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Factors Associated With Human IgG Antibody Response to *Anopheles albimanus* Salivary Gland Extract, Artibonite Department, Haiti, 2017

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Serological data can provide estimates of human exposure to both malaria vector and parasite based on antibody responses. A multiplex bead-based assay was developed to simultaneously detect IgG to *Anopheles albimanus* salivary gland extract (SGE) and 23 *Plasmodium falciparum* antigens among 4185 participants enrolled in Artibonite department, Haiti in 2017. Logistic regression adjusted for participant- and site-level covariates and found children under 5 years and 6–15 years old had 3.7- and 5.4-fold increase in odds, respectively, of high anti-SGE IgG compared to participants >15 years. Seropositivity to *P. falciparum* CSP, Rh2_2030, and SEA-1 antigens was significantly associated with high IgG response against SGE, and participant enrolment at elevations under 200 m was associated with higher anti-SGE IgG levels. The ability to approximate population exposure to malaria vectors through SGE serology data is very dependent by age categories, and SGE antigens can be easily integrated into a multiplex serological assay.

Keywords. *Anopheles albimanus*; multiplex serology; mosquito saliva; immunoglobulin G; *Plasmodium falciparum*.

Malaria transmission continues in tropical areas of the world despite widespread efforts at control and elimination. In the Caribbean, malaria is endemic only to the island of Hispaniola, composed of Haiti and the Dominican Republic [1]. Malaria transmission in Haiti is low, often not exceeding 1% prevalence of infection in many areas across the country [2], with *Plasmodium falciparum* the predominant malaria species and *Anopheles albimanus* is the most prevalent vector [3, 4]. In low-transmission settings such as Haiti, accurate estimation of human exposure both to the vector and the parasite becomes especially crucial for obtaining accurate and precise estimates.

Serologic methods, which leverage the human antibody response against antigens in vector and parasite alike, have become a useful tool for obtaining these exposure estimates. Compared to single-target assays like enzyme-linked immunosorbent assay (ELISA), the multiplex bead-based assay (MBA) provides capacity for higher throughput data collection by assaying multiple analytes at once from a single specimen [5]. The MBA has recently been utilized to detect antibodies against a variety of *Plasmodium* antigens as evidence of both long- and short-term malaria exposure [6]. Serologic methods have also been employed for detecting antibody responses to anopheline malaria vectors as biomarkers of exposure, and have been used for understanding vector contact for individuals and populations [7–9]. Immunoglobulin G (IgG) response to *Anopheles* salivary antigens can serve as a biomarker of exposure to mosquito bites in areas where *An. albimanus* is endemic, such as Haiti [10–12].

This study used cross-sectional data collected in 2017 from an easy access group (EAG) survey conducted in Artibonite department, Haiti with participant enrollment at places of congregation [13]. Persons of all ages were enrolled, given a questionnaire, and provided a blood sample for testing of active *P. falciparum* infection and later serological assays. An MBA was developed and deployed to detect IgG against a panel of 23 *P. falciparum* antigens in blood samples collected from study participants as well as IgG response against *An. albimanus* whole salivary gland extract (SGE). The primary objectives of this study were to investigate associations between anti-SGE IgG and individual and environmental factors, as well as determine if persons with greater *P. falciparum* exposure had evidence of increased vector exposure through this serological data.

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METHODS

Human Subjects

As described previously [13, 14], EAG surveys received approval from the Haitian Ministry of Public Health and Population Bioethics Committee (*Comité National de Bioéthique*), the Institutional Review Boards of Tulane University and the London School of Hygiene and Tropical Medicine. This activity did not constitute engagement in human subjects research as determined by the US Centers for Disease Control and Prevention's Center for Global Health human subjects office (2016–135a). Consent for school EAGs was collected from school directors and following community meetings to inform parents and allow opt-out. At health facility and church EAGs, adult participants provided consent directly; consent for children (<18 years) was provided by a parent or guardian, and children above 6 years gave assent to participant. Individuals aged 16 or 17 who were married, head of household, or a parent were considered mature minors and consented directly. Thumbprint consent or assent (countersigned by a witness) was used for illiterate participants. Individuals under 6 months of age or requiring immediate medical attention were excluded. A single finger-prick was performed on consenting participants to collect capillary blood for the high-sensitivity rapid diagnostic test (hsRDT, also known as ultrasensitive RDT, Alere Malaria Ag P.f.; 05FK141; Standard Diagnostics), and dried blood spots (DBS) on filter paper (Whatman 903; GE Healthcare).

Participant Enrollment

The Verrettes and La Chapelle communes of the Artibonite department in Haiti are characterized by mountainous terrain with the Artibonite river forming the northern border of both communes. Enrollment sites encompassed rural, semirural, and urban populations within the Artibonite valley as well as the higher-elevation sides of the valley. Participants were enrolled at 3 different types of venues: health facilities, schools, and churches, with further detail in Supplementary Material. A sample of 21 schools and 9 churches was selected using stratified random sampling in Artibonite (Figure 1). As previously reported, among individuals for whom household data were available, the median distance traveled to schools was 1.1 km (interquartile range [IQR], 0.5–1.8 km) and to health facilities was 2.4 km (IQR, 0.7–4.9 km) [13]. Data collection was performed in April and May 2017, which is the beginning of the rainy season in Haiti. A questionnaire was administered to all participants to gather information about their history of fever, treatment-seeking practices, travel, and sociodemographic characteristics. DBS were shipped immediately to the national laboratory in Port-au-Prince, Haiti for serological data collection from May to June 2017.

Mosquito Dissection, SGE Preparation, and Bead Conjugation

Three- to 7-day-old unfed *An. albimanus* mosquitoes (laboratory strain STECLA) had whole salivary glands dissected and frozen for later use. Whole salivary glands were homogenized with glass tissue grinder in phosphate buffered saline pH 7.2, and freeze-thawed twice for further protein dissociation. Total protein concentration of this SGE homogenate was determined by bicinchoninic acid (BCA) assay (Pierce BCA Protein Assay Kit; ThermoFisher).

Malaria antigens and SGE homogenate proteins were covalently bound to magnetic microbeads (Luminex) through the sulfo-NHS/EDC (N-hydroxysulfosuccinimide/1-ethyl-3-(3-dimethylaminopropyl) carbodiimide)intermediate reaction as described previously [15, 16], and fully described in Supplementary Material.

Assay for Anti-SGE and *P. falciparum* IgG by Multiplex Bead Assay

DBS processing and IgG detection assay was performed in flat-bottom BioPlex Pro 96-well plates (Bio-Rad) as described previously [16], and fully outlined in Supplementary Material. Background mean fluorescence intensity (MFI) from wells incubated with buffer B was subtracted from each sample to give a final value of MFI background. Positive and negative IgG controls were added to each assay plate to ensure performance and identify if assay plates needed rerunning as described previously [17]. To optimize the protein coupling concentration to provide the most robust anti-SGE IgG assay signal, a random panel of samples from 78 Haitian participants was tested against beads coupled to different concentrations of anti-SGE homogenate (Supplementary Figure 1).

Statistical Analyses

Data analysis was performed using SAS version 9.4. Log-transformed MFI background values represent anti-SGE IgG. Student *t* test, ANOVA, and Tukey pairwise comparisons assessed differences in anti-SGE IgG response by participant-level covariates. Nonparametric Mann-Whitney, Kruskal-Wallis, and Dunn tests of comparisons assessed differences by enrollment site-level environmental covariates using an empirical Bayes estimate of mean anti-SGE IgG by site. Remotely sensed data for environmental covariates were obtained from outside sources and values for each site were sampled using QGIS (3.20.3-Odense) (Supplementary Table 1). A multilevel logistic regression model estimated odds of an above-the-median anti-SGE IgG response by adjusting for participant- and enrollment site-level covariates. An α level of .05 was used for all statistical tests.

RESULTS

SGE Bead Conjugation Optimization

Five different protein concentrations of SGE homogenate (7.5, 15, 30, 60, and 120 µg/mL) were conjugated to different bead regions to directly compare IgG assay signal among these concentrations and choose the best for large-scale bead

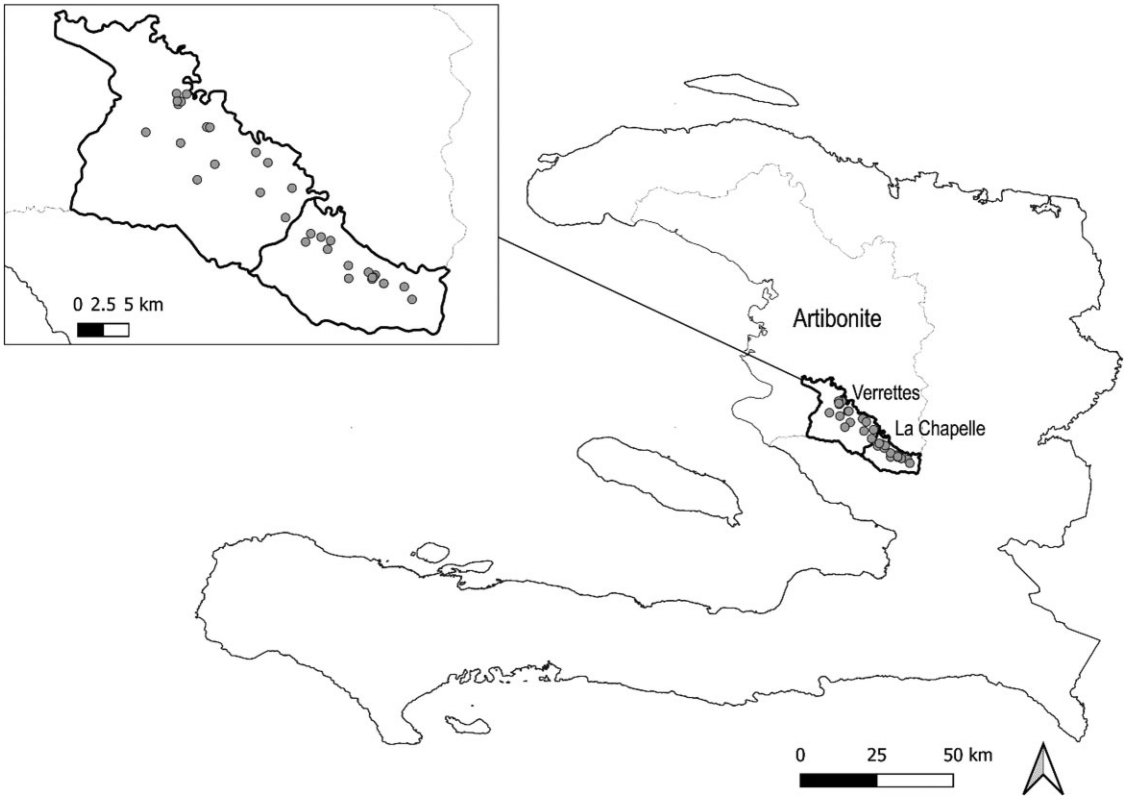

Figure 1. Study enrollment sites in the communes of Verrettes and La Chapelle in Artibonite, Haiti, 2017.

conjugation. As shown in Supplementary Figure 1, among the panel of 78 random Haitian blood samples, the 30 μg/mL SGE homogenate concentration consistently gave the highest MFI background assay signal, and thus was chosen for the bead conjugation for the remainder of the study.

Study Population

Within the Artibonite EAG, the median age of study participants was 14 years and 60.5% of participants were female (Table 1). Participants enrolled with the highest frequency at health facilities (40.9%), compared to schools (34.6%) and churches (24.5%). As indicated by a positive hsRDT result, 0.9% of participants had active *P. falciparum* malaria infection at the time of enrollment. In total, 2.9% of all participants had a fever at the time of data collection, while 14.0% had a fever within the 2 weeks prior to collection; among those with a positive hsRDT, 7.7% had current fever and 36.1% had a history of fever. Most participants (58.0%) indicated they had used a bed net the night before enrollment. For the 1021 participants for whom these data were available, anti-SGE IgG was higher among those who used a bed net (mean = 6.91, SD = 2.36) versus those who had not (mean = 6.68, SD = 2.24); however, this difference was not statistically significant ($t = -1.60$, $P = .11$).

Individual Covariates

Median log SGE IgG MFI background level of the study population was 6.0 with a strong peak for IgG response occurring between the ages of 6 and 7 years (Figure 2). The 6 to 7-year age group showed a median value of 9.3, while the lowest median value, 4.9, occurred in the 6-month to 1-year age group (Figure 2*A*). Beyond age 30 years, SGE responses remained consistently low for all ages with a median of 5.1 for >30 year olds (Figure 2*B*). The mean anti-SGE IgG value of males was significantly higher than that of females in both Verrettes ($t = 4.09$, $P < .0001$) and La Chapelle ($t = 3.25$, $P = .001$) (Supplementary Figure 2*A*). Differences in anti-SGE IgG response by household size (categories of 1–4, 5–7, or more than 7 people) were significant in La Chapelle ($F = 4.60$, $P = .01$), but not in Verrettes ($F = 1.86$, $P = .16$; Supplementary Figure 2*B*). Mean SGE IgG level was not significantly different based on malaria infection ($t = 0.46$, $P = .65$; Supplementary Figure 2*C*). There were no significant differences in mean anti-SGE IgG by sex based on age group ($t_{\text{age }0-5} = -1.52$, $P = .13$; $t_{\text{age }5-10} = 0.76$, $P = .45$; $t_{\text{age }10-15} = -0.62$, $P = .53$; $t_{\text{age }>15} = -0.01$, $P = .99$; Supplementary Figure 2*D*). Distribution of anti-SGE IgG levels by commune of enrollment and sex are shown in Supplementary Figure 3.

Table 1. Characteristics of the Study Population, Artibonite, Haiti, 2017

Characteristic	Participants (n = 4185)	hsRDT Positive (n = 39)
Age (n = 4180 for all)		
6 m to 5 y	620 (14.8)	1 (2.6)
6–15 y	1667 (40.0)	16 (41.0)
>15 y	1893 (45.3)	22 (56.4)
Median age (years)	14.0 (8.0–32.0)	19.0 (11.0–44.0)
Female sex (n = 4142 for all)	2506 (60.5)	17 (47.2)
Household size (number persons)		
1–4	1636 (39.5)	10 (27.8)
5–7	1863 (45.0)	18 (50.0)
>7	641 (15.5)	8 (22.2)
Median size (number persons)	5.0 (4.0–7.0)	6.0 (4.0–7.0)
History of fever[a] (n = 4120 for all)	575 (14.0)	13 (36.1)
Current fever[b] (n = 4183 for all)	123 (2.9)	3 (7.7)
Used individual bed net[c] (n = 1021 for all)	593 (58.0)	1 (25.0)
Commune		
La Chapelle	1888 (45.1)	20 (51.3)
Verrettes	2297 (54.9)	19 (48.7)
Venue		
Church	1027 (24.5)	6 (15.4)
Health facility	1711 (40.9)	19 (48.7)
School	1447 (34.6)	14 (35.9)
Positive hsRDT (n = 4184 for all)	39 (0.9)	…

Data are No. (%) except where indicated.

Abbreviation: hsRDT, high sensitivity rapid diagnostic test.

[a]Ill with fever at any point during the past 2 weeks

[b]Temperature >37.5°C

[c]Use of a bed net the prior night.

Environmental Covariates

SGE IgG by site of enrollment ranged from 4.8 to 8.0 (Figure 3*A*). Anti-SGE IgG monotonically declined with increasing elevation ($r_s = -0.22$, $P = .21$; Figure 3*B*). When elevation was categorized into 3 levels (<100 m, 100–200 m, >200 m), anti-SGE IgG at elevations between 100 and 200 m was significantly higher compared to the levels seen at enrollment sites above 200 m ($P = .01$; Figure 3*C*). When comparing all sites below 200 m to any sites above 200 m, this difference was statistically significant ($P = .03$). Differences in anti-SGE IgG by other environmental covariates were also examined based on each covariate's median value (listed in Supplementary Table 2). Anti-SGE IgG was significantly higher at sites that were less than 2.3 km away from the nearest water body compared to those farther than 2.3 km ($P = .04$). When categorizing distance to the nearest water body into quartiles (0.9–2.0 km, >2.0–2.3 km, >2.3–2.6 km, and >2.6 km), the only significant difference in pairwise comparisons was for sites with water bodies 2.0–2.3 km away versus those with water bodies greater than 2.6 km away ($P = .002$). There was no difference in anti-SGE IgG based on population density above versus below the median of 424 people/km^2 ($P = .71$), by normalized difference vegetation index above versus below 0.55 ($P = .34$), by air temperature above versus below 25.9°C ($P = .54$), or by average rainfall above versus below 297 mm ($P = .10$).

Multilevel Logistic Regression

When controlling for other covariates, only age group and seropositivity to 3 *P. falciparum* antigen targets (circumsporozoite protein [CSP], reticulocyte binding-like protein homologue 2 [Rh2_2030], and schizont egress antigen [SEA-1]) were significantly associated with a high anti-SGE IgG response (Table 2 and Supplementary Table 3). The adjusted odds of a high IgG response to SGE for participants aged 6 months to 5 years were nearly 4-fold greater (adjusted odds

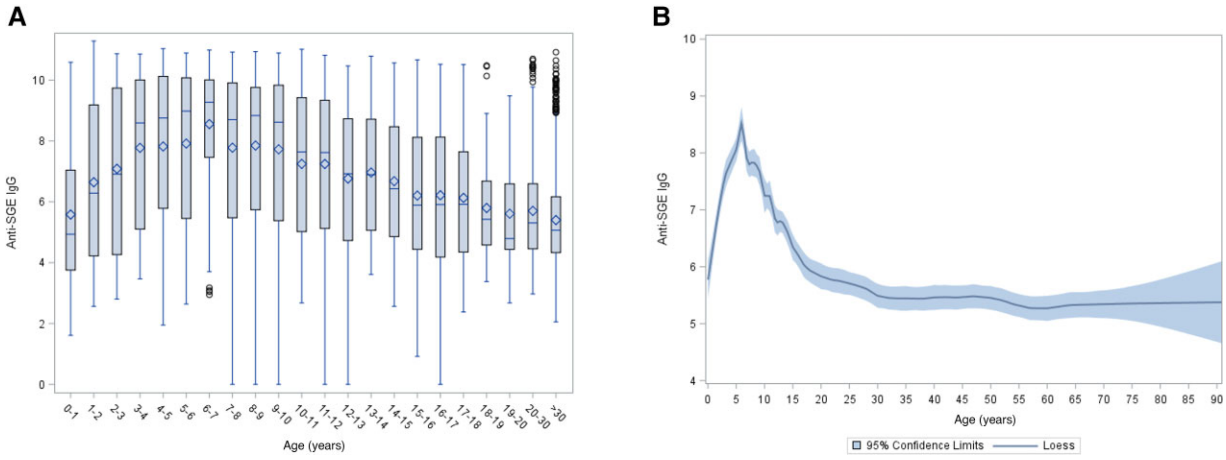

Figure 2. Relationship between age of enrolled participants and anti-salivary gland extract (SGE) immunoglobulin G (IgG) levels. *A*, Boxplots of log-transformed SGE IgG levels by age group (in years). Boxes represent the interquartile range (IQR) of anti-SGE IgG values for each age group; the horizontal line in each box is the median anti-SGE IgG value for each age group, and the diamond represents each group's mean anti-SGE IgG value. Whiskers extend 1.5 × IQR above and below boxes, and circles represent outlier anti-SGE IgG values outside of 1.5 × IQR. *B*, Loess curve of the relationship between anti-SGE IgG and age. The line represents a fitted smooth curve between age and anti-SGE IgG. Shading depicts the range of values we are 95% confident contain the true range of anti-SGE IgG values by age.

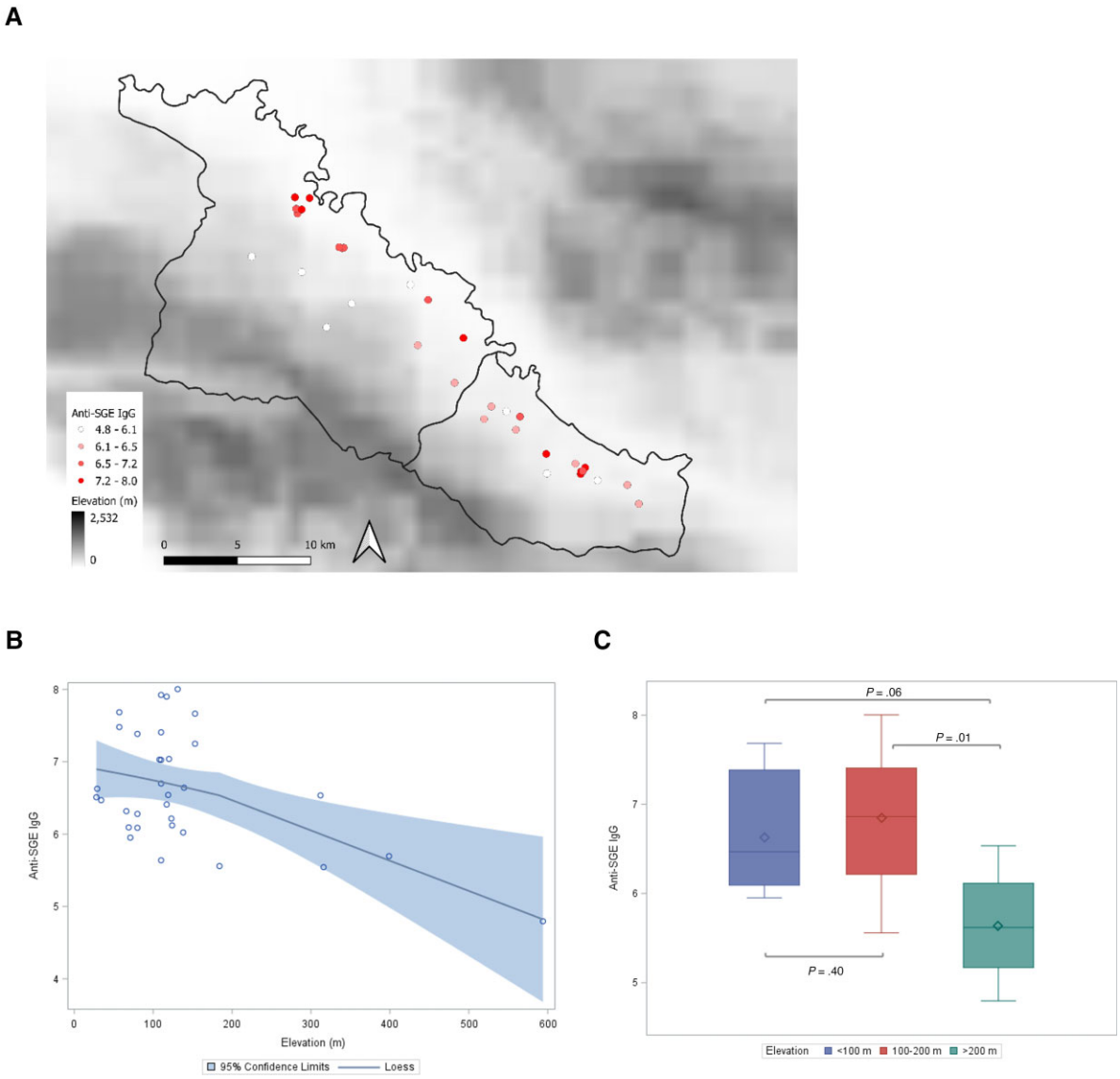

Figure 3. Association between anti-salivary gland extract (SGE) immunoglobulin G (IgG) and elevation of enrollment sites. *A*, Study enrollment sites in La Chapelle and Verrettes by quartile of anti-SGE IgG. Darker shading represents higher elevation, while lighter shading represents lower elevations. Circles represent each of 33 study enrollment sites with darker color indicating higher anti-SGE IgG value for the site. *B*, Scatter plot with smoothed Loess line of the relationship between anti-SGE IgG by site and elevation. Shading depicts the range of values we are 95% confident contain the true range of anti-SGE IgG values by elevation. *C*, Boxplots of anti-SGE IgG by site for elevations <100 m, 100–200 m, or >200 m. Boxes represent the interquartile range (IQR) of anti-SGE IgG values for each elevation level; the horizontal line in each box is the mean anti-SGE IgG value for each level, and the diamond represents each elevation level's mean anti-SGE IgG value. Whiskers extend 1.5 × IQR above and below boxes, and circles represent outlier anti-SGE IgG values outside of 1.5 × IQR. Horizontal lines above boxes show *P* values from Dunn test.

ratio [aOR], 3.72; 95% confidence interval [CI], 2.29–6.04) when compared to participants over the age of 15 years. For 6–15 year olds, odds of an elevated IgG response were over 5 times higher (aOR, 5.40; 95% CI, 3.68–7.93) compared to >15 year olds. Among the 3 *P. falciparum* antigens, seropositivity to CSP and SEA-1 were both positively associated with increased odds of high anti-SGE IgG compared to those who were not seropositive to each antigen. Seropositivity to Rh2_2030 was associated with approximately 60% decreased odds of the high SGE IgG when compared to those not seropositive to Rh2_2030 (aOR, 0.39; 95% CI, .22–.69). All 3 antigens showed significant differences in mean anti-SGE IgG levels when each antigen's log-transformed MFI background values were categorized into quartiles (Supplementary Figure 4).

DISCUSSION

This study examined the relationship between human IgG responses to a mixture of proteins from the *An. albimanus*

Table 2. Parameter Estimates of Odds Ratios for Main Effects of Multilevel Logistic Regression

Effect	Estimate	Odds Ratio (95% CI)	*P* Value
Age, y (ref. >15)			
0–5	**1.31**	**3.72 (2.29–6.04)**	**<.0001**
6–15	**1.69**	**5.40 (3.68–7.93)**	**<.0001**
Sex (ref. female)	0.26	1.30 (.97–1.74)	.08
History of fever (ref. none)	0.26	1.30 (.83–2.04)	.26
Current fever (ref. none)	0.023	1.02 (.43–2.41)	.96
Individual bed net usage (ref. none)	0.11	1.12 (.84–1.50)	.44
Positive hsRDT (ref. negative)	−1.50	0.22 (.02–2.28)	.21
Plasmodium falciparum seropositivity (ref. seronegative)			
*Pf*AMA1	0.12	1.13 (.76–1.67)	.56
MSP2 CH150/9	0.01	1.01 (.55–1.84)	.98
CSP	**0.55**	**1.74 (1.03–2.95)**	**.04**
MSP2 Dd2	0.28	1.32 (.67–2.59)	.42
E140	−0.03	0.97 (.58–1.62)	.91
E175	0.20	1.22 (.55–2.71)	.62
E181	−0.22	0.80 (.36–1.80)	.60
Etramp 4 Ag 2	0.32	1.38 (.77–2.48)	.28
Etramp 5 Ag 1	0.09	1.09 (.56–2.11)	.80
GEXP18	0.12	1.13 (.61–2.09)	.70
*Pf*GLURP R0	0.81	2.24 (.98–5.13)	.06
*Pf*GLURP R2	0.16	1.17 (.68–2.01)	.57
H103	−0.21	0.81 (.27–2.44)	.71
HRP2	0.39	1.48 (.78–2.83)	.23
HSP40 Ag 1	−0.08	0.92 (.41–2.11)	.85
Hyp 2	−0.21	0.81 (.38–1.73)	.58
LSA-1	−0.10	0.91 (.39–2.10)	.82
*Pf*MSP-1_{19}	0.02	1.02 (.70–1.47)	.94
Rh5.1	0.20	1.23 (.63–2.38)	.55
Rh4.2	−0.03	0.97 (.53–1.81)	.94
Rh2_2030	**−0.95**	**0.39 (.22–0.69)**	**.001**
SBP1	0.15	1.16 (.84–1.62)	.37
SEA-1	**0.45**	**1.57 (1.03–2.39)**	**04**
Elevation, m	−0.00	1.00 (1.00–1.00)	.15
Air temperature, °C >25.9 °C (ref. ≤25.9 °C)	0.28	1.32 (.99–1.75)	.06
Distance, m, to nearest water body >2311 m (ref. ≤2311 m)	0.12	1.13 (.80–1.58)	.49
Population density, per km^2	−0.00	1.00 (1.00–1.00)	.82
Normalized difference vegetation index	−0.13	0.88 (.15–5.17)	.89
Average rainfall, mm	0.00	1.00 (1.00–1.01)	.35

Bold indicates a statistically significant association with high anti-SGE IgG level.

Abbreviations: CI, confidence interval; hsRDT, high sensitivity rapid diagnostic test; ref. reference.

laboratory strain STECLA salivary glands and other individual or environmental covariates. Participants were enrolled in a 2017 EAG survey in Haiti, and also had multiplex data collected for IgG response to an extensive panel of *P. falciparum* antigens. This region of Haiti is known to be of low to moderate malaria endemicity and harbors multiple anopheline species [18]. Results of this analysis point to a significant elevation in anti-SGE IgG responses among children of approximately 4–10 years of age, and elevated IgG in persons enrolled at venues below 200 m in elevation. Additionally, IgG against certain *P. falciparum* antigens CSP, Rh2_2030, and SEA-1 were also found to have significant associations with anti-SGE IgG level in adjusted models. These data for both vector and parasite exposure may be utilized to identify subpopulations of persons who are at greater risk of exposure, and better optimize future intervention efforts.

While an extensive body of literature exists on the human antibody response to *Plasmodium* parasites, very few studies have examined human antibody response to *Anopheles* salivary antigens, with most choosing the *An. gambiae* gSG6 single target [10–12, 19–22]. Various groups have also examined the relationship between environmental factors and mosquito salivary antigens in other mosquito species or other anopheline salivary components [7, 23, 24]. As anopheline mosquitoes are ubiquitous throughout Haiti, there would be no reason to assume anyone enrolled in this current study would have not been bitten by these mosquitoes at some point in their life, and attempts were not made to dichotomize the anti-SGE IgG assay signal into seropositive and seronegative categories. Even so, it is still interesting to note the bimodal distribution of log-transformed SGE IgG signal, pointing towards potential subsets of persons who get bitten more, or from some individuals' more-robust IgG response against salivary antigens. From ages of 6 months to 7 years, a rapid increase in SGE IgG was observed in this study population, with peak anti-SGE IgG response around ages 6–7 years and monotonic declines after age 7 years. This finding likely illustrates the accumulation of B-cell responses to mosquito bites as children age. With increased age there is an increase in mobility, including participation in outdoor activities, as well as later bedtimes and thus less time under a bed net—all of which can provide more opportunity for mosquitoes to bite. However, the decrease of anti-SGE IgG in older children onwards was a surprising finding and may point towards immune tolerance to salivary antigens. If IgG against salivary antigens is used to assess vector exposure, these age differences are crucial for interpretation of findings and provide a rationale for age restriction to younger children for analysis.

When adjusting for other covariates, children aged 6 months to 5 years and those aged 6–15 years with any *P. falciparum* antigen seropositivity had 4- to 5-fold higher odds, respectively, of an elevated anti-SGE IgG response compared to individuals older than 15 years. Unlike findings reported here, other studies have reported conflicting results on antisalivary IgG responses, including no association between age and antibody response [25–27], a decrease in antibodies with increasing age [12, 28], and higher antibody levels in adults compared to children [29, 30]. The relationship between these current findings for age and antibody response SGE is difficult to interpret when comparing to previous studies conducted in various malaria

transmission settings and utilizing various salivary components (or different anopheline species). Although the inclusion of the crude salivary gland homogenate in this study ensures numerous proteins are available for IgG binding, it is also not possible to specify from this current study which specific mosquito proteins are capturing the human IgG.

Previous literature points to environmental factors that contribute to spatial variation in anopheline mosquito breeding sites and feeding habits, including vegetation, temperature, rainfall, elevation, population density, and proximity to water bodies [31–34]. Although anopheline mosquitoes are thought to bite primarily at night, there is growing evidence of both diurnal and crepuscular biting behavior [35, 36] that could lead to participants being bitten not just at home, but also at nearby EAG enrollment sites to which environmental data were matched; as such, these sites serve as appropriate proxies for locations of bite exposure. This study's results suggested that higher elevation and increased distance from water bodies are associated with lower levels of anti-SGE IgG. *An. albimanus* breeding site preference for altitudes under 500 meters has been reported before [37], and reduction in elevation would provide better possibilities for the warmer temperatures and standing water needed for these mosquitoes to complete their life cycle. Anopheline mosquitoes also use smaller water habitats such as containers, puddles, and ditches as breeding sites; proximity to such sites would presumably increase odds of exposure to mosquito bites [38, 39]. However, this study used distance to the nearest river, lake, or stream as an estimate of proximity to water bodies, which may not have provided enough granularity to capture a significant association in an adjusted model. Further investigation of these findings is needed using individual participant-level IgG data rather than aggregated mean IgG values, and additionally covariates collected at the individual level that may further explain the relationship between environmental factors and mosquito exposure.

Previous studies have reported various associations between IgG against *P. falciparum* antigens and antisalivary antigen IgG. Previous findings focus on gSG6 which, like SGE, is useful for examining *Anopheles* exposure in low-transmission settings [40]. A study performed on the Thailand-Myanmar border found that individuals with a very high response against gSG6 had increased odds of having a positive antibody response to both *Pf*CSP and *Pf*MSP-1 [20]. In Colombia, however, no association was found between *An. darlingi* SGE and *Pf*MSP-1 [11]. This is in line with this current study's results as well as those of other groups, which have not observed consistently significant associations between antisalivary IgG and IgG against *Pf*MSP-1 [41, 42]. Whole schizont extract, which comprises SEA-1, was found to result in a greater increase in antibody response with higher exposure to gSG6 among those with malaria infection in Côte d'Ivoire [41]. This study found significant associations between SGE and seropositivity to *P. falciparum* antigens CSP, SEA-1, and Rh2_2030. By default, host exposure to mosquito SGE antigens and the *P. falciparum* CSP antigen (on sporozoites) would happen at the same time when the mosquito takes a blood meal [43]. However, persons would also be exposed to SGE antigens when being bitten by mosquitoes not infected with *Plasmodium*. Based on how quickly sporozoites flow into the vascular system, host immune response to sporozoites is partially based on already circulating antibodies [44]. It is possible that an elevated immune response against mosquito saliva forms part of this anticipated defense. The SEA-1 and Rh2_2030 antigens are only produced by blood-stage merozoites, and because the invasion of erythrocytes by *Plasmodium* merozoites is partially mediated by Rh2_2030, it follows that antibody response against this antigen leads to the blockage of that invasion system [45]. Though consensus is lacking about its function, SEA has been reported to act as an indirect, upstream regulator to merozoite egress from infected erythrocytes [46]. It would be unclear why IgG responses to the blood-stage antigens SEA and Rh2_2030 would have an association with anti-SGE IgG levels, and this should be investigated among other vector, parasite, and host populations.

This study had several limitations. Because participant enrollment was limited to Artibonite, a region of low malaria endemicity, the main limitation is generalizability of our results to other areas of Haiti or beyond. As IgG serological data are a proxy for history of exposure, another limitation to this study is the association between anti-SGE IgG and environmental covariates by study enrollment site. Because anopheline mosquito bites could occur anywhere, it would be difficult to know the true location where exposure to SGE antigens was occurring for study participants. In addition, as this study only enrolled persons in a relatively small geographical area in Artibonite, Haiti, investigating associations among environmental covariates and anti-SGE IgG would be more robust with a wider geographic distribution of enrollment sites.

In conclusion, in Haitian participants enrolled in the Artibonite department in 2017, the IgG response to *An. albimanus* SGE was observed to be strongly associated with age, and significantly associated with select *P. falciparum* antigens found at various points in the parasite life cycle. General trends were also seen with higher anti-SGE IgG responses from persons enrolled at lower elevations. Characterization of factors associated with IgG response to SGE can further understanding of how anopheline salivary antigens can be used to predict malaria vector exposure.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials

are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Data availability. All anonymized data and SAS scripts used for this analysis can be made available by contacting the corresponding author under reasonable request.

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