Naturally acquired antibody response to \textit{Plasmodium falciparum} describes heterogeneity in transmission on islands in Lake Victoria

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As markers of exposure anti-malaria antibody responses can help characterise heterogeneity in malaria transmission. In the present study antibody responses to \textit{Plasmodium falciparum} AMA-1, MSP-1\textsubscript{19} and CSP were measured with the aim to describe transmission patterns in meso-endemic settings in Lake Victoria. Two cross-sectional surveys were conducted in Lake Victoria in January and August 2012. The study area comprised of three settings: mainland (Ungoye), large island (Mfangano) and small islands (Takawiri, Kibuogi, Ngodhe). Individuals provided a finger-blood sample to assess malaria infection by microscopy and PCR. Antibody response to \textit{P. falciparum} was determined in 4,112 individuals by ELISA using eluted dried blood from filter paper. The overall seroprevalence was 64.0\% for AMA-1, 39.5\% for MSP-1\textsubscript{19}, and 12.9\% for CSP. Between settings, seroprevalences for merozoite antigens were similar between Ungoye and Mfangano, but higher when compared to the small islands. For AMA-1, the seroconversion rates (SCRs) ranged from 0.121 (Ngodhe) to 0.202 (Ungoye), and were strongly correlated to parasite prevalence. We observed heterogeneity in serological indices across study sites in Lake Victoria. These data suggest that AMA-1 and MSP-1\textsubscript{19} sero-epidemiological analysis may provide further evidence in assessing variation in malaria exposure and evaluating malaria control efforts in high endemic area.

In sub-Saharan Africa, malaria remains one of the leading causes of morbidity and mortality, with 191 million cases and over 390 thousand deaths reported in 2016\textsuperscript{1}. Nevertheless, with scaling up of malaria prevention, diagnosis and treatment, the prevalence of \textit{Plasmodium falciparum} infection in many parts of sub-Saharan Africa declined by 50\%, and the incidence of clinical disease fell by 40\% between 2000 and 2015\textsuperscript{2}.

In Kenya, 65\% (26 million) of the population live in areas where \textit{P. falciparum} parasite rate for the population aged 2–10 years (P\textsubscript{f}PR\textsubscript{2–10}) is below 1\%\textsuperscript{3}. Despite significant increases in coverage of long-lasting insecticide-treated nets (LLINs) through free-mass distribution campaigns\textsuperscript{4} and the change in first-line treatment to artemisinin-based combination therapy (ACT)\textsuperscript{5}, 10.6\% (4.3 million) of the population still live in areas with P\textsubscript{f}PR\textsubscript{2–10} \geq 40\%, mainly counties adjacent to Lake Victoria in western Kenya\textsuperscript{6}. In addition, increased paediatric

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\textsuperscript{Received: 7 April 2017}
\textsuperscript{Accepted: 25 July 2017}
\textsuperscript{Published online: 22 August 2017}
malaria hospital admissions in 2009\(^7\) and high prevalence of malaria infection among school children in 2014\(^8\) highlight the challenge of effective malaria control in this highly endemic region of Kenya.

Understanding the intensity of malaria transmission in a community is fundamental to the design and evaluation of malaria control and elimination programmes. The most widely used metric of malaria transmission...
intensity is parasite prevalence, measured through cross-sectional surveys. In recent years, antibody responses to one or more malaria parasite-specific antigens have been explored as alternative means to estimate malaria transmission intensity9, 10. As a proxy measure of malaria transmission, serological responses to P. falciparum antigens have shown a robust and consistent correlation with estimates of entomological inoculation rate (EIR)10, and thus have increasingly been incorporated in cross-sectional and longitudinal studies to monitor changes in transmission11–15 and identify hotspots in transmission16, 17.

Whilst several sero-epidemiological studies have been conducted in the low-transmission western highlands of Kenya18–20, no such study has been carried out in the adjacent Lake Victoria basin where prevalence is moderate to high with significant local heterogeneity21. In the present study, antibody responses to P. falciparum blood-stages antigens apical membrane antigen 1 (AMA-1), merozoite surface antigen-1 19 (MSP-1 19) and circumsporozoite antigen (CSP) were measured to assess malaria exposure and transmission on islands in Lake Victoria. Results from this study provide baseline data to evaluate the planned malaria elimination programme in the study area.

Results
Characteristics and parasite rates of the study participants. A total of 5044 participants were enrolled from five different settings (336–1947 individuals per site) in January and August 2012. Population coverage varied among settings: 10.5% in Mfangano, 35.7% in Ungoye and 48–90.6% in the small islands. Gender and age distributions were similar across the five settings. The majority of participants were children and adolescents ≤15 years old (73.0%, 95% CI: 71.7–74.2) and came from the islands (75.4%, 95% CI: 74.2–76.6). At enrolment, 5.9% (95% CI: 5.2–6.5) of the population were febrile (axillary temperature >37.5 °C), and 20.8% (95% CI: 19.7–22.0) were anaemic (haemoglobin [Hb] level <12 g/dL), 5.9% (95% CI: 5.2–6.5) of the population were febrile (axillary temperature >37.5 °C), and 20.8% (95% CI: 19.7–22.0) were anaemic (haemoglobin [Hb] level <12 g/dL). Of all children 12 years and below (n = 3045), 1261 (41.4%; 95% CI: 39.7–43.2) were found to have an enlarged spleen. The prevalence of febrile illness, anaemia, and enlarged spleen varied significantly by study sites (P < 0.001). Further details on the study population are shown in Table 1.

The prevalence of P. falciparum infection by microscopy and PCR in the study sites is shown in Fig. 1. P. falciparum parasite prevalence ranged between 4.1 and 32.1% by microscopy, and between 11.2 and 56.2% by PCR. Parasite prevalence was significantly higher in Ungoye than other sites, regardless of detection method (P < 0.001 for all comparisons). Parasite prevalence by PCR generally peaked in the 11–15 years group and declined thereafter in all study sites (Fig. 2A). There were no statistically significant differences in mean parasite density among study sites after adjusting for age (P = 0.091). Geographic heterogeneities in malaria prevalence, sub-microscopic infections, and distribution of Plasmodium spp. in the study area have been reported previously21.

Naturally acquired antibody responses against malaria antigens. AMA-1 antibodies. Antibody responses to AMA-1, whether evaluated as OD level, antibody titre or SCR, generally increased with increasing malaria prevalence (Table 2). The overall seroprevalence was 64% (95% CI: 62.5–65.5). Seroprevalence was significantly higher (P < 0.001 for all comparisons) in Ungoye (68.6%; 95% CI: 65.3–71.6) and Mfangano (66.3%; 95% CI: 63.9–68.7) than Takawiri (60.5%; 95% CI: 56.0–64.0), Kibuogi (58.5%; 95% CI: 52.9–63.9) and Ngodhe (58.4%; 95% CI: 54.3–62.5) (Fig. 1). Seroprevalence of males and females in all study areas were comparable. Antibody responses increased with age (P < 0.001) and antibody prevalence rapidly rose in children aged 6–10 years in all study areas (Fig. 2B). More than 75% of participants became seropositive by 20 years of age (Fig. 2A),

Table 1. Demographic characteristics of all surveyed population. *No gender and age recorded, n = 6; Body temperature, N = 5038; Hb level, N = 5035; Spleen test, N = 3045; IQR, interquartile range; SD; standard deviation.

| Characteristic                  | Overall     | Study site |
|--------------------------------|-------------|------------|
|                                |            | Ungoye    | Mfangano | Takawiri | Kibuogi | Ngodhe |
| Total number of study participants | 5044       | 1239      | 1947      | 888      | 336     | 634     |
| Female gender, n (%)           | 2623 (52.2%) | 628 (50.7) | 998 (51.3) | 468 (52.9) | 194 (57.7) | 344 (54.3) |
| Age, median (IQR), years       | 10 (6–18)   | 10 (5–13) | 11 (6–18) | 10 (5–20) | 10 (5–20) | 12 (7–23) |
| 0–5                            | 1211 (24.0) | 345 (27.9) | 396 (23.4) | 233 (26.3) | 102 (30.4) | 135 (21.3) |
| 6–10                           | 1319 (26.2) | 362 (29.2) | 526 (27.0) | 228 (25.8) | 72 (21.4) | 131 (20.7) |
| 11–15                          | 1146 (22.8) | 298 (24.1) | 502 (25.8) | 171 (19.3) | 41 (12.2) | 134 (21.2) |
| 16–30                          | 756 (15.0)  | 123 (9.9)  | 266 (13.7) | 160 (18.1) | 84 (25.0) | 123 (19.4) |
| >30                            | 606 (12.0)  | 110 (8.9)  | 256 (13.2) | 93 (10.5)  | 37 (11.0) | 110 (17.4) |
| Axillary temperature, Mean ± SD, °C | 36.9 ± 0.6 | 36.9 ± 0.7 | 36.9 ± 0.6 | 36.8 ± 0.5 | 36.7 ± 0.5 | 36.8 ± 0.6 |
| Temperature > 37.5°C at time of survey, n (%) | 295 (5.9) | 118 (9.5) | 105 (5.4) | 32 (3.6) | 9 (2.7) | 31 (4.9) |
| Haemoglobin (Hb) level, Mean ± SD, g/dL | 12.5 ± 2.1 | 12.5 ± 1.8 | 12.6 ± 2.0 | 12.1 ± 2.3 | 12.7 ± 2.1 | 12.2 ± 2.2 |
| Proportion anaemic (<11 g/dL), n (%) | 1049 (20.8) | 214 (17.3) | 368 (18.9) | 245 (27.7) | 66 (19.7) | 156 (24.6) |
| Enlarged spleen, n (%)         | 1261 (41.4) | 584/948 (61.6) | 438/1160 (37.8) | 129/548 (23.5) | 73/180 (40.6) | 37/209 (17.7) |
indicating rapid acquisition of antibodies with repeated infection. Estimated EIRs based on SCRs were 12, 7, 4, 4, and 3 infectious bites per person per year (ib/p/yr) for Ungoye, Mfangano, Takawiri, Kibuogi, and Ngodhe, respectively (Table 2). The age-seroprevalence curves did not indicate more than one force of infection over time; this was checked by allowing the SCR to differ at a single time point (Fig. 3A). Across the study sites, SCRs correlated significantly with \( P. falciparum \) parasite rates by microscopy (Correlation of determination, \( R^2: 0.9402, P = 0.009 \)) and PCR (\( R^2: 0.9601, P < 0.001 \)) (Fig. 4).

**MSP-1\(_{19}\) antibodies.** Median MSP-1\(_{19}\) antibody OD levels and titres, as well as seroprevalence were lower than their corresponding AMA-1 measures across the five study sites (Fig. 1, Table 2). The overall seroprevalence was 39.5% (95% CI: 37.9–41.0). Seropositivity to MSP-1\(_{19}\) was similar (\( P = 0.382 \)) between Ungoye (43.4%) and Mfangano (45.4%), and significantly lower on the three small islands (\( P < 0.001 \) for all comparisons). Seroprevalence between genders showed a higher prevalence in females in Ungoye (\( P = 0.023 \)) and Mfangano (\( P = 0.024 \)), but the differences were comparable in the small islands. Antibody level and seroprevalence increased significantly with age (\( P < 0.001 \)) in all study areas (Fig. 2B). The acquisition of antibodies to MSP-1\(_{19}\) was moderate, with only 40% of participants becoming seropositive by 20 years of age (Fig. 3B). MSP-1\(_{19}\) SCRs were approximately 0.08 for Ungoye and Mfangano and 0.05 for the small islands, which corresponded to calculated aEIRs of 7 ib/p/yr in Ungoye and Mfangano, but only fewer than 3 ib/p/yr on the small islands (Fig. 3B, Table 2). Nevertheless, SCRs were not statistically significant across the study sites as evidenced by the overlapping confidence intervals. Similar to AMA-1, MSP-1\(_{19}\) SCRs showed significant correlation with \( P. falciparum \) parasite rates (microscopy, \( R^2: 0.8875, P = 0.017 \); PCR, \( R^2: 0.9446, P = 0.007 \)) (Fig. 4).

**CSP antibodies.** The overall seroprevalence for CSP was 12.9% (95% CI: 11.9–13.9). The different serological outcomes for CSP all produced similar trends; similar between the study areas, albeit the differences in antibody responses were statistically lower than AMA-1 and MSP-1\(_{19}\) (\( P < 0.001 \) for all comparisons) (Fig. 1, Table 2). Seroprevalence of males and females in all study areas were comparable. Antibody level and seroprevalence increased with age group in all study sites (\( P < 0.001 \)) (Fig. 2B), and the acquisition of antibodies to CSP was slow, with <20% of participants becoming seropositive by 20 years of age (Fig. 3C). SCRs were not significantly different among study sites and were not significantly correlated with \( P. falciparum \) parasite rate (microscopy, \( P = 0.869 \); PCR, \( P = 0.986 \)) (Fig. 4A).

**Antibody responses among \( P. falciparum \)-infected populations.** Figure 5 shows age-adjusted antibody responses to parasite antigens among PCR-confirmed \( P. falciparum \) infected individuals. Antibody responses did not differ significantly between the febrile and the non-febrile groups in all study sites, but the proportions of AMA-1 seropositive individuals were significantly higher (\( P < 0.05 \)) in the non-febrile groups in Ungoye and Mfangano (Fig. 5A). When stratified by anaemia (Hb level < 11 g/dL), total anti-AMA-1 IgG levels were significantly higher...
all three parasite antigens in Ungoye (P < 0.001) restricted to children 12 years and below, enlarged spleen was significantly associated with seropositivity to MSP-1 19 (P < 0.001). Enlarged spleen was also significantly associated with seropositivity to AMA-1 (P = 0.037) on Ngodhe. Significant association between anaemia and MSP-1 19 seropositivity was observed in Ungoye (P = 0.012) and Mfangano (P = 0.03) only (Table 4).

### Table 2. Estimates of current malaria infection and exposure.

| Category | Characteristic | Study site |
|----------|----------------|------------|
|          |                | Ungoye     | Mfangano   | Takawiri   | Kibuogi    | Ngodhe     |
| Sample size | Number of sample for microscopy | 1238 | 1938 | 883 | 536 | 633 |
|            | Number of sample for PCR | 1237 | 1938 | 883 | 536 | 632 |
|            | Number of sample for ELISA | 868 | 1574 | 770 | 325 | 575 |
| Blood smear microscopy | Positive for Plasmodium spp., n (%) | 403 (32.6) | 393 (20.3) | 38 (4.3) | 21 (6.3) | 39 (6.2) |
|            | Geometric mean parasite density, parasites/µl (95% CI) | 1101 (942–1286) | 1045 (877–1245) | 1458 (841–2526) | 1665 (641–4326) | 1229 (657–2298) |
|            | P. falciparum parasite rate in children 2–10 years, n/N (%) | 283/669 (42.3) | 211/864 (24.9) | 24/419 (5.7) | 11/146 (7.5) | 25/234 (10.7) |
|            | P. falciparum gametocyte rate, n/N (%) | 41/397 (10.3) | 34/358 (9.5) | 2/36 (5.6) | 5/21 (23.8) | 3/39 (7.7) |
| PCR | Positive for Plasmodium spp., n (%) | 1048 (84.7) | 1033 (53.3) | 153 (17.3) | 60 (17.9) | 82 (13.0) |
|            | P. falciparum parasite rate in children 2–10 years, n/N (%) | 433/669 (64.7) | 366/847 (42.5) | 62/420 (14.8) | 26/146 (17.8) | 35/234 (15.0) |
| ELISA | Median AMA-1 OD (IQR) | 0.89 (0.48–1.08) | 0.79 (0.33–1.06) | 0.35 (0.09–0.82) | 0.48 (0.14–0.92) | 0.40 (0.13–0.83) |
|            | Median AMA-1 antibody titre (IQR) | 1579 (358–5564) | 1001 (189–4083) | 194 (40–1082) | 265 (44–1418) | 225 (47–1025) |
|            | AMA-1 SCR, (95% CI) | 0.204 (0.181–0.228) | 0.166 (0.153–0.181) | 0.139 (0.123–0.156) | 0.136 (0.113–0.165) | 0.122 (0.106–0.141) |
|            | AMA-1 estimate EIR† (95% CI) | 12.1 (8.9–16.2) | 7.1 (5.7–8.9) | 4.4 (3.2–6.0) | 4.2 (2.6–7.0) | 3.2 (2.2–4.6) |
|            | Median MSP-1 19 OD (IQR) | 0.30 (0.13–0.81) | 0.33 (0.17–0.79) | 0.19 (0.11–0.43) | 0.20 (0.12–0.51) | 0.18 (0.11–0.43) |
|            | Median MSP-1 19 antibody titre (IQR) | 96 (33–580) | 118 (45–530) | 43 (24–162) | 52 (28–217) | 41 (22–131) |
|            | MSP-1 19 SCR, (95% CI) | 0.079 (0.070–0.089) | 0.075 (0.069–0.082) | 0.043 (0.037–0.049) | 0.048 (0.039–0.060) | 0.046 (0.039–0.054) |
|            | MSP-1 19 estimate EIR† (95% CI) | 7.1 (5.5–9.2) | 6.4 (5.4–7.7) | 2.0 (1.4–2.6) | 2.5 (1.6–4.0) | 2.3 (1.6–3.2) |
|            | Median CSP OD (IQR) | 0.44 (0.22–0.68) | 0.41 (0.26–0.72) | 0.24 (0.12–0.41) | 0.29 (0.20–0.44) | 0.24 (0.14–0.40) |
|            | Median CSP antibody titre (IQR) | 385 (207–834) | 446 (246–946) | 221 (106–406) | 245 (153–433) | 219 (123–422) |
|            | CSP SCR, (95% CI) | 0.008 (0.006–0.010) | 0.011 (0.010–0.013) | 0.008 (0.006–0.010) | 0.010 (0.007–0.014) | 0.009 (0.007–0.011) |

**Factors associated with seropositivity.** In the adjusted model, age was significantly (P < 0.005) associated with seropositivity to AMA-1, MSP-1 19 and CSP in all five study sites. Anaemia was significantly associated with seronegativity to AMA-1 on Mfangano (P = 0.018), Kibuogi (P = 0.008), and Ngodhe (P = 0.037) (Table 3). When restricted to children 12 years and below, enlarged spleen was significantly associated with seropositivity to all three parasite antigens in Ungoye (P < 0.01), Mfangano (P < 0.001), and Takawiri (P < 0.005). Enlarged spleen was also significantly associated with seropositivity to AMA-1 (P = 0.005) on Kibuogi, and seropositivity to MSP-1 19 (P < 0.001) and CSP (P = 0.002) on Ngodhe. Age was significantly (P < 0.001) associated with AMA-1 seropositivity in all five study sites, MSP-1 19 seropositivity in Ungoye (P < 0.001), and CSP seropositivity on Ngodhe (P = 0.024). Significant association between anaemia and MSP-1 19 seropositivity was observed in Ungoye (P = 0.012) and Mfangano (P = 0.03) only (Table 4).
Discussion
This study describes data on parasite prevalence and antibodies responses to AMA-1, MSP-1<sub>19</sub>, and CSP antigens of *P. falciparum* in 5,044 individuals living on a coastal area and four different islands in Lake Victoria, Kenya. The present study demonstrates a clear relation of serological outcomes for AMA-1 and MSP-1<sub>19</sub> with parasite prevalence and serology-derived EIR in heterogeneity of malaria transmission in the study area. AMA-1 exhibited the highest seroprevalence of the three antigens tested; AMA-1 seroprevalence was typically two- to four-fold greater than that for MSP-1<sub>19</sub> and CSP between each study area. The relationship between age and parasite prevalence was similar in all five areas, increasing with age until peaking by 11–15 years and then decreasing with

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**Figure 3.** Annual probability of seroconversion rate for specific malaria antigen by age in each setting. Maximum-likelihood fits from reversible catalytic equilibrium model from each setting are shown. λ, the area-specific annual rate of seroconversion. (A) AMA-1, (B) MSP-1<sub>19</sub>, (C) CSP. The model was constrained to fit a single value for the annual probability of a common seroreversion rate (ρ), which was estimated; AMA-1: 0.0377 (95% CI 0.0312–0.0456); MSP-1<sub>19</sub>: 0.0433 (95% CI 0.0330–0.0568); CSP: 0.0011 (95% CI 0.0000–0.61138). Points indicated observed seroprevalence and solid lines show model-predicted seroprevalence. Broken lines are 95% confidence intervals.

**Figure 4.** Association between the annual rate of seroconversion and *P. falciparum* parasite rate in the study area. Plot of estimate of seroconversion rates (λ) (calculated as for Table 2) against *P. falciparum* parasite rates by (A) microscopy, and (B) PCR. Un = Ungoye, Mf = Mfangano Island, Ta = Takawiri Island, Ki = Kibuogi Island, and Ng = Ngodhe Island.
age. Seroprevalence and seroconversion data showed clear increases in seroreactivity with age and independently associated with enlarged spleen in children below 12 years old.

We examined the cross-sectional data to investigate the capability of antigens tested to pick up current differences in malaria prevalence. We observed heterogeneity in age-seropositivity between the study areas. Although seroprevalence showed less marked difference in \( P. falciparum \) exposure between the study areas, SCR showed a distinct variation in transmission intensity. SCRs for \( P. falciparum \) merozoite antigens (i.e. AMA-1, MSP-1\(_{19} \))

**Figure 5.** Age-adjusted antibody responses among \( P. falciparum \) infected individual (positive by PCR) for AMA-1, MSP-1\(_{19} \), and CSP in each setting. (A) Presented with or without fever; stratified into non-febrile (axillary temperature \( \leq 37.5 ^\circ C \)) and febrile (\( > 37.5 ^\circ C \)) groups. (B) Presented with or without anaemia; stratified into anaemic (Hb level <11 g/dL) and non-anaemic (Hb level \( \geq 11 \) g/dL) groups. (C) Presented with or without enlarged spleen among children \( \leq 12 \) years old by Hackett’s method; stratified into enlarged spleen and normal groups. Data are presented in box plots (with the median shown as a line within the box and interquartile value (IQR, 25th–75th percentile) at the edge of box) and bar graphs (with Error bar represents 95% confidence interval (CI)). Any outlier values exceeding the interquartile range are shown as circles. Differences between the two groups of antibody levels were analysed by linear regression adjusting values for age (antibody level) and Chi-square test (proportion). *, ** and *** indicates significance at \( P < 0.05 \), \( P < 0.01 \) and \( P < 0.001 \), respectively.
higher than those for MSP-119 switching39, which may explain some of the differences in findings in our study areas. Nevertheless, antibodies in malaria areas, when analysis was restricted in children between AMA-1 than for MSP-1 19. This antigen into circulation37. In consequence, the availability of CSP to immune system is of much shorter dura- race, possibly reflecting their inherent immunogenicity, subclass dependent half-life, polymorphism etc. AMA-1 appears to be more immunogenic than MSP-1199, 10 and anti-AMA-1 titers tend to be higher than those for MSP-119, suggesting that seroconversion may be faster and seroreversion may be slower for AMA-1 than for MSP-119.

Despite differences in immunogenicity, both *P. falciparum* AMA-1 and MSP-119 antibody prevalence have been previously demonstrated as useful surrogate markers for malaria transmission intensity in areas of low transmission22, 59–31. At low transmission intensities, SCR has high sensitivity since the longevity of the antibody response toward blood-stage antigens generates higher seroprevalence rates than equivalent parasite rates25. As a measure that integrates exposure over time and reflects cumulative exposure rather than single current infection (e.g. microscopy), SCR based on serology can provide a more robust picture of the malaria transmission dynamics in an endemic area. In this setting, in the area with lack evidence of infections by microscopy, serological and molecular tools enabled a more complete understanding of the ongoing transmission and allowed for an examination of risk factors. Furthermore, as a metric of malaria transmission intensity, EIR is notoriously difficult to measure and the heterogeneous distribution of mosquito bites among individuals within a population makes it even more difficult to standardize32. Previously developed log-log calibration curves that relate SCRs to EIR values can provide a straightforward low-cost measure of malaria transmission9, 10. These serology-based EIR estimates, especially those based on MSP-119 SCR, have shown a robust correlation with measured EIR10, and thus allow for clear differentiation of low-, moderate-, and high-transmission areas.

Antibody responses to CSP did not follow the same pattern as those for merozoite antigens; neither sero-prevalence nor SCR showed significant difference among settings. CSP has been previously reported to give reliable estimates of malaria endemicity in hyper-endemic areas and reflect the seasonal dynamics of transmission25–39, making it a suitable marker for monitoring historical changes in transmission intensity11. AMA-1 and MSP-119 are erythrocytic-stage antigens; they are produced continuously and in relative abundance because the erythrocytic-stage parasites go through several cycles of multiplication. In contrast, CSP is a pre-erythrocytic-stage antigen and is available to the immune system when a small number of sporozoites shed this antigen into circulation27. In consequence, the availability of CSP to immune system is of much shorter duration, which may explain its inability to detect subtle differences in exposure and transmission among settings in this study.

Factors associated to seroreactivity were identified by multivariable analyses in each study area. In all five areas, *P. falciparum* antibody prevalence increased with age. When analysed as a whole population, only antibody responses to AMA-1 were negatively associated with anaemia in Mfangano, Takawiri and Ngondo. In contrast, when analysis was restricted in children ≤12 years, antibody responses to MSP-119 were significantly associated with anaemia in Ungoye and Mfangano, whilst all the antigens tested were independently associated with enlarged spleen in all areas. Associations between measured antibody responses to *P. falciparum* antigens and the risk of malaria have shown to be inconsistent in multiple studies38. Potential reasons for these inconsistencies include differences in the intensity and stability of transmission, allelic variation of specific antigens and IgG subclass switching39, which may explain some of the differences in findings in our study areas. Nevertheless, antibodies to AMA-1 have been associated with protection from clinical malaria in some studies38, 40, 41, but not others42, 43. Some studies have also documented independent association between antibodies to MSP-119 and protection from clinical malaria32, 44, whilst others have not43. Similar to the responses to blood-stage antigens, antibodies to CSP was associated with a higher odds of having an enlarged spleen, and age-adjusted antibody levels showed apparent high responses in children with enlarged spleen in each study area (Fig. 5C). This lack of protection to clinical malaria is consistent with prior studies45, 46 but in contrast with others47.
suggests that immune responses are less stable in this age group, fluctuating with concurrent infections. In high transmission settings where reductions are most difficult to achieve and sustain. This is promising information for future research, particularly in evaluating control interventions for malaria elimination.

The use of serology in assessing naturally acquired immune responses to malaria can provide useful information to understand the heterogeneity in malaria transmission between a relatively restricted geographical area. Measurement of parasite prevalence and serology enabled us to compare malariometric indices across different endemicity settings and confirm the heterogeneity in malaria transmission between a relatively restricted geographical area. Nevertheless, sub-microscopic infections will provide an antigenic stimulus to maintain immune responses, but the role of sub-microscopic infections in maintaining immunity is yet to be quantified.

This study has several limitations. First, the convenience sampling method used in this study has inherent selection bias. Most surveys were conducted in schools, meaning that children of school age (i.e. 6–15 years) were disproportionately represented (Table 1). Although this approach is valid in obtaining an estimate of antimalarial antibody prevalence, over-representation of this age group among our samples likely overestimated the true malaria prevalence in the study area. Second, our surveys undersampled adult males that could have led to an underestimation of overall seroprevalence. Many adult males in the study area are engaged in fishing activities during daytime hours when our surveys were held. Malaria infections and immune status in this mobile and hard-to-reach group are not well characterised and warrant further investigation. Third, the survey design did not permit georeferencing of individual household locations and complete climate data from each study setting was unavailable. Therefore, no spatiotemporal analysis was possible to investigate patterns within individual sites or settings. A recent study screened over 800 Plasmodium antigens has identified serological markers of recent malaria infection. Some of these markers may perform considerably better for the purpose of monitoring changes in transmission intensity.

Together these observations collectively suggest that serological analysis could be effective as an adjunct tool used in combination with parasite prevalence for surveillance, control and elimination of malaria in high endemic area. This study has demonstrated the potential of malaria antigens, namely AMA-1 and MSP-1₁₀₀, as important markers for assessing differences in malaria transmission in a moderate to high endemic area. The concurrent measurement of parasite prevalence and serology enabled us to compare malariometric indices across different endemicity settings and confirm the heterogeneity in malaria transmission between a relatively restricted geographical area. The use of serology in assessing naturally acquired immune responses to malaria can provide promising information for future research, particularly in evaluating control interventions for malaria elimination in high transmission settings where reductions are most difficult to achieve and sustain.

### Methods

#### Study area

The present study was conducted on four islands in Lake Victoria (Mfangano, Takawiri, Kibuogi, Ngodhe) and one mainland lakeshore area (Ungoye) in Homa Bay County, western Kenya (Fig. 1). Homa Bay County has an area of 4,267 square kilometres (km²). The population in the study area consists mainly of people of the Luo ethnic group with Dholuo is primarily spoken, as well as the national language of Kiswahili. Population estimates were 18,600 for Mfangano, 1,000 for Takawiri, 700 each for Kibuogi and Ngodhe (Nagasaki University-Mbita Health and Demographic Surveillance System), and 3,471 for Ungoye (2009 Kenya Population and Housing Census). The area is predominantly rural with most residents depend on fishing and traditional small-scale farming as the main occupations. Most houses are typically made of mud walls with thatched or corrugated iron roofs.

The climate in Lake Victoria is tropical, with temperatures ranging from a mean annual minimum of 17.7 °C to a mean annual maximum of 34.8 °C, and humidity is relatively high. This region generally experiences a bimodal climate with two seasons: the short rains and long rains.
pattern of rainfall, with the longer rainy season starting in March and ending in July and the shorter rainy season from November to December. In Lake Victoria basin, peak malaria transmission occurs 1–2 months after the rainy season and the mean monthly anopheline vector abundance has been reported to increase by 6- to 8-fold in the rainy season compared to the dry season. Malaria in the study area is meso-endemic but geographically heterogeneous, with prevalence estimates ranging from 14.6% on islands to 44.2% in the coastal mainland. The major vector responsible for malaria transmission in Lake Victoria is Anopheles gambiae s.l. and estimated annual EIR from serological markers were reported as high as 50 infectious bites per person per year. Plasmodium vivax and Plasmodium ovale are less common, and no Plasmodium falciparum infections are observed.

In this study, seven catchment areas on Mfangano (i.e. large island) were selected to represent the different local environments found on the island. Gulwe and Kagungu are situated in the central highland. On the other hand, Ramba, Wakinga, Mrongo, Ugina and Wakula are situated along the coastal lowlands of the island. Across the island, there are six government health facilities including one health centre and five dispensaries. Meanwhile, on each small island, population is mainly distributed between two main settlements: Kamarach and Konga on Takawiri Island, Kibuogi A and Kibuogi B on Kibuogi Island, and Bonde and Luanda on Ngodhe Island. Takawiri and Kibuogi are close to Mfangano while Ngodhe is situated to the north of Rusinga Island. Takawiri and Ngodhe are each served by a dispensary, but no public health facility is available on Kibuogi. Ungoye, a small village on the mainland was included for comparative purposes due to its similarity to the islands in environmental characteristics, infrastructure, and access to the health facilities. It is served by a government dispensary. The village is connected by an unpaved road to the nearby towns of Sindo and Mbita, each with a sub-district hospital.

Ethical consideration. This study was carried out in full accordance with all international, Kenyan and Swedish accepted guidelines as written informed consent was obtained from all participants. The Ethics Committee reviewed and approved all the consent procedures. This study was approved by the Kenyatta National Hospital/University of Nairobi-Ethics and Research Committee in Kenya (No.P7/1/2012) and the Committee on the Ethics of Human Research of Karolinska Institutet in Sweden (Dnr 2012/7139--31/4).

Sample collections. Two cross-sectional surveys were performed in January and August 2012, after the short and long rainy seasons, respectively. Both surveys were conducted approximately 2 months after the rainy seasons to coincide with the periods of heaviest malaria burden. In our effort to ensure a high coverage of sampled population, each survey was conducted on different parts of the five study settings. The convenience sampling strategy was used in this study, whereby residents were asked to come to selected survey points such as community-based beach management unit (BMU) halls or school for study participation. Island and village leaders were sensitised to study by trained field workers and together provided information to community members at community meetings. Adult community members willing to participate were asked to read and provide signature on an informed consent form. In case of illiteracy, consent by thumb print was obtained in the presence of an independent literate adult witness. For children ≤12 years of age, consent was obtained from parents or guardians.

Different catchment areas were targeted in each survey (Fig. 1). On Mfangano Island, three (i.e. Gulwe, Ramba, Wakinga) and four (i.e. Kagungu, Mrongo, Ugina, Wakula) catchment areas were surveyed in January and August, respectively. Except for Wakula and Ugina where surveys were conducted in BMU halls, primary school surveys were carried out in other catchment areas on Mfangano. The intra-island epidemiology of malaria in this island has been described in detail elsewhere. On each small island, surveys were conducted separately between the two main settlements: Kamarach, Kibuogi A and Bonde in January and Kongata, Kibuogi B and Luanda in August. All surveys on small islands were conducted at BMU halls, which are typically connected to marketplaces.

To ensure a balanced representation of all age groups, five age categories were defined per study area: one to five years, six to ten years, 11–15 years, 16–30 years, and >30 years. Age and gender of each participant were recorded. Axillary body temperature was determined using a digital thermometer (Terumo, New Jersey, US), and no fever or malaria status was recorded. A 3 mm disk was punched from each dried blood spot and serum was eluted in reconstitution buffer in 0.5 ml deep well plates (Corning Costar, PA, USA) as described previously. The reconstituted blood spot solution, equivalent to a 1/200 dilution of serum, was stored at 4 °C until used for antibody test.
All sera were tested for IgG antibodies by indirect quantitative enzyme-linked immunosorbent assay (ELISA) to two recombinant blood-stage *P. falciparum* malaria antigens namely apical membrane antigen-1 (AMA-1, 3D7) and merozoite surface protein-1 (MSP-1,19, Wellcome genotype), as well as to the *P. falciparum* sporozoite antigen NAP-1 repeat peptide circumsporozoite protein (CSP) (Alpha Diagnostic International, USA). Briefly, antigens were coated on NUNC-Immuno plates (Sigma) at concentrations of 0.5 µg/ml and 1.0 µg/ml in coating buffer (50 µl per well) for recombinant blood-stage antigens (AMA-1 and MSP-1,19) and CSP, respectively. The plates were washed in PBS with 0.05% Tween 20 (PBS/T) and blocked using 1% (w/v) skimmed milk solution (Sigma, USA) in PBS/T for three hours. After washing, 50 µl of reconstituted blood spot solution were added in duplicate at final dilutions of 1:1000 for both MSP-1,19 and CSP, and 1:2000 for AMA-1 and incubated overnight at 4 °C. A positive control consisting of a pool of hyper-immune sera was included in each plate. The plates were washed and 50 µl of horse-radish peroxidase (HRP)-conjugated rabbit anti-human IgG antibody (DAKO, Denmark) were added to all wells at a dilution of 1:15,000 in PBS/T and incubated for three hours. After further series of washes, 100 µl of the substrate solution 3,3′,5,5′-tetramethylbenzidine (TMB) (tebu-bio laboratories, France) were added. Reactions were stopped after 15 minutes with 50 µl per well of 0.2 M H₂SO₄. The optical density (OD) at 450 nm was read using Multiskan Go ELISA reader (Thermo Fisher Scientific, USA).

**Data analysis.** Statistical analysis was performed using STATA/SE version 13.1 (StataCorp, TX, USA) and GraphPad Prism Software version 5.03 (GraphPad Software Inc., CA, USA). Duplicate ELISA OD values were averaged and normalised against values from blank wells as previously described to adjust for background reactivity5. Seropositivity was determined by fitting a mixture model to normalised OD values assuming two Gaussian distributions, one for sero-negative individuals and another for sero-positive individuals58,59. The mean OD plus three standard deviations associated with the sero-negative group was used as the cut-off value for seropositivity. A separate cut-off was generated for each antigen. Differences in parasite prevalence and seroprevalence estimates between study areas and age categories were performed using a two-sided test for proportions9,10. The titre of antibody responses was estimated using the equation dilution/maximum OD/(OD test serum – minimum OD) – 1; the median titre and interquartile range (IQR) are given. Differences in antibody responses were assessed using age-adjusted linear regression. Seroprevalence was stratified into yearly age groups and then analysed using a reverse catalytic modelling approach under a binomial sampling assumption, as described elsewhere9,10,11. This method provides estimates of the mean annual rates of conversion to seropositive (seroconversion rate, SCR [λ]) and reversion to seronegative (seroreversion rate, SRR [µ]) status, averaged over the age of the population. The common SCR estimates of the mean annual rates of conversion to seropositive (seroconversion rate, SCR [λ]) at 4 °C. A positive control consisting of a pool of hyper-immune sera was included in each plate. The plates were washed and 50 µl of horse-radish peroxidase (HRP)-conjugated rabbit anti-human IgG antibody (DAKO, Denmark) were added to all wells at a dilution of 1:15,000 in PBS/T and incubated for three hours. After further series of washes, 100 µl of the substrate solution 3,3′,5,5′-tetramethylbenzidine (TMB) (tebu-bio laboratories, France) were added. Reactions were stopped after 15 minutes with 50 µl per well of 0.2 M H₂SO₄. The optical density (OD) at 450 nm was read using Multiskan Go ELISA reader (Thermo Fisher Scientific, USA).

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**Acknowledgements**

First and foremost, we would like to extend our gratitude to the communities and community leaders for their support and participation in the surveys. We wish to sincerely thank all members in field team (Charles Owino, Peter Obudho, Stephen Omondi, Juliet Ndage, Debby Cheptoo, Kevin Omondi, Alphone Otunga, Lilian Wang’a, Everlyne Akinyi, Daphne Atieno, Debra Sang, Caroline Avoga, Amisi Kevin, Irene Oguta, Opollo Joy, Winnie Ochieng, Wycliffe Ochieng, Mercy Ochieng, Nyabanda Abraham, Lucy Oketch, Martin Shikuku, Raymond Oketch), microscopists (Irene Awour, George Orwa, Moses Adie, Kennedy Odok, Sophie Yogo, Fredrick Ooko, Daniel Nanzai, Pamela Omega, Adel), local community health workers (Vincent Opoto, Elekia Odida, Kennedy Obiero, Samwer Ocheing, John Ooko, Steve Okoth, Kesia Achola, Pamela Auma), and boat coxswains (Kennedy Ochieng, Anwar Oreng, Steve Omondi, Duncan). We are grateful to Rie Isozumi, Mayumi Fukui and Ikuo Kusuda from Osaka City University who helped in running the PCR assays. We also thank Tomomi Kuwana, Yukie Saito, and Mitsuo Takato for their assistance in management and logistics of the surveys. Finally, we specially thank Prof. Yoshio Ichinose for his overall supports to our research activities in Kenya. This work (P.I.: A.K.) was supported by Swedish Research Council grants (523-2009-3233, 348-2012-6346, and 348-2013-6311), Japan Society for Promotion of Science (JSPS) Core-to-Core Programme B, Asia-Africa Science Platforms JSPS KAKENHI grant numbers (24390141 and 26257504), Health Labor Sciences Research Grant, Research on Global Health issues, and the collaborative research grant of Nagasaki University Institute of Tropical Medicine. Z.M.I. was supported by a fellowship from the Academic Training Scheme for Lecturers (SLAI) administrated by the Ministry of Higher Education, Malaysia.

**Author Contributions**

Z.M.I., C.D. and A.K. conceived and designed the study. Z.M.I., C.W.H., J.K., J.L., J.G. and A.K. participated in field study and sample collection. Z.M.I. and T.H. performed the laboratory assays. Z.M.I. analysed the data. C.D. and A.K. contributed reagents/materials/analysis tools. Z.M.I. wrote the first draft of the manuscript. All authors read and approved the final manuscript.

**Additional Information**

**Supplementary information** accompanies this paper at doi:10.1038/s41598-017-09585-4

**Competing Interests:** The authors declare that they have no competing interests.

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