79	99.0
TA40	10	202–292	26	0.84	97.0
POLY α	4	125–191	20	0.88	93.5
ARA2	11	59–80	8	0.80	98.5
PfG377	12	90–105	6	0.63	98.5
TA109	6	148–208	18	0.83	99.0
TA87	6	75–126	16	0.86	96.5
TA42	5	179–233	8	0.35	89.0
2490	10	75–90	5	0.40	91.5
TA81	5	103–136	11	0.84	98.0
PfPK2	12	153–192	14	0.83	94.5

A = number of alleles per locus; H_e = expected heterozygosity.

SUPPLEMENTAL TABLE 3

Microsatellite allele frequencies and sample sizes (n) for 12 microsatellite loci

Marker	bp	All infections (N = 200)		Dominant infections (N = 74)		
		Frequency	Frequency	Frequency	Frequency	
TA1	151	0.012	0.016			
	154	0.006	–			
	157	0.006	0.016			
	160	0.084	0.082			
	163	0.145	0.131			
	166	0.187	0.197			
	169	0.151	0.098			
	172	0.133	0.131			
	175	0.072	0.049			
	178	0.066	0.082			
	181	0.036	0.082			
	184	0.024	–			
	187	0.018	0.016			
	190	0.018	0.033			
	193	0.030	0.033			
	196	0.006	0.016			
	202	0.006	0.016			
n		166	61			
TA60	66	0.020	0.027			
	72	0.278	0.284			
	75	0.222	0.230			
	78	0.030	0.027			
	81	0.248	0.176			
	84	0.152	0.162			
	87	0.030	0.054			
	90	0.005	0.014			
	93	0.010	0.014			
	99	0.005	0.014			
	n		198	74		
	TA40	202	0.010	–		
205		0.010	0.014			
208		0.021	0.028			
211		0.010	–			
214		0.010	0.014			
217		0.010	0.014			
220		0.067	0.085			
223		0.021	0.028			
226		0.077	0.113			
229		0.005	0.014			
235		0.026	0.042			
238		0.021	0.028			
241		0.253	0.183			
244		0.278	0.211			
247		0.057	0.085			
250		0.010	0.028			

(continued)

SUPPLEMENTAL TABLE 3

Continued

Marker	bp	All infections (N = 200)		Dominant infections (N = 74)		
		Frequency	Frequency	Frequency	Frequency	
POLY α	253	0.026	0.014			
	256	0.016	0.028			
	259	0.005	–			
	262	0.005	0.014			
	265	0.005	0.014			
	274	0.005	0.014			
	283	0.005	–			
	286	0.026	0.028			
	289	0.010	–			
	292	0.010	–			
	n		194	71		
	125	0.005	0.014			
	131	0.011	–			
	137	0.005	–			
	140	0.005	–			
	143	0.043	0.043			
	146	0.027	0.029			
149	0.054	0.043				
152	0.267	0.257				
155	0.155	0.129				
158	0.107	0.071				
161	0.091	0.071				
164	0.043	0.057				
167	0.043	0.071				
170	0.032	0.014				
173	0.043	0.071				
176	0.037	0.071				
179	0.011	0.014				
185	0.011	0.014				
188	0.005	0.014				
191	0.005	0.014				
n		187	70			
ARA2	59	0.0812	0.081			
	62	0.066	0.066			
	65	0.3401	0.340			
	68	0.1929	0.193			
	71	0.1827	0.183			
	74	0.0863	0.086			
	77	0.0305	0.031			
	80	0.0203	0.020			
	n		197	74		
	PfG377	90	0.025	0.014		
93		0.046	0.081			
96		0.279	0.311			

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SUPPLEMENTAL TABLE 3
Continued

Marker	bp	All infections (N = 200)	Dominant infections (N = 74)
		Frequency	Frequency
TA109	99	0.543	0.500
	102	0.096	0.081
	105	0.010	0.014
	<i>n</i>	197	74
	148	0.005	0.014
	151	0.005	–
	154	0.010	–
	157	0.005	–
	160	0.172	0.176
	163	0.192	0.203
	166	0.015	0.014
	169	0.015	0.027
	172	0.182	0.176
	175	0.237	0.284
	178	0.015	–
	181	0.005	–
	184	0.056	0.054
	187	0.010	0.014
	190	0.005	–
	196	0.061	0.041
199	0.005	–	
208	0.005	–	
<i>n</i>	198	74	
TA87	75	0.010	–
	81	0.005	0.014
	84	0.005	–
	87	0.005	–
	90	0.031	–
	93	0.062	0.110
	96	0.150	0.164
	99	0.171	0.123
	102	0.218	0.219
	105	0.140	0.151
	108	0.098	0.110
	111	0.067	0.069
	114	0.005	0.014
	117	0.010	0.014
	120	0.010	–
	126	0.010	0.014
	<i>n</i>	193	73
TA42	179	0.006	–
	185	0.803	0.779
	188	0.017	0.029
	194	0.006	–
	197	0.140	0.162
	200	0.006	–
	209	0.017	0.029
	233	0.006	–
	<i>n</i>	178	68
2490	75	0.011	0.014
	81	0.175	0.143
	84	0.760	0.743
	87	0.033	0.071
	90	0.022	0.029
<i>n</i>	183	70	
TA81	103	0.005	–
	106	0.020	0.055
	109	0.036	0.027
	112	0.077	0.069
	115	0.235	0.233
	118	0.214	0.164
	121	0.194	0.192
	124	0.092	0.110

SUPPLEMENTAL TABLE 3
Continued

Marker	bp	All infections (N = 200)	Dominant infections (N = 74)
		Frequency	Frequency
PfPK2	127	0.097	0.096
	130	0.026	0.055
	136	0.005	–
	<i>n</i>	196	73
	153	0.005	0.014
156	0.027	0.014	
159	0.164	0.167	
162	0.233	0.292	
165	0.259	0.181	
168	0.122	0.153	
171	0.058	0.097	
174	0.016	–	
177	0.032	0.014	
180	0.042	0.028	
183	0.016	0.014	
186	0.005	–	
189	0.016	0.028	
192	0.005	–	
<i>n</i>	189	72	

(continued)

SUPPLEMENTAL TABLE 4
Patterns of genetic diversity in Bongo District for dominant infections

Population	<i>n</i>	<i>h</i>	<i>A</i> ± SE	<i>R_s</i> ± SE	<i>H_e</i> ± SE
Vea/Gowrie catchment area	42	42	8.67 ± 3.34	8.45 ± 3.24	0.76 ± 0.17
Vea	26	26	7.42 ± 2.81	6.14 ± 2.01	0.76 ± 0.13
Gowrie	16	16	5.75 ± 2.09	5.45 ± 1.94	0.72 ± 0.23
Soe catchment area	32	32	8.00 ± 3.59	7.97 ± 3.58	0.75 ± 0.19
Soe Sanabisi	10	10	5.08 ± 1.93	5.07 ± 1.92	0.72 ± 0.23
Soe Boko	22	22	7.08 ± 2.81	5.96 ± 2.09	0.74 ± 0.17
Total	74	74	10.50 ± 4.82	10.44 ± 4.78	0.76 ± 0.18

A = number of alleles per locus; *h* = number of haplotypes; *H_e* = expected heterozygosity; *n* = number of isolates; *R_s* = allelic richness estimate.

SUPPLEMENTAL TABLE 5
Multilocus linkage disequilibrium in Bongo District for one marker/chromosome for 55 dominant infections with no missing data.

chr5	chr6	chr10	chr12	<i>I_A^S</i> (<i>P</i> value)
TA42	TA109	TA40	PFG377	0.0120 (0.066)
TA42	TA109	2490	PFG377	0.0105 (0.145)
TA42	TA109	TA40	PfPK2	0.0083 (0.127)
TA42	TA109	2490	PfPK2	0.0062 (0.254)
TA42	TA87	TA40	PFG377	0.0106 (0.091)
TA42	TA87	2490	PFG377	0.0077 (0.209)
TA42	TA87	TA40	PfPK2	0.0088 (0.108)
TA42	TA87	2490	PfPK2	0.0050 (0.288)
TA42	TA1	TA40	PFG377	0.0168 (0.022)*
TA42	TA1	2490	PFG377	0.0166 (0.055)
TA42	TA1	TA40	PfPK2	0.0151 (0.028)*
TA42	TA1	2490	PfPK2	0.0141 (0.070)
TA81	TA109	TA40	PFG377	0.0046 (0.230)
TA81	TA109	2490	PFG377	0.0097 (0.103)
TA81	TA109	TA40	PfPK2	0.0030 (0.312)
TA81	TA109	2490	PfPK2	0.0077 (0.138)
TA81	TA87	TA40	PFG377	0.0028 (0.311)
TA81	TA87	2490	PFG377	0.0066 (0.178)
TA81	TA87	TA40	PfPK2	0.0036 (0.276)
TA81	TA87	2490	PfPK2	0.0066 (0.171)
TA81	TA1	TA40	PFG377	0.0034 (0.294)
TA81	TA1	2490	PFG377	0.0112 (0.074)
TA81	TA1	TA40	PfPK2	0.0037 (0.272)
TA81	TA1	2490	PfPK2	0.0111 (0.061)

* *P* < 0.05