Anopheles salivary antigens as serological biomarkers of vector exposure and malaria transmission: A systematic review with multilevel modelling

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Abstract

Background: Entomological surveillance for malaria is inherently resource-intensive and produces crude population-level measures of vector exposure which are insensitive in low-transmission settings. Antibodies against *Anopheles* salivary proteins measured at the individual-level may serve as proxy biomarkers for vector exposure and malaria transmission, but their relationship is yet to be quantified.

Methods: A systematic review of studies measuring antibodies against *Anopheles* salivary antigens (PROSPERO: CRD42020185449). Multilevel modelling (to account for multiple study-specific observations (level-one), nested within study (level-two), and study nested within country (level-three)) estimated associations between seroprevalence with *Anopheles* human biting rate (HBR) and malaria transmission measures.

Results: From 3981 studies identified in literature searches, 42 studies across 16 countries were included contributing 393 study-specific observations of anti-*Anopheles* salivary antibodies determined in 42,764 samples. A positive association between HBR (log transformed) and seroprevalence was found; overall a 2-fold (100% relative) increase in HBR was associated with a 23% increase in odds of seropositivity (OR: 1.23, 95%CI: 1.10-1.37, $p<0.001$). The association between HBR and *Anopheles* salivary antibodies was strongest with concordant, rather than discordant *Anopheles* species. Seroprevalence was also significantly positively associated with established epidemiological measures of malaria transmission: entomological inoculation rate, *Plasmodium* spp. prevalence, and malarial endemicity class.

Conclusions: *Anopheles* salivary antibody biomarkers can serve as a proxy measure for HBR and malaria transmission, and could monitor malaria receptivity of a population to sustain malaria transmission. Validation of *Anopheles* species-specific biomarkers are important given the global heterogeneity in the distribution of *Anopheles* species. Salivary biomarkers have the potential to transform surveillance by replacing impractical, inaccurate entomological investigations, especially in areas progressing towards malaria elimination.

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Introduction

Sensitive and accurate tools to measure and monitor changes in malaria transmission are essential to track progress towards malaria control and elimination goals. Currently, the gold standard measurement of malaria transmission intensity is the entomological inoculation rate (EIR), a population-measure defined as the number of infective *Anopheles* mosquito bites a person receives per unit of time. EIR is calculated as the human biting rate (HBR; measured at the population-level by entomological vector-sampling methodologies (gold standard: human landing catch)) multiplied by the sporozoite index (proportion of captured *Anopheles* with sporozoites present in their salivary glands). However, estimation of EIR and HBR via entomological investigations are inherently labour and resource intensive, requiring trained collectors, specialised laboratories and skilled entomologists. Furthermore, these approaches provide a crude population-level estimate of total vector exposure at a particular time and location, precluding investigation of heterogeneity and natural transmission dynamics of individual-level vector-human interactions [1]. For example, indoor human landing catches provide poor estimates of outdoor biting and thus total vector exposure [2]. The sensitivity of EIR is further compromised in low transmission settings where the number of *Plasmodium*-infected specimens detected is low and often zero.

Evaluation of the human antibody response to *Anopheles* spp. salivary proteins has the potential to be a logistically practical approach to estimate levels of exposure to vector bites at an individual-level. Several *Anopheles* salivary proteins have been shown to be immunogenic in individuals naturally exposed to the bites of *Anopheles* vectors and have been investigated as serological biomarkers to measure *Anopheles* exposure [3-11], malaria transmission [12-14] and as an outcome for vector control intervention studies [4-6, 14, 15]. However, a major short-coming of the literature is that studies are largely descriptive and do not quantify the association between entomological and malariometric measures and anti-*Anopheles* salivary antibody responses. We undertook a systematic review with multilevel modelling, to quantify the association between HBR, EIR, and other markers of malaria transmission, with anti-*Anopheles* salivary antibody responses and to understand how these

associations vary according to transmission setting and dominant *Anopheles* vectors which can exhibit different biting behaviours. In particular, we were interested in comparing the African context (where *An. gambiae* and *P. falciparum* predominates) to non-African settings (where *An. gambiae* is absent and where both *P. falciparum* and *P. vivax* are prevalent). This knowledge is pertinent to advance the use of salivary antibody biomarkers as a vector and malaria transmission sero-surveillance tool.

Methodology

Search strategy and selection criteria

We performed a systematic review with multilevel modelling according to the MOOSE and PRISMA guidelines [16, 17] (Reporting Standards Document). Five databases were searched for published studies investigating antibodies to *Anopheles* salivary antigens as a biomarker for mosquito exposure or malaria transmission published before 30th of June 2020. The protocol (Appendix 1) was registered with PROSPERO (CRD42020185449).

The primary criteria for inclusion in this systematic review was the reporting of estimates of seroprevalence or total levels of Immunoglobulin (Ig) in human sera against *Anopheles* salivary antigens. We considered for inclusion: cross-sectional, cohort, intervention and case-control studies of individuals or populations living in all geographies with natural exposure to *Anopheles* mosquitoes. Studies that were solely performed in participants not representative of the wider naturally exposed population (*i.e.* mosquito allergic patients, soldiers, returned travellers) were excluded.

Measures

Outcomes

The primary outcome of our systematic review was antibodies (seroprevalence or levels, including all Ig isotypes and subclasses) against any *Anopheles* salivary antigens (full-length recombinant proteins, peptides and crude salivary extract). Study reported salivary antibody data was extracted at the most granular level (*i.e.* for each site; time point), with each observation of seroprevalence or levels

included as a study-specific salivary antibody observation. As measurement of antibody levels does not produce a common metric between studies only values of seroprevalence could be included in multilevel modelling analyses. Therefore, to maximise data, authors of studies that reported only antibody levels were contacted and asked to classify their participants as 'responders' or 'non-responders' according to seropositivity (antibody level relative to unexposed sera). Studies that provided antibody levels or categorised seropositivity based upon arbitrary cut offs are included in narrative terms only.

Exposures

The primary exposures of interest were the entomological metrics HBR (average number of bites received per person per night) and EIR (infectious bites received per person per year). Secondary exposures included study-reported prevalence of *Plasmodium* spp. infection (confirmed by either microscopy, rapid diagnostic test, or polymerase chain reaction (PCR)) and seroprevalence of antimalarial antibodies against pre-erythrocytic and blood-stage *Plasmodium* spp. antigens. Where exposure estimates were not provided, we attempted to source data from other publications by the authors, or using the site geolocation (longitude and latitude) and year to obtain estimates of EIR from the Pangaea dataset [18], *P. falciparum* rates in 2-10 year olds ($Pf\mathrm{PR}_{2\text{-}10}$) and dominant vector species (DVS) from the Malaria Atlas Project (MAP) [19]. Malarial endemicity classes were derived by applying established endemicity cut-offs to MAP $Pf\mathrm{PR}_{2\text{-}10}$ estimates [20]. For the purposes of the modelling analyses we defined DVS as where *An. gambiae sensu lato* (*s.l.*) was the only DVS, where *An. gambiae s.l.*, was present with additional DVS, or where *An. gambiae s.l.* was absent. Studies of salivary antigens where exposure variables could not be sourced and data could not be extracted were excluded.

Statistical analysis

Where observations of the seroprevalence of antibodies against the same salivary antigen and exposure of interest were reported in more than one study, generalised linear multilevel modelling (mixed-effects, logistic) was used to quantify associations between the exposures of interest and

124 salivary antibody seroprevalence measurements [21]. Random intercepts for study and country were
125 estimated to account for nested dependencies induced from multiple study-specific salivary antibody
126 observations (level-one) from the same study (level-two) and studies from the same country (level-
127 three). Additionally, study-level random slopes for the entomological and malariometric exposure
128 parameters were estimated to model study-specific heterogeneity in the effect of the exposure of
129 interest (HBR/EIR/malaria prevalence/antimalarial antibody seroprevalence). The associations
130 between the various exposures and the different salivary antigens were analysed separately, however
131 observations of IgG seroprevalence against the recombinant full-length protein (gSG6) and synthetic
132 peptide (gSG6-P1, the one peptide determined in all studies utilising peptides) form of the gSG6
133 antigen were analysed together.

134 Potential effect modification of the associations between exposures and anti-*Anopheles* salivary
135 antibody responses were explored. In analyses quantifying the associations between HBR, as well as
136 EIR, and seropositivity, we included an interaction term with DVS and for vector collection method
137 (human landing catch or other indirect measures *e.g.* light traps, spray catches, etc.). For the
138 association between *Plasmodium* spp. prevalence and seropositivity, interaction terms with malaria
139 detection methodology (light microscopy or PCR) and malarial species (*P. falciparum* only, or *P.*
140 *falciparum* and *P. vivax*) were estimated.

141 For the exposure measures (HBR, EIR, malaria prevalence and antimalarial antibody seroprevalence),
142 the data were log transformed since there were non-linear associations between the exposure measures
143 on the original scale and seroprevalence - supported empirically by superior model fit as indicated by
144 Akaike's information criterion (AIC) and Bayesian information criterion (BIC) fit indices (Appendix 1
145 – Table 1). To aid interpretation, we present our results as a relative increase in the odds of the gSG6
146 IgG seropositivity for a 2-fold or in other words a 100% relative increase in the exposures. Intraclass
147 correlation coefficients (ICCs) were estimated for country- and study-specific heterogeneity using
148 estimated model variance components. In order to explore the presence of study-level influence in
149 (HBR and EIR) effect estimate modelling, the Generalised Linear Latent and Mixed Models (gllamm)
150 package [22] was used to produce Cooks distance statistics [23] at the study-level from the

generalised linear multilevel models. A conservative cut-off threshold for Cooks distance (4/*n*) was used to guide sensitivity analyses, where studies were excluded, in-turn, to assess outlier influence. All statistical analyses were performed using STATA v15.1.

Risk of bias in individual studies

Risk of bias was assessed by one reviewer using the Risk of Bias in Prevalence Studies tool [24]. The risk of bias pertains to the reported observations of anti-*Anopheles* salivary antibody seroprevalence included in the multilevel modelling.

Results

Literature searches identified 158 potentially relevant studies, of which 42 studies were included in the systematic review (Figure 1) and are described in Table 1. From these studies we extracted n=393 study-specific observations of anti-*Anopheles* salivary antibodies determined from antibody measurements in a total of 42,764 sera samples. These studies were performed in 16 countries mostly in hypo or mesoendemic areas of Africa (32 studies), with a minority performed in South America (4 studies), Asia (4 studies), and the Pacific (2 studies). Studies were classified according to their DVS which reflected the region where the study was conducted. *An. gambiae s.l.* was a DVS in all African study sites (n=151 study-specific observations from 23 studies where *An. gambiae s.l.* was the only DVS and n=68 from 16 studies where *An. gambiae s.l.* was present with additional DVS (*i.e. An. funestus, An. pharoensis*)), with the exception of one study, which together with the 10 non-African studies contributed n=174 study-specific estimates where *An. gambiae s.l.* was absent. Most observations came from cross-sectional (n=191 from 16 studies) or repeated cross-sectional studies (n=137 from 18 studies), with n=60 from cohort studies (6 studies) and n=5 from case-control studies (2 studies).

The salivary antigen most commonly assessed was *An. gambiae* Salivary Gland 6 (gSG6), as a full-length protein (n=67 from 8 studies) and synthetic peptide (*An. gambiae* Salivary Gland 6 Peptide 1; gSG6-P1; n=270 from 24 studies). Additional salivary antigens assessed included *An. gambiae* gSG6-

P2 (n=119 from 3 studies), recombinant cE5 (n=15 from 2 studies), g-5'nuc (n=3 from 1 study), and recombinant *An. funestus* fSG6 (n=6 from 2 studies) and f-5'nuc (n=3 from 1 study). Seven studies measured antibodies to whole salivary gland extracts from *An. gambiae* (n=24 from 4 studies), *An. darlingi* (n=5 from 2 studies), *An. albimanus* (n=2 from 1 study), and *An. dirus* (n=3 from 1 study), while one study assessed antibodies against synthetic peptides of *An. albimanus* (n=2) (Table 1). All studies investigated total IgG and only five determined an additional isotype or subclass [7, 25-28]. The paucity of studies investigating these latter-mentioned antibody types and *Anopheles* salivary biomarkers precluded extensive multilevel analyses; instead, we present their associations in narrative terms in Appendix 10. Analyses reported below focus on quantifying the relationships between HBR, EIR and markers of malaria transmission with total IgG to *An. gambiae* gSG6. The distributions of exposure observations were: HBR (n=197 from 24 studies, median: 3.0 bites per person per night, IQR: 0.9-12.1; range: 0-121.4), EIR (n=60 from 8 studies, median: 7.3 infectious bites received per person per year, IQR: 0-36.4; range: 0-585.6), and *Plasmodium* spp. prevalence (n=266 from 22 studies, median: 9.1%; IQR: 4-22%; range: 0-94.6%).

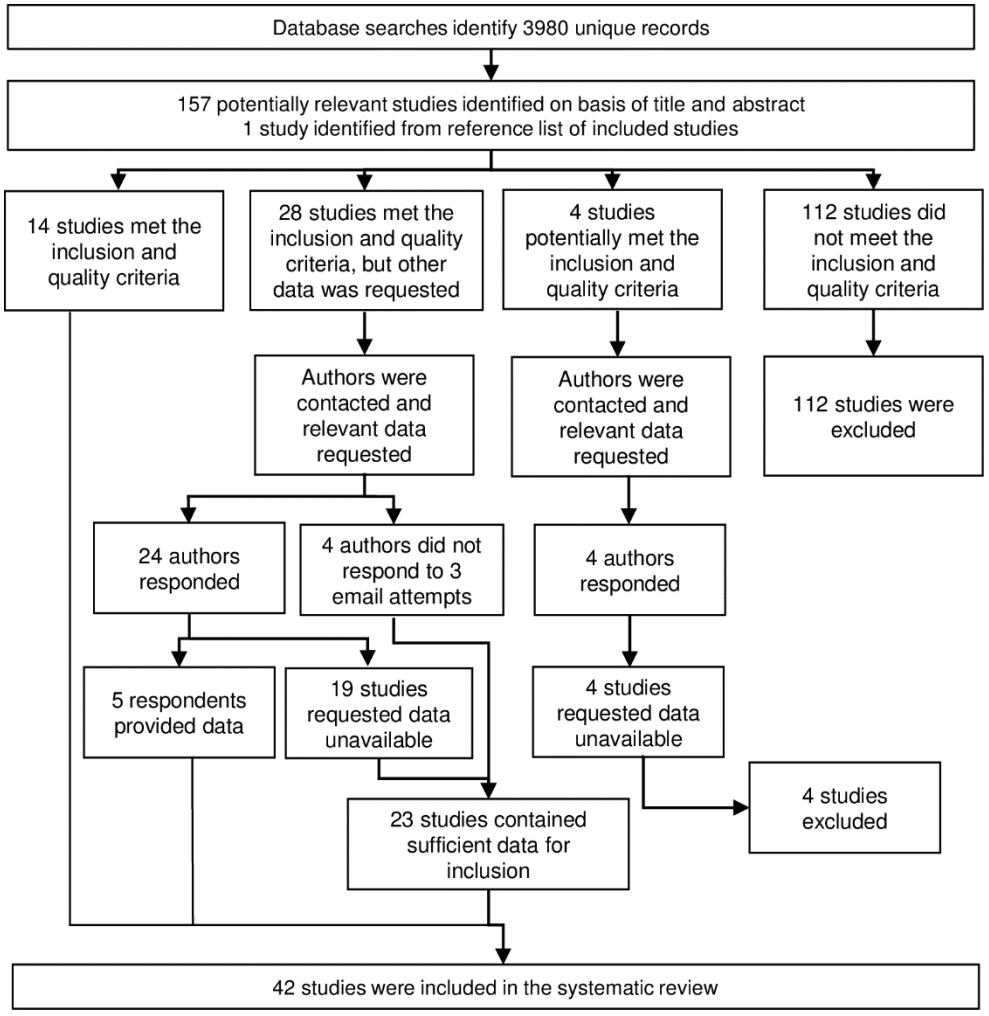


Figure 1. Flow diagram of study identification. Excluded studies are detailed in Appendix 3.

193 **Table 1: Key descriptive information from included studies**

Study year	Country	Malarial endemicity class	Dominant malaria vector species	Study design	No. participants (samples)	Study-specific n	Vector and malariometric variables	Salivary antibody outcomes (Seroprevalence[%];[L]evels)
Africa								
Brosseau 2012 [29]	Angola	Hypoendemic; Mesoendemic	*An. gambiae s.l.; An. funestus*	Cross-sectional‡	- (1584)	6	*Plas*+LM; *Pf*PR	*g*SGE IgG [L]
Drame 2010 [5]	Angola	Hypoendemic	*An. gambiae s.l.*	Cohort	105 (1470)	12	HBR; *Plas*+LM; *Pf*PR	*g*SG6-P1 IgG [%;L]
Drame 2010 [6]	Angola	Hypoendemic	*An. gambiae s.l.*	Cohort	109 (1279)	12	HBR; *Plas*+LM; *Pf*PR	*g*SGE IgG [L]
Marie 2015 [30]	Angola	Hypoendemic	*An. gambiae s.l.*	Cohort	71 (852)	12	HBR; *Pf*PR	*g*cE5 IgG [L]
Drame 2015 [7]	Benin	Hyperendemic	*An. gambiae s.l.; An. funestus*	Cohort ‡	133 (532)	4	HBR; *Pf*PR	*g*SG6-P1 IgG & IgM [%;L]
Rizzo 2011 [9]	Burkina Faso	Hyperendemic*	*An. gambiae s.l.*	Repeated cross-sectional	- (2066)	14	HBR; EIR; *Plas*+LM§	*g*SG6 IgG [%;L]
Rizzo 2011 [8]	Burkina Faso	Hyperendemic*	*An. gambiae s.l.*	Repeated cross-sectional	335 (335)	3	HBR	*f*SG6 IgG [%;L]
Rizzo 2014 [26]	Burkina Faso	Hyperendemic*	*An. gambiae s.l.*	Repeated cross-sectional	- (359)	3	HBR	*g*cE5 IgG [%;L]; IgG1 & IgG4 [L]
Rizzo 2014 [27]	Burkina Faso	Hyperendemic*	*An. gambiae s.l.*	Repeated cross-sectional	270 (270)	6	HBR	*g*SG6 IgG1 & IgG4 [L]
Soma 2018 [31]	Burkina Faso	Mesoendemic	*An. gambiae s.l.*	Cross-sectional	1728 (273)	6	HBR; EIR; *Plas*+LM; *Pf*PR	*g*SG6-P1 IgG [%;L]
Koffi 2015 [32]	Cote d'Ivoire	Hypoendemic; Mesoendemic	*An. gambiae s.l.; An. funestus*†	Cross-sectional	94 (94)	3	*Plas*+LM; *Pf*-IgG; *Pf*PR	*g*SG6-P1 IgG [%;L]
Koffi 2017 [33]	Cote d'Ivoire	Hypoendemic	*An. gambiae s.l.; An. funestus*†	Repeated cross-sectional	234 (234)	5	*Pf*-IgG; *Pf*PR	*g*SG6-P1 IgG [%;L]
Traoré 2018 [34]	Cote d'Ivoire	Hypoendemic	*An. gambiae s.l.*	Repeated cross-sectional‡	89 (178)	4	HBR; *Plas*+LM; *Pf*PR	*g*SG6-P1 IgG [L]
Traoré 2019 [35]	Cote d'Ivoire	Hypoendemic	*An. gambiae s.l.; An. funestus*†	Repeated cross-sectional‡	- (442)	6	HBR; *Plas*+LM; *Pf*PR	*g*SG6-P1 IgG [%;L]
Sadia-Kacou	Cote	Mesoendemic	*An. gambiae s.l.*	Repeated cross-	775	8	*Pf*PR	*g*SG6-P1 IgG [L]

2019 [36]	d'Ivoire			sectional‡	(775)			
Badu 2015 [37]	Ghana	Mesoendemic	*An. gambiae s.l.;* *An. funestus*†	Repeated cross-sectional‡	295 (885)	3	*Plas*+LM; *Pf*-IgG; *Pf*PR	*g*SG6-P1 IgG [%;L]
Badu 2012 [3]	Kenya	Hypoendemic; Mesoendemic	*An. gambiae s.l.*	Repeated cross-sectional	- (1366)	5	EIR; *Plas*+LM§; *Pf*PR	*g*SG6-P1 IgG [%;L]
Sagna 2013[38]	Senegal	Hypoendemic; Mesoendemic	*An. gambiae s.l.*	Cohort‡	265 (1325)	25	HBR; *Plas*+LM§; *Pf*PR	*g*SG6-P1 IgG [%;L]
Drame 2012 [11]	Senegal	Hypoendemic	*An. gambiae s.l.*	Cross-sectional	1010 (1010)	16	HBR; *Pf*PR	*g*SG6-P1 IgG [%;L]
Poinsignon 2010 [39]	Senegal	Hypoendemic	*An. funestus*	Cohort‡	87 (261)	3	HBR; *Plas*+LM§; *Pf*PR	*g*SG6-P1 IgG [L]
Sarr 2012 [40]	Senegal	Hypoendemic; Mesoendemic	*An. gambiae s.l.;* *An. funestus*†	Repeated cross-sectional‡	- (401)	4	HBR; *Plas*+LM§; *Pf*-IgG; *Pf*PR	*g*SG6-P1 IgG [%;L]
Lawaly 2012 [25]	Senegal	Mesoendemic	*An. gambiae s.l.;* *An. funestus*†	Cohort	387 (711)	4	HBR; *Plas*+LM§; *Pf*PR	*g*SGE IgG, IgG4 & IgE [L]
Ali 2012 [41]	Senegal	Hypoendemic;* Mesoendemic;* Hyperendemic*	*An. gambiae s.l.;* *An. funestus;* *An. pharoensis*	Cross-sectional	- (134)	3	HBR; EIR	*g*SG6 IgG [%;L] *f*SG6 IgG [%;L]; *f*5'nuc IgG [%;L]; *g*5'nuc IgG [%;L]
Ambrosino 2010 [42]	Senegal	Hypoendemic;* Mesoendemic;* Hyperendemic*	*An. gambiae s.l.;* *An. funestus;* *An. pharoensis*	Cross-sectional	- (123)	3	EIR; *Pf*-IgG	*g*SG6-P1 IgG [%]; *g*SG6-P2 IgG [%]
Perraut 2017 [43]	Senegal	Hypoendemic; Mesoendemic	*An. gambiae s.l.;* *An. funestus*	Repeated cross-sectional	- (798)	4	EIR; *Plas*+LM; *Plas*+PCR; *Pf*-IgG; *Pf*PR	*g*SG6-P1 IgG [%]
Poinsignon 2008 [44]	Senegal	Mesoendemic	*An. gambiae s.l.*	Cross-sectional‡	241 (241)	3	HBR; *Pf*PR	*g*SG6-P1 IgG [L]; *g*SG6-P2 IgG [L]
Poinsignon 2009 [45]	Senegal	Mesoendemic	*An. gambiae s.l.*	Repeated cross-sectional‡	61 (122)	2	HBR; *Plas*+LM§; *Pf*PR	*g*SG6-P1 IgG [L]
Remoue 2006 [46]	Senegal	Mesoendemic	*An. gambiae s.l.*	Cross-sectional‡	448 (448)	4	HBR; *Plas*+LM§; *Pf*PR	*g*SGE IgG [%;L]
Sagna 2019 [47]	Senegal	Hypoendemic	*An. gambiae s.l.*	Cross-sectional‡	809 (809)	4	*Pf*PR	*g*SG6-P1 IgG [L]
Stone 2012 [10]	Tanzania	Mesoendemic; Hyperendemic	*An. gambiae s.l.*	Cross-sectional‡	636 (636)	16	HBR; *Pf*-IgG; *Pf*PR	*g*SG6 IgG [%;L]

Yman 2016 [48]	Tanzania	Mesoendemic; Holoendemic*	*An. gambiae s.l.; An. funestus*	Repeated cross-sectional‡	668 (668)	16	*Pf*-IgG; *Pf*PR	*g*SG6 IgG [%]
Proietti 2013 [49]	Uganda	Mesoendemic	*An. gambiae s.l.; An. funestus*†	Repeated cross-sectional	509 (509)	3	*Pf*-IgG; *Pf*PR	*g*SG6 IgG [%]
South America								
Andrade 2009 [50]	Brazil	Eliminating; Hypoendemic	*An. darlingi*	Cross-sectional	204 (204)	3	*Plas*+^{LM¶}; *Plas*+^{PCR¶}; *Pf*PR	*d*SGE IgG [L$^{‖}$]
Londono-Renteria 2015 [12]	Colombia		*An. albimanus*	Cross-sectional	42 (42)	2	*Plas*+^{PCR¶}	*g*SG6-P1 IgG [L$^{‖}$]
Londono-Renteria 2020 [51]	Colombia	Eliminating	*An. albimanus*	Cross-sectional	337 (337)	2	*Plas*+^{PCR}; *Pf*PR	*a*PEROX-P1, P2 & P3 IgG [L]; *a*TRANS-P1 & P2 IgG [L]
Montiel 2020 [52]	Colombia	Eliminating	*An. albimanus*	Case-control	113 (113)	2	*Plas*+^{LM}; *Plas*+^{PCR¶}; *Pf*PR	*g*SG6-P1 IgG [L$^{‖}$]; *d*SGE IgG [L$^{‖}$]; *a*STECLA SGE IgG [L$^{‖}$]; *a*Cartagena SGE IgG [L$^{‖}$]
Asia								
Kerkhof 2016 [53]	Cambodia	Hypoendemic	*An. dirus*	Cross-sectional	- (8438)	113	*Plas*+^{PCR}; *Pf*-IgG; *Pv*-IgG; *Pf*PR	*g*SG6-P1 IgG [%;L]; *g*SG6-P2 IgG [%;L]
Charlwood 2017 [54]	Cambodia	Eliminating	*An. dirus*	Repeated cross-sectional	454 (1180)	6	HBR; *Plas*+^{PCR}; *Pf*-IgG; *Pf*PR	*g*SG6 IgG [L]
Ya-Umphan 2017 [13]	Myanmar	Eliminating	*An. minimus; An. maculatus; An. dirus s.l.*	Repeated cross-sectional	2602 (9425)	28	HBR; EIR; *Plas*+^{PCR}; *Pf*-IgG; *Pf*PR	*g*SG6-P1 IgG [%;L]
Waitayakul 2006 [28]	Thailand		*An. dirus*	Case-control	139 (139)	3	*Plas*+^{LM}	*dir*SGE IgG & IgM [L$^{‖}$]
Pacific								
Pollard 2019 [55]	Solomon Islands	Eliminating; Hypoendemic	*An. farauti*	Repeated cross-sectional	686 (791)	9	HBR; EIR; *Pf*PR	*g*SG6-P1 IgG [%;L]
Idris 2017 [15]	Vanuatu	Eliminating; Hypoendemic; Mesoendemic	*An. farauti*	Repeated cross-sectional	905 (905)	3	*Plas*+^{LM}; *Pf*-IgG; *Pv*-IgG; *Pf*PR	*g*SG6 IgG [%;L]

Data are given as: study, year of publication, country, malarial endemicity class, malarial dominant vector species (DVS), study design (‡ indicate that study was performed solely in children), number of participants and number of samples, number of study-specific salivary antibody outcome observations (Study-specific n), entomological and malariometric parameters and salivary

antibody outcomes assessed. Malarial endemicity class (categorical) is derived from *P. falciparum* prevalence rate in 2-10 year olds (*Pf*PR) extracted from Malaria Atlas Project (MAP) using site geolocations and year of study, and applying established cut offs reported in Bhatt *et al.* [20]. If *Pf*PR data were not available (*e.g.* surveys prior to 2000; or unable to determine study site geolocation and year), endemicity class is given as stated in the study (indicated by *). DVS is as stated in the study or extracted from MAP (indicated by †). Of note, *An. gambiae sensu lato* (*s.l.*) includes both *An. gambiae sensu stricto* and *An. arabiensis.* Entomological and malariometric parameters include human biting rate (HBR), entomological inoculation rate (EIR), prevalence estimates of *Plasmodium* spp. (*Plas*+): detected by light microscopy (LM) or polymerase chain reaction (PCR), with § indicating prevalence of *P. falciparum* only and ¶ indicating prevalence of *P. vivax* only (no footnote indicates *P. falciparum* and *P. vivax* co-endemic), as well as *Pf*PR extracted from MAP [56]. Salivary antibody outcomes are indicated as either seroprevalence [%] or levels [L], or both [%;L], with ‖ indicating that studies reported results stratified by malarial infection status. Salivary antigens include recombinant full-length proteins, synthetic peptides and whole salivary gland extracts (SGE). Italicised prefix of salivary antigen indicates species: *An. gambiae* (*g*), *An. funestus* (*f*), *An. darlingi* (*d*), *An. albimanus* (*a*), *An. dirus* (*dir*).

Generalised linear multilevel modelling (mixed-effects, logistic) of n=132 study-specific observations from 12 studies estimated a positive association between *Anopheles* spp.-HBR (log transformed) and seroprevalence of IgG to *An. gambiae* gSG6 salivary antigen [5, 7, 8, 10, 11, 13, 31, 35, 38, 40, 41, 55] (Figure 2 and Appendix 4 – Table 1). As we have log transformed HBR to account for the non-linear relationship between HBR and log odds of gSG6 IgG seropositivity, we have presented estimated odds ratios for different incremental per cent increases in HBR (Figure 2 – Supplement 1). For example, the magnitude of the association was such that a 2-fold (100% relative) increase in HBR was associated with a 23% increase (OR: 1.23; 95%CI: 1.10-1.37, $p<0.001$) in the odds of anti-gSG6 IgG seropositivity (Figure 2). Heterogeneity in the effect of HBR on gSG6 across studies was observed (likelihood ratio $\chi^2(1) = 109.25$, $p<0.001$); the 95% reference range of study-specific effects for a 2-fold increase in HBR ranged from a 12% reduction to a 70% increase in odds (OR:0.88-1.70). There was no evidence that the association between HBR and gSG6 IgG varied according to vector collection method (human landing catch or other indirect methods; $p=0.443$) or study design (longitudinal cohort or cross-sectional/repeated cross-sectional; $p=0.138$). Given the global heterogeneity in the distribution of *Anopheles* species, we sought to quantify the extent to which the association between *An. gambiae* gSG6 IgG seropositivity and HBR is moderated by DVS. We observed that the magnitude of the association between *An. gambiae* gSG6 IgG seropositivity and HBR was greatest in African studies where *An. gambiae s.l.* was the only dominant vector ($p<0.001$, Appendix 5); a 2-fold increase in HBR was associated with a 37% increase (OR: 1.37; 95%CI: 1.19-1.58; $p<0.001$) in the odds of gSG6 IgG seropositivity, compared to an attenuated association for African studies where *An. gambiae s.l.* was not the only DVS (OR: 1.14 per 2-fold increase in HBR; 95%CI: 0.98-1.33; $p=0.079$) and non-African studies where *An. gambiae s.l.* was absent (OR: 1.05 per 2-fold increase in HBR; 95%CI: 1.03-1.08; $p<0.001$). In order to quantify the relationship between gSG6 IgG seroprevalence and HBR, for given HBR values we estimated gSG6 IgG seroprevalence by producing model-based predicted probabilities overall and by DVS (Figure 3). In African studies where *An. gambiae s.l* is the only DVS, predicted seroprevalence of *An. gambiae* gSG6 ranged from 8% (95%CI: 0-22%) to 86% (95%CI: 67-100%) for an HBR of 0.01 to 100 bites per person per night respectively (Figure 3 and Figure 3 – Supplement 1).

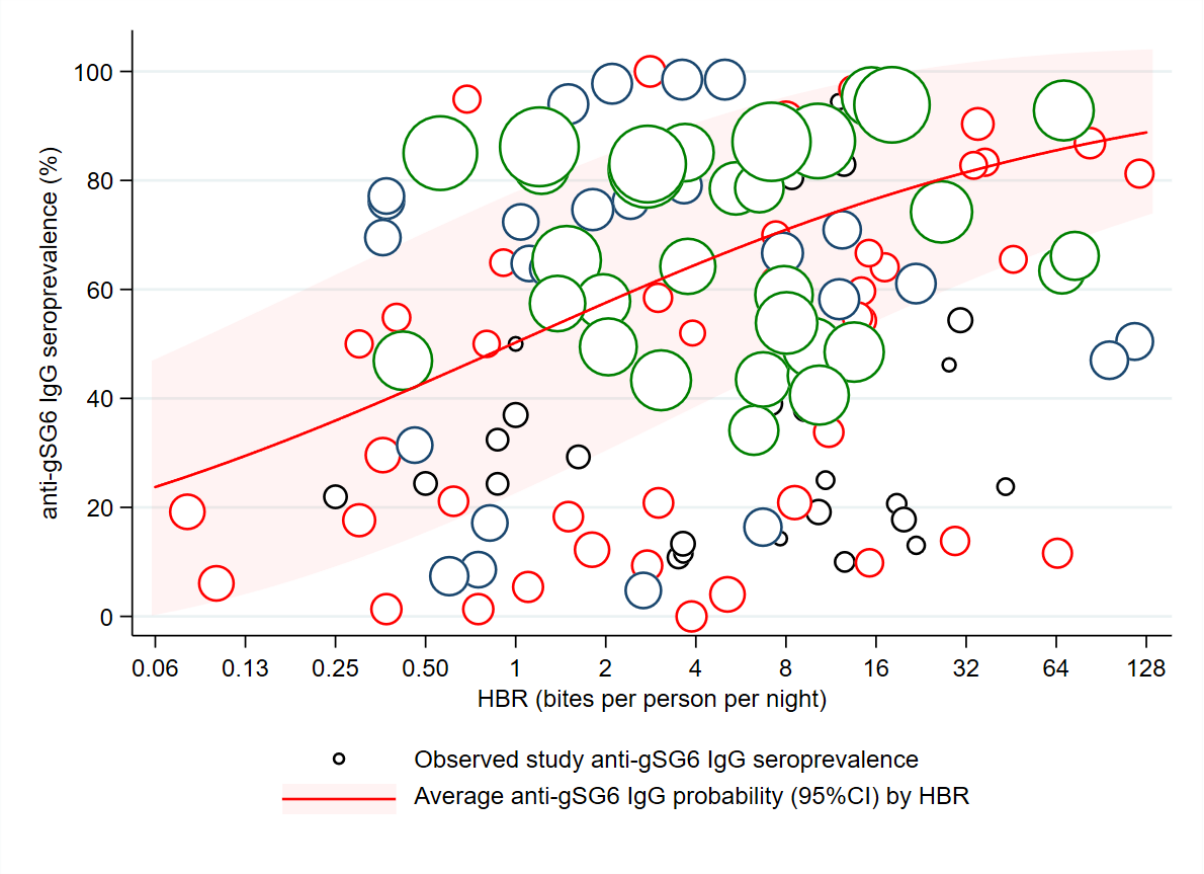
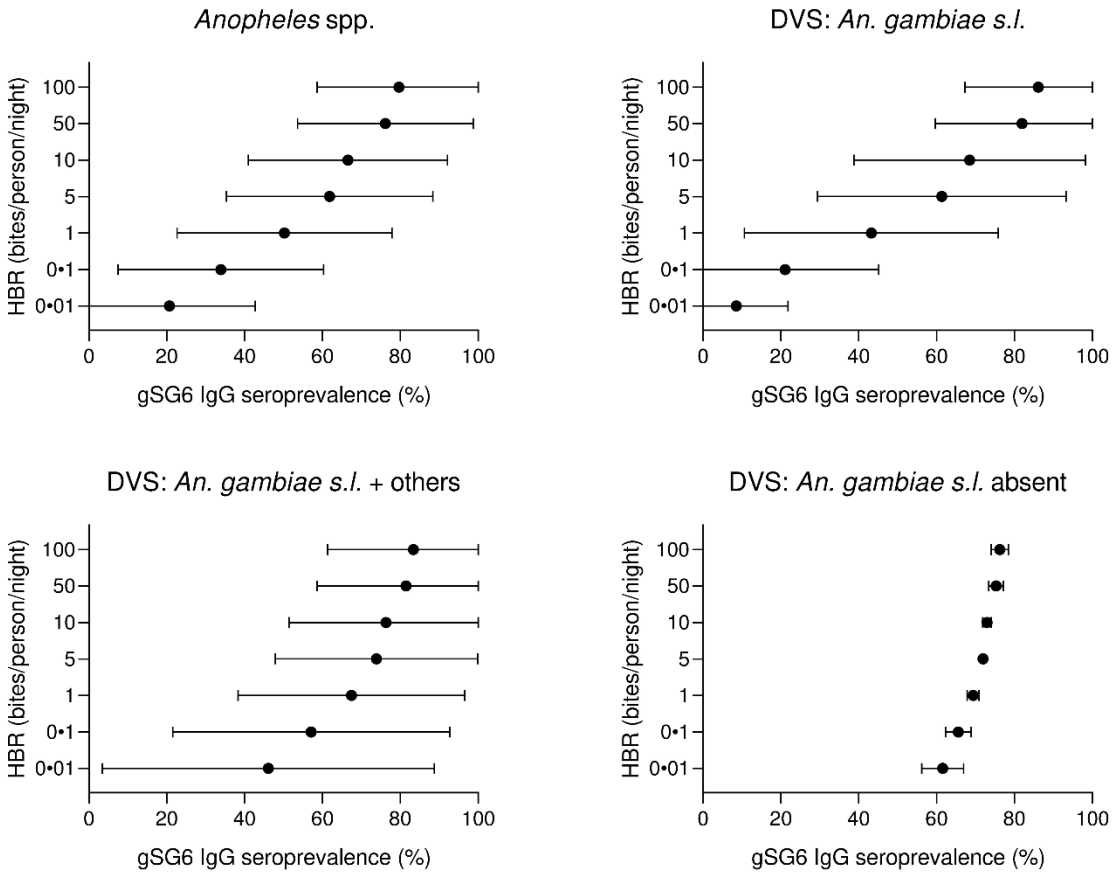


Figure 2. Association between anti-gSG6 IgG seroprevalence and $\log_2$ human biting rate (HBR). Figure shows the observed anti-gSG6 IgG (either recombinant or peptide form) seroprevalence (%) and HBR for each study-specific observation, as well as the predicted average anti-gSG6 IgG seroprevalence (predicted probability for the average study and country) with 95% confidence intervals (95%CI). Circles are proportional to the size of the sample for each study-specific observation, with colours indicating sample size: black (<50), red (50-100), navy (100-150) and green (>150). Association estimated using generalised linear multilevel modelling (mixed-effects, logistic) to account for the hierarchical nature of the data, where study-specific anti-gSG6 IgG observations, are nested within study and study is nested within country (model output shown in Appendix 4; p<0.001).



244

245 **Figure 3. Forest plots of predicted anti-gSG6 IgG seroprevalence (%) and *Anopheles* species-**
246 **specific human biting rate (HBR).** Panels show the predicted average anti-gSG6 IgG seroprevalence
247 (predicted probability for the average study and country) with 95% confidence intervals for given
248 HBR, for all *Anopheles* spp. (using model output from Appendix 4) and for specific-dominant vector
249 species (DVS): where *An. gambiae s.l.* is the only DVS, where other DVS were present in addition to
250 *An. gambiae s.l.* and where *An. gambiae s.l.* was absent (using model output from Appendix 5).

A positive association was also found between seroprevalence of anti-gSG6 IgG antibodies and EIR in analysis of n=38 study-specific observations from eight studies (Figure 4, Appendix 6) [3, 9, 13, 31, 41-43, 55]. For a 2-fold increase in EIR, the odds of anti-gSG6 IgG seropositivity increased by 11% (OR: 1.11; 95%CI: 1.05-1.17; $p<0.001$), with heterogeneity in the study-specific effects (95% reference range: 1.00-1.24; likelihood ratio χ^2 (1) = 15.02, $p<0.001$). There was no evidence of effect modification by either vector collection method ($p=0.095$) or DVS ($p=0.080$) on the association between seroprevalence of anti-gSG6 IgG and EIR.

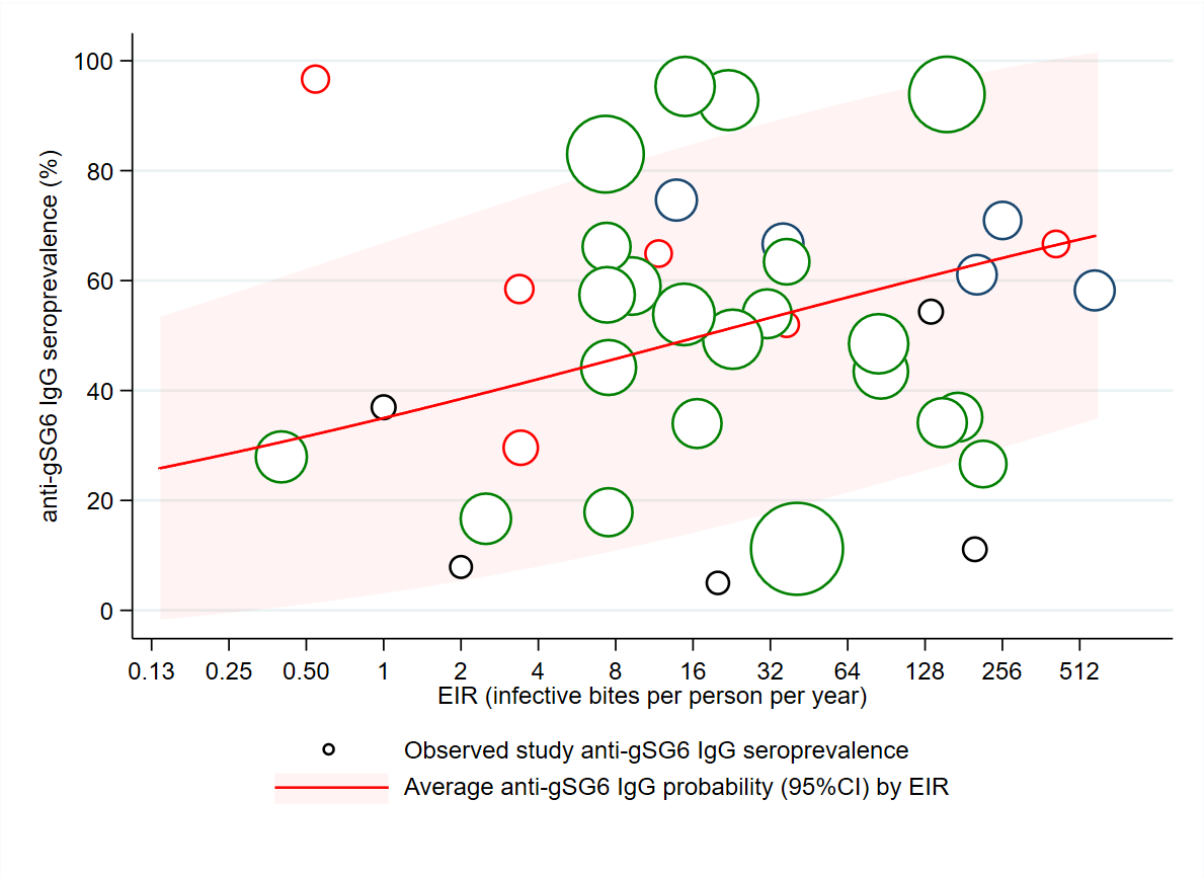
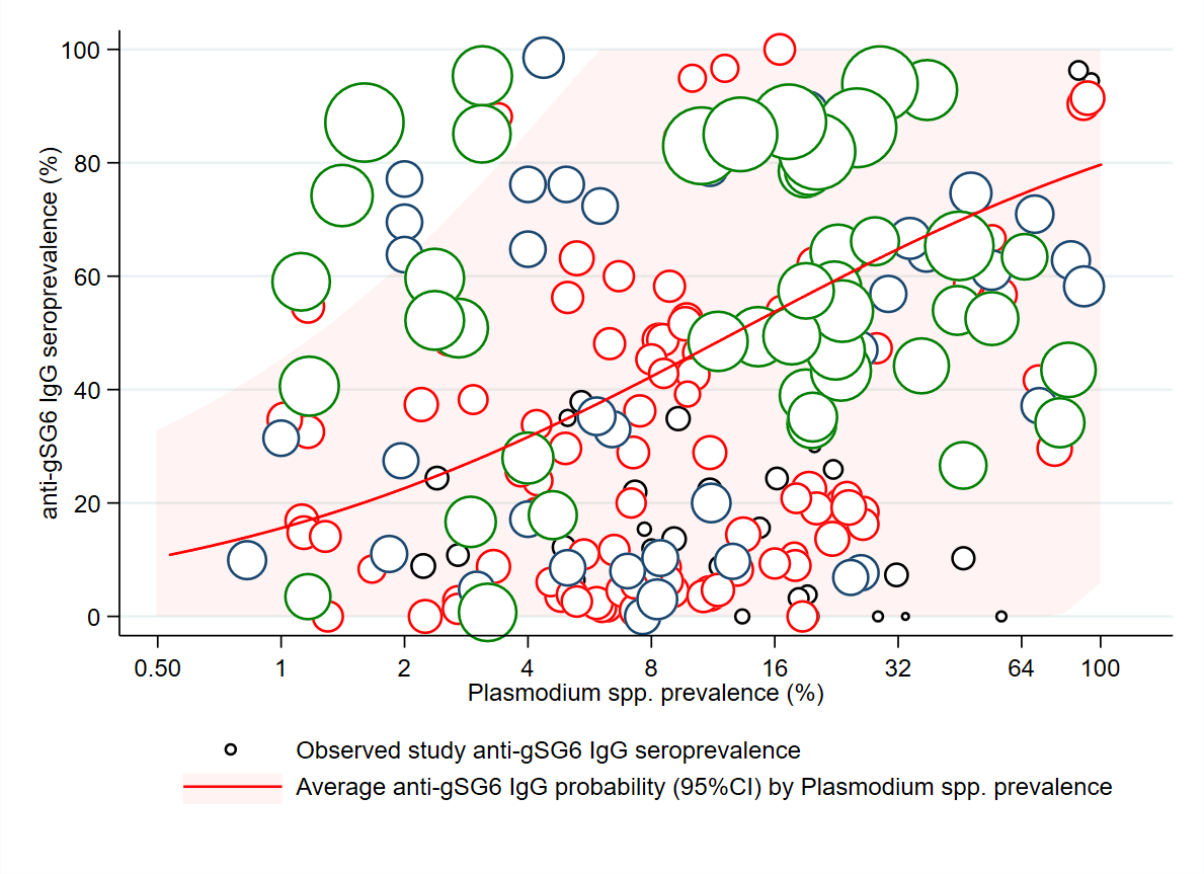


Figure 4. Association between anti-gSG6 IgG seroprevalence and log_2 entomological inoculation rate (EIR). Figure shows the observed anti-gSG6 IgG (either recombinant or peptide form) seroprevalence (%) and EIR for each study-specific observation, as well as the predicted average anti-gSG6 IgG seroprevalence (predicted probability for the average study and country) with 95% confidence intervals (95%CI). Circles are proportional to the size of the sample for each study-specific estimate, with colours indicating sample size: black (<50), red (50-100), navy (100-150) and green (>150). Association estimated using generalised linear multilevel modelling (mixed-effects, logistic) to account for the hierarchical nature of the data, where study-specific anti-gSG6 IgG observations, are nested within study and study is nested within country (model output shown in Appendix 6; $p<0.001$).

Similar positive associations were also found between anti-gSG6 IgG levels, HBR and EIR in 11 studies [7-11, 31, 38, 39, 41, 44, 54] and three studies [9, 13, 41] respectively but seven studies showed no association between HBR and levels of IgG to gSG6 [5, 13, 34, 35, 40, 45, 55].

The association between anti-gSG6 IgG seroprevalence and population-level prevalence of *Plasmodium* spp. infection was investigated. Generalised linear multilevel modelling (mixed-effects, logistic) of n=212 from 14 studies that measured *Plasmodium* spp. prevalence contemporaneously in their study [3, 5, 9, 13, 15, 31, 32, 35, 37, 38, 40, 43, 49, 53] showed that for a 2-fold increase in the prevalence of *Plasmodium* spp. infection the odds of gSG6 IgG seropositivity increased by 38%, although the confidence intervals were wide (OR: 1.38; 95%CI: 0.89-2.12; p=0.148) and heterogeneity in the study-specific effects was observed (95% reference range: 0.30-6.37; likelihood ratio χ^2 (1) = 235.5, p<0.001) (Figure 5 and Appendix 7). In the association between gSG6 IgG seropositivity and *Plasmodium* spp. infection, there was no evidence for a moderating effect of *Plasmodium* spp. detection method (light microscopy, or PCR, p=0.968), or species (African studies with *P. falciparum* versus non-African studies where *P. falciparum* and *P. vivax* are co-prevalent, p=0.538).



285

286 **Figure 5. The association between anti-gSG6 IgG seroprevalence (%) and $\log_2$ *Plasmodium* spp.**
287 **prevalence (%).** Figure shows the observed anti-gSG6 IgG (either recombinant or peptide form)
288 seroprevalence (%) and prevalence of any *Plasmodium* spp. infection (%) for each study-specific
289 observation, as well as the predicted average anti-gSG6 IgG seroprevalence (predicted probability for
290 average study) with 95% confidence intervals (95%CI). Circles are proportional to the size of the
291 sample for each study-specific observation, with colours indicating sample size: black (<50), red (50-
292 100), navy (100-150) and green (>150). Association estimated using generalised linear multilevel
293 modelling (mixed-effects, logistic) to account for the hierarchical nature of the data, where study-
294 specific anti-gSG6 IgG observations are nested within study. See Appendix 7 for model output.

Additionally, 14 studies reported observations of anti-gSG6 IgG levels and the prevalence of *Plasmodium* spp. infections measured contemporaneously in their study. The median anti-gSG6 IgG antibody levels increased with increasing *Plasmodium* spp. prevalence in six of these studies [5, 13, 15, 39, 40, 53], or in *Plasmodium* spp. infected compared to non-infected individuals [12, 52], but showed no association in eight studies [9, 31, 32, 34, 35, 37, 38, 45]. Furthermore, we also investigated associations with serological measures of malaria exposure and found that for a 2-fold increase in pre-erythrocytic and blood-stage stage antigen seroprevalence there was a 2.19-fold (OR: 2.19; 95%CI: 1.18-4.04, p=0.013) and 41% to 5.69-fold (OR range: 1.41 to 5.69; *p* range: <0.001 to 0.523) increase in the odds of anti-gSG6 IgG seropositivity, respectively (Appendix 8).

To give epidemiological context we estimated anti-gSG6 seroprevalence by producing model-based predicted probabilities by malarial endemicity class (a categorical variable derived by applying established cut off values for the $Pf\text{PR}_{2\text{-}10}$ extracted from MAP). Generalised linear multilevel modelling (mixed-effects, logistic) on 297 study-specific salivary antibody observations from 22 studies shows that the estimated anti-gSG6 IgG seroprevalence is higher for the higher endemicity classes (eliminating malaria: 20% (95%CI: 8-31%); hypoendemic: 34% (95%CI: 19-49%); mesoendemic: 52% (95%CI: 35-68%); hyperendemic settings: 47% (95%CI: 27-64%); holoendemic: 78% (95%CI: 67-90%); $p<0.001$; Table 2). Interactions with DVS or region (Africa/non-Africa) could not be explored due to collinearity with malaria endemicity class. Therefore, in addition using Bayes Best-Linear-Unbiased Predictions (BLUPs) we estimated country-specific gSG6 IgG seroprevalence from an intercept only multilevel model fitted to 301 study-specific salivary antibody observations from 22 studies. It showed that IgG seroprevalence to *An. gambiae* gSG6 was lowest in countries in the Pacific Region where *An. gambiae* is absent (Vanuatu (31%) and Solomon Islands (32%)) and highest in countries where *An. gambiae* is a DVS (Benin (72%) and Burkina Faso (65%); Appendix 9).

Table 2: Association between gSG6 IgG seroprevalence (%) and malarial endemicity (*Pf*PR$_{2-10}$).

Malaria Endemicity Class[a]	OR	95%CI	*p*-value	Predicted gSG6 IgG seroprevalence (%)	95%CI
Eliminating malaria$_{(Pf\text{PR }<1\%)}$	**Ref.**			20.0	8.3, 31.7
Hypoendemic$_{(Pf\text{PR }1\text{-}10\%)}$	**2.04**	1.43, 2.90	<0.001	33.7	18.9, 48.5
Mesoendemic$_{(Pf\text{PR }10\text{-}50\%)}$	**4.19**	2.80, 6.08	<0.001	51.5	34.6, 67.7
Hyperendemic$_{(Pf\text{PR }50\text{-}75\%)}$	**3.36**	1.98, 5.71	<0.001	46.5	27.4, 63.8
Holoendemic$_{(Pf\text{PR }>75\%)}$	**14.4**	9.72, 21.36	<0.001	78.2	66.8, 89.7

Table shows the odds ratio (OR), 95% confidence interval (95%CI), p-value, as well as the predicted gSG6 IgG seroprevalence and associated 95%CI[b] for associations between endemicity class (categorical: derived from *P. falciparum* parasite rates in 2-10 year olds (*Pf*PR)) and anti-gSG6 IgG seropositivity.

[a] Generalised linear multilevel modelling (mixed-effects, logistic) estimating the association between anti-gSG6 IgG seropositivity and endemicity class with random-effects for study-specific heterogeneity in gSG6 IgG. Model fitted to N=297 study-specific observations from XX studies. Of note, 9 studies that measured *Plasmodium* spp. prevalence and IgG antibodies to gSG6 were excluded from this analysis as 8 only reported gSG6 IgG levels and 1 was a case control study. Endemicity class membership is derived from *Pf*PR from MAP, using cut-offs taken from Bhatt *et al.* [20], or where MAP data were unavailable, endemicity was included as indicated in the study.

[b] Predicted gSG6 IgG seroprevalence (predicted probability in the average study) is estimated from generalised linear multilevel modelling (mixed-effects, logistic).

Assessments of internal and external study validity revealed there was a moderate risk of selection bias (Appendix 2) due to the study-specific inclusion criteria of populations at higher risk of malaria which contributed gSG6 seroprevalence observations. Sensitivity analyses exploring potential study-level outlier influence on the estimated associations between anti-gSG6 IgG seroprevalence, HBR and EIR showed no evidence of bias (effect estimates for each sensitivity analysis were consistent with model estimates overall) for studies identified as exhibiting potential influence (HBR: n=6; EIR: n=6).

Discussion

This systematic review and multilevel modelling analysis provides the first quantification of a positive non-linear association between seroprevalence of *An. gambiae* gSG6 IgG antibodies and HBR and demonstrated that its magnitude varied with respect to the DVS present in the area. Importantly, this review identified a paucity of studies conducted outside of Africa, as well as investigating salivary antigens representing different *Anopheles* spp. and antigenic targets. gSG6 antibodies were positively associated with the prevalence of *Plasmodium* spp. infection as well as established epidemiological measures of malaria transmission: malaria endemicity class and EIR. Overall, our results demonstrate that antibody seroprevalence specific for *Anopheles* spp. salivary antigens has the potential to be an

effective measure of vector exposure and malaria transmission at the population- and, potentially, individual-level.

An. gambiae gSG6 IgG seropositivity increased with increasing HBR, although these increases had diminishing impact on *An. gambiae* gSG6 IgG seropositivity at higher levels of HBR (approximately greater than 2 bites per person per night). In our study, 17 studies performed across Africa (Angola, Benin, Burkina Faso, Cote d'Ivoire, Senegal) and the Asia Pacific (Cambodia, Myanmar, and the Solomon Islands) reported an HBR <2 demonstrating that the applicability of gSG6 as a biomarker of HBR across a broad range of malaria endemic regions. We also observed that the association was strongest in areas where *An. gambiae s.l.* was the only DVS (that is concordant *An. gambiae* species-specific HBR with *An. gambiae* gSG6 antibodies). Associations, albeit weaker, were also observed between discordant species-specific HBR and gSG6, most likely because the *An. gambiae SG6* gene shares moderate sequence identity with vector species that are dominant in other regions (Africa: 80% *An. funestus*; Asia: 79% *An. stephensi* and *An. maculatus*; 54% *An. dirus*; Pacific: 52.5% *An. farauti*), and is absent from the DVS of the Americas (*An. albimanus* and *An. darlingi*) [57]. The generalisability of *An. gambiae* gSG6 IgG as a biomarker of exposure to other *Anopheles* spp. may therefore be limited. However, our review also identified a paucity of studies investigating additional salivary antigenic targets and *Anopheles* species not present in Africa. The identification of novel salivary antigens that are species-specific will be valuable in quantifying exposure to the other *Anopheles* vectors that share limited identity with *An. gambiae SG6* (such as *An. farauti* and *An. dirus*), as well as *Anopheles* spp. which lack *SG6* (as done for *An. albimanus* and *An. darlingi* [51, 58]). An *Anopheles* species-specific serological platform could advance vector surveillance by more accurately capturing exposure to DVS in the South American and Asia Pacific regions which exhibit diverse biting behaviours and vector competence (DVS typically bite outdoors during the night and day respectively [19, 59-63]), as well as the increasing threat of urban malaria from *An. stephensi* in Africa [64, 65].

This review demonstrated that the prevalence of *Anopheles* salivary antibodies increased with increasing prevalence of *Plasmodium* spp. infection (although confidence intervals were wide and we

observed heterogeneity in the effect between studies) as well as established epidemiological measures of malaria transmission: malaria endemicity class and EIR. Anti-salivary antibodies, such as SG6 IgG, may therefore have the potential to serve as a proxy measure for receptivity of a population to sustain malaria transmission. Their application could be particularly relevant in pre-elimination areas, or non-endemic areas under threat of imported malaria, where *Anopheles* salivary antibodies are more readily detectable than parasites; salivary antibodies were predicted to be prevalent (20%) in areas defined as eliminating malaria (<1% $Pf PR_{2-10}$). Furthermore, if SG6 IgG seroprevalence can be effectively combined with a measurement of the sporozoite index, salivary antibodies as a marker of HBR could help overcome sensitivity limitations of EIR in low transmission areas. Additional measures could include estimates of malaria prevalence or serological biomarkers that are species- or life stage-specific (e.g. *Plasmodium* spp. pre-erythrocytic antigens as biomarkers for recent parasite inoculation). Indeed, positive associations between antibodies specific for *Plasmodium* spp. pre-erythrocytic and blood-stage antigens with gSG6 were demonstrated in analyses of data from diverse malaria endemic areas. Serological tools combining salivary antigens with antigens specific for the different *Plasmodium* spp. could be easy to employ and complement malaria surveillance programs. These tools may be particularly useful in the Asia Pacific, a region of relatively low malaria transmission with goals of elimination, but the highest burden of *P. vivax* malaria where blood-stage infection can be caused by relapses from dormant liver stages. In these areas, parasite prevalence may therefore overestimate ongoing malaria transmission, making vector surveillance tools essential to informing elimination strategies in the Asia Pacific and other regions where *P. vivax* is endemic.

The gold standard entomological measures HBR and EIR provide crude population-level estimates of vector and malaria exposure that are specific in space and time and preclude investigation of individual-level heterogeneity and natural transmission dynamics. Our study demonstrated that salivary biomarkers measured at the individual-level, such as gSG6 IgG, can be used to quantify total vector exposure at the population-level, without requiring laborious entomological experiments. However, validating an individual-level serological measure, which demonstrates considerable individual-level variation, against the imperfect population-level gold standards of HBR and EIR is

challenging and reflected in the variation in study-specific estimates in the association between gSG6 IgG and HBR in modelling analyses. However, the accuracy of salivary antibodies to measure individual-level exposure to *Anopheles* bites is yet to be validated; literature searches identified no studies investigating this association at the individual-level. Without detailed measurements of individual-level vector exposure, or a detailed knowledge of the half-life of *Anopheles* salivary antibodies post biting event, the true accuracy of salivary antibodies, such as SG6 IgG, to measure individual-level HBR remains unknown. This knowledge is particularly pertinent where *Anopheles* salivary biomarkers might be applied to assess the effectiveness of a vector control intervention or used to measure temporal changes in malaria transmission; particularly in areas or populations where there is considerable heterogeneity in individual-level risk of *Anopheles* exposure (*e.g.* unmeasured outdoor biting due to occupational exposure for forest workers [66]).

The broad nature of our inclusion and quality criteria was a key strength of our systematic review, which aimed to provide a comprehensive analysis of all *Anopheles* salivary biomarkers and determine their associations with entomological and malariometric measures of transmission. However, this review has two main limitations. First, despite the inclusive nature, assessment of the external validity of the review revealed a moderate risk of bias; some studies exhibited a high risk of selection bias as they were performed in specific high-risk populations not representative of the overall population (*i.e.* children only). This is accounted for to some degree by specification of a random effect (*i.e.* intercept) for study, which accounts for unmeasured study-specific factors that may introduce study-specific measurement error to measurement of the outcome. Second, with respect to internal validity, there may be potential selection bias introduced by the exclusion of studies reporting zero HBR (seven observations from three studies [9, 38, 55]), EIR (22 observations from three studies [9, 13, 31]) and malaria prevalence (15 observations from three studies [15, 38, 53]) estimates, given we modelled the log of these factors. However, adding a small constant (*e.g.* 0.001) to a zero value to permit modelling of a log estimate can also introduce considerable bias (*i.e.* seemingly small differences between values become very large on the log scale). In light of this, we also chose to provide estimates of association and gSG6 IgG seroprevalence according to a selected range of epidemiologically relevant

hypothetical HBR's (no widely accepted HBR classification exists in the literature) and according to widely accepted, discrete, endemicity classes according to MAP estimates (which permitted inclusion of all studies) to provide epidemiological context. However, there is the potential for misclassification of malarial endemicity class derived from geospatially extracted MAP predictions of *Pf*PR_{2-10} which increase in uncertainty in areas with scarce data. Similarly, we used MAP vector occurrence data to inform DVS categories for 7 (out of 42) studies. Any misclassification events may cause us to underestimate the standard error in the effect of malaria endemicity class and DVS on gSG6 IgG.

Conclusions

In order to advance progress towards malaria elimination the World Health Organisation has called for innovative tools and improved approaches to enhance vector surveillance and monitoring and evaluation of interventions [67]. Our systematic review has provided evidence that *Anopheles* salivary antibodies are serological biomarkers of vector and malaria exposure, by quantifying their positive association with *Anopheles*-HBR and established epidemiological measures of malaria transmission. These salivary biomarkers have the potential to replace crude population-level estimates of entomological indices with a precise and scalable tool that measures *Anopheles* vector exposure at the individual-level. This approach could be expanded into a sero-surveillance tool to assess the effectiveness of vector control interventions, define heterogeneity in malaria transmission and inform efficient resource-allocation, that would ultimately accelerate progress towards elimination.

Declaration of interests

We declare no competing interests.

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References

1. Monroe A, Moore S, Okumu F, Kiware S, Lobo NF, Koenker H, Sherrard-Smith E, Gimnig J, Killeen GF. Methods and indicators for measuring patterns of human exposure to malaria vectors. Malar J. 2020;19(1):207. doi: 10.1186/s12936-020-03271-z.
2. Mathenge EM, Misiani GO, Oulo DO, Irungu LW, Ndegwa PN, Smith TA, Killeen GF, Knols BGJ. Comparative performance of the Mbita trap, CDC light trap and the human landing catch in the sampling of *Anopheles arabiensis*, *An. funestus* and culicine species in a rice irrigation in western Kenya. Malar J. 2005;4. doi: 10.1186/1475-2875-4-7. PubMed PMID: 15667666.
3. Badu K, Siangla J, Larbi J, Lawson BW, Afrane Y, Ong'echa J, Remoue F, Zhou G, Githeko AK, Yan G. Variation in exposure to *Anopheles gambiae* salivary gland peptide (gSG6-P1) across different malaria transmission settings in the western Kenya highlands. Malar J. 2012;11:318. doi: 10.1186/1475-2875-11-318.
4. Drame PM, Diallo A, Poinsignon A, Boussari O, Dos Santos S, Machault V, Lalou R, Cornelie S, LeHesran JY, Remoue F. Evaluation of the effectiveness of malaria vector control measures in urban settings of Dakar by a specific *Anopheles* salivary biomarker. PLoS One. 2013;8(6):e66354. doi: 10.1371/journal.pone.0066354.
5. Drame PM, Poinsignon A, Besnard P, Cornelie S, Le Mire J, Toto JC, Foumane V, Dos-Santos MA, Sembène M, Fortes F, Simondon F, Carnevale P, Remoue F. Human antibody responses to the *Anopheles* salivary gSG6-P1 peptide: a novel tool for evaluating the efficacy of ITNs in malaria vector control. PLoS One. 2010;5(12):e15596. doi: 10.1371/journal.pone.0015596.
6. Drame PM, Poinsignon A, Besnard P, Le Mire J, Dos-Santos MA, Sow CS, Cornelie S, Foumane V, Toto JC, Sembene M, Boulanger D, Simondon F, Fortes F, Carnevale P, Remoue F. Human antibody response to *Anopheles gambiae* saliva: an immuno-epidemiological biomarker to evaluate the efficacy of insecticide-treated nets in malaria vector control. Am J Trop Med Hyg. 2010;83(1):115-21. doi: 10.4269/ajtmh.2010.09-0684.
7. Drame PM, Poinsignon A, Dechavanne C, Cottrell G, Farce M, Ladekpo R, Massougbodji A, Cornélie S, Courtin D, Migot-Nabias F, Garcia A, Remoué F. Specific antibodies to *Anopheles* gSG6-P1 salivary peptide to assess early childhood exposure to malaria vector bites. Malar J. 2015;14:285. doi: 10.1186/s12936-015-0800-6.
8. Rizzo C, Ronca R, Fiorentino G, Mangano VD, Sirima SB, Nèbiè I, Petrarca V, Modiano D, Arcà B. Wide cross-reactivity between *Anopheles gambiae* and *Anopheles funestus* SG6 salivary proteins supports exploitation of gSG6 as a marker of human exposure to major malaria vectors in tropical Africa. Malar J. 2011;10:206. doi: 10.1186/1475-2875-10-206.
9. Rizzo C, Ronca R, Fiorentino G, Verra F, Mangano V, Poinsignon A, Sirima SB, Nèbiè I, Lombardo F, Remoue F, Coluzzi M, Petrarca V, Modiano D, Arcà B. Humoral response to the *Anopheles gambiae* salivary protein gSG6: a serological indicator of exposure to Afrotropical malaria vectors. PLoS One. 2011;6(3):e17980. doi: 10.1371/journal.pone.0017980.
10. Stone W, Bousema T, Jones S, Gesase S, Hashim R, Gosling R, Carneiro I, Chandramohan D, Theander T, Ronca R, Modiano D, Arcà B, Drakeley C. IgG responses to *Anopheles gambiae* salivary antigen gSG6 detect variation in exposure to malaria vectors and disease risk. PLoS One. 2012;7(6):e40170. doi: 10.1371/journal.pone.0040170.
11. Drame PM, Machault V, Diallo A, Cornélie S, Poinsignon A, Lalou R, Sembène M, Dos Santos S, Rogier C, Pagès F, Le Hesran JY, Remoué F. IgG responses to the gSG6-P1 salivary peptide for evaluating human exposure to *Anopheles* bites in urban areas of Dakar region, Sénégal. Malar J. 2012;11:72. doi: 10.1186/1475-2875-11-72.
12. Londono-Renteria B, Drame PM, Weitzel T, Rosas R, Gripping C, Cardenas JC, Alvares M, Wesson DM, Poinsignon A, Remoue F, Colpitts TM. *An. gambiae* gSG6-P1 evaluation as a proxy for human-vector contact in the Americas: a pilot study. Parasit Vectors. 2015;8:533. doi: 10.1186/s13071-015-1160-3.
13. Ya-Umphan P, Cerqueira D, Parker DM, Cottrell G, Poinsignon A, Remoue F, Brengues C, Chareonviriyaphap T, Nosten F, Corbel V. Use of an *Anopheles* salivary biomarker to assess malaria transmission risk along the Thailand-Myanmar Border. J Infect Dis. 2017;215(3):396-404. doi: 10.1093/infdis/jiw543.
14. Noukpo MH, Damien GB, Elanga-N'Dille E, Sagna AB, Drame PM, Chaffa E, Boussari O, Corbel V, Akogbéto M, Remoue F. Operational assessment of long-lasting insecticidal nets by using an *Anopheles* salivary biomarker of human-vector contact. Am J Trop Med Hyg. 2016;95(6):1376-82. doi: 10.4269/ajtmh.15-0541.
15. Idris ZM, Chan CW, Mohammed M, Kalkoa M, Taleo G, Junker K, Arcà B, Drakeley C, Kaneko A. Serological measures to assess the efficacy of malaria control programme on Ambae Island, Vanuatu. Parasit Vectors. 2017;10(1):204. doi: 10.1186/s13071-017-2139-z.

16. Moher D, Liberati A, Tetzlaff J, Altman DG, The PG. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med. 2009;6(7):e1000097. doi: 10.1371/journal.pmed.1000097.
17. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA. 2000;283(15):2008-12. Epub 2000/05/02. doi: 10.1001/jama.283.15.2008. PubMed PMID: 10789670.
18. Yamba EI, Tompkins AM, Fink AH, Ermert V, Djouda A, Amekudzi LK, Briët OJT. Monthly entomological inoculation rates for studying malaria transmission seasonality in Africa. PANGAEA; 2018.
19. The Malaria Atlas Project [Internet]. 2017. Available from: https://malariaatlas.org/.
20. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, Battle K, Moyes CL, Henry A, Eckhoff PA, Wenger EA, Briët O, Penny MA, Smith TA, Bennett A, Yukich J, Eisele TP, Griffin JT, Fergus CA, Lynch M, Lindgren F, Cohen JM, Murray CLJ, Smith DL, Hay SI, Cibulskis RE, Gething PW. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature. 2015;526(7572):207-11. Epub 2015/09/16. doi: 10.1038/nature15535. PubMed PMID: 26375008.
21. Song P, Rudan D, Zhu Y, Fowkes FJI, Rahimi K, Fowkes FGR, Rudan I. Global, regional, and national prevalence and risk factors for peripheral artery disease in 2015: an updated systematic review and analysis. Lancet Glob Health. 2019;7(8):e1020-e30. doi: 10.1016/S2214-109X(19)30255-4.
22. Rabe-Hesketh S, Pickles A, Taylor C. Generalized linear latent and mixed models. Stata Technical Bulletin. 2000;53(sg129):293-307.
23. Cook RDJT. Detection of influential observation in linear regression. 1977;19(1):15-8.
24. Hoy D, Brooks P, Woolf A, Blyth F, March L, Bain C, Baker P, Smith E, Buchbinder R. Assessing risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement. J Clin Epidemiol. 2012;65(9):934-9. doi: 10.1016/j.jclinepi.2011.11.014.
25. Lawaly R, Konate L, Marrama L, Dia I, Diallo D, Sarr FD, Schneider BS, Casademont I, Diallo M, Brey PT, Sakuntabhai A, Mecheri S, Paul R. Impact of mosquito bites on asexual parasite density and gametocyte prevalence in asymptomatic chronic *Plasmodium falciparum* infections and correlation with IgE and IgG titers. Infect Immun. 2012;80(6):2240-6. doi: 10.1128/iai.06414-11. PubMed PMID: WOS:000304387700031.
26. Rizzo C, Lombardo F, Ronca R, Mangano V, Sirima SB, Nèbiè I, Fiorentino G, Modiano D, Arcà B. Differential antibody response to the *Anopheles gambiae* gSG6 and cE5 salivary proteins in individuals naturally exposed to bites of malaria vectors. Parasit Vectors. 2014;7:549. doi: 10.1186/s13071-014-0549-8.
27. Rizzo C, Ronca R, Lombardo F, Mangano V, Sirima SB, Nèbiè I, Fiorentino G, Troye-Blomberg M, Modiano D, Arcà B. IgG1 and IgG4 antibody responses to the *Anopheles gambiae* salivary protein gSG6 in the sympatric ethnic groups Mossi and Fulani in a malaria hyperhendemic area of Burkina Faso. PLoS One. 2014;9(4):e96130. doi: 10.1371/journal.pone.0096130.
28. Waitayakul A, Somsri S, Sattabongkot J, Looareesuwan S, Cui L, Udomsangpetch R. Natural human humoral response to salivary gland proteins of *Anopheles* mosquitoes in Thailand. Acta Trop. 2006;98(1):66-73. doi: 10.1016/j.actatropica.2006.02.004.
29. Brosseau L, Drame PM, Besnard P, Toto JC, Foumane V, Le Mire J, Mouchet F, Remoue F, Allan R, Fortes F, Carnevale P, Manguin S. Human antibody response to *Anopheles* saliva for comparing the efficacy of three malaria vector control methods in Balombo, Angola. PLoS One. 2012;7(9):e44189. doi: 10.1371/journal.pone.0044189.
30. Marie A, Ronca R, Poinsignon A, Lombardo F, Drame PM, Cornelie S, Besnard P, Le Mire J, Fiorentino G, Fortes F, Carnevale P, Remoue F, Arcà B. The *Anopheles gambiae* cE5 salivary protein: a sensitive biomarker to evaluate the efficacy of insecticide-treated nets in malaria vector control. Microbes Infect. 2015;17(6):409-16. doi: 10.1016/j.micinf.2015.01.002.
31. Soma DD, Kassié D, Sanou S, Karama FB, Ouari A, Mamai W, Ouédraogo GA, Salem G, Dabiré RK, Fournet F. Uneven malaria transmission in geographically distinct districts of Bobo-Dioulasso, Burkina Faso. Parasit Vectors. 2018;11(1):296. doi: 10.1186/s13071-018-2857-x.
32. Koffi D, Touré AO, Varela ML, Vigan-Womas I, Béourou S, Brou S, Ehouman MF, Gnamien L, Richard V, Djaman JA, Perraut R. Analysis of antibody profiles in symptomatic malaria in three sentinel sites of Ivory Coast by using multiplex, fluorescent, magnetic, bead-based serological assay (MAGPIX™). Malar J. 2015;14:509. doi: 10.1186/s12936-015-1043-2.
33. Koffi D, Varela ML, Loucoubar C, Beourou S, Vigan-Womas I, Touré A, Djaman JA, Touré AO, Perraut R. Longitudinal analysis of antibody responses in symptomatic malaria cases do not mirror parasite transmission in peri-urban area of Cote d'Ivoire between 2010 and 2013. PLoS One. 2017;12(2):e0172899. doi: 10.1371/journal.pone.0172899.
34. Traoré DF, Sagna AB, Adja AM, Zoh DD, Lingué KN, Coulibaly I, N'Cho Tchiekoi B, Assi SB, Poinsignon A, Dagnogo M, Remoue F. Evaluation of malaria urban risk using an immuno-epidemiological

570 biomarker of human exposure to *Anopheles* bites. Am J Trop Med Hyg. 2018;98(5):1353-9. doi:
571 10.4269/ajtmh.17-0231.
572 35. Traoré DF, Sagna AB, Adja AM, Zoh DD, Adou KA, Lingué KN, Coulibaly I, Tchiekoi NB, Assi SB,
573 Poinsignon A, Dagnogo M, Remoue F. Exploring the heterogeneity of human exposure to malaria vectors in an
574 urban setting, Bouaké, Côte d'Ivoire, using an immuno-epidemiological biomarker. Malar J. 2019;18(1):68. doi:
575 10.1186/s12936-019-2696-z.
576 36. Sadia-Kacou CAM, Yobo CM, Adja MA, Sagna AB, Ndille EE, Poinsignon A, Tano Y, Koudou BG,
577 Remoue F. Use of *Anopheles* salivary biomarker to assess seasonal variation of human exposure to *Anopheles*
578 bites in children living near rubber and oil palm cultivations in Côte d'Ivoire. Parasite Epidemiol Control.
579 2019;5:e00102. doi: 10.1016/j.parepi.2019.e00102.
580 37. Badu K, Gyan B, Appawu M, Mensah D, Dodoo D, Yan G, Drakeley C, Zhou G, Owusu-Dabo E,
581 Koram KA. Serological evidence of vector and parasite exposure in Southern Ghana: the dynamics of malaria
582 transmission intensity. Parasit Vectors. 2015;8:251. doi: 10.1186/s13071-015-0861-y.
583 38. Sagna AB, Sarr JB, Gaayeb L, Drame PM, Ndiath MO, Senghor S, Sow CS, Poinsignon A, Seck M,
584 Hermann E, Schacht A-M, Faye N, Sokhna C, Remoue F, Riveau G. gSG6-P1 salivary biomarker discriminates
585 micro-geographical heterogeneity of human exposure to *Anopheles* bites in low and seasonal malaria areas.
586 Parasit Vectors. 2013;6(1):68. doi: 10.1186/1756-3305-6-68.
587 39. Poinsignon A, Samb B, Doucoure S, Drame PM, Sarr JB, Sow C, Cornelie S, Maiga S, Thiam C,
588 Rogerie F, Guindo S, Hermann E, Simondon F, Dia I, Riveau G, Konate L, Remoue F. First attempt to validate
589 the gSG6-P1 salivary peptide as an immuno-epidemiological tool for evaluating human exposure to *Anopheles*
590 *funestus* bites. Trop Med Int Health. 2010;15(10):1198-203. doi: 10.1111/j.1365-3156.2010.02611.x.
591 40. Sarr JB, Samb B, Sagna AB, Fortin S, Doucoure S, Sow C, Senghor S, Gaayeb L, Guindo S, Schacht
592 AM, Rogerie F, Hermann E, Dia I, Konate L, Riveau G, Remoue F. Differential acquisition of human antibody
593 responses to *Plasmodium falciparum* according to intensity of exposure to *Anopheles* bites. Trans R Soc Trop
594 Med Hyg. 2012;106(8):460-7. doi: 10.1016/j.trstmh.2012.05.006.
595 41. Ali ZM, Bakli M, Fontaine A, Bakkali N, Vu Hai V, Audebert S, Boublik Y, Pagès F, Remoué F,
596 Rogier C, Fraisier C, Almeras L. Assessment of *Anopheles* salivary antigens as individual exposure biomarkers
597 to species-specific malaria vector bites. Malar J. 2012;11:439. doi: 10.1186/1475-2875-11-439.
598 42. Ambrosino E, Dumoulin C, Orlandi-Pradines E, Remoue F, Toure-Baldé A, Tall A, Sarr JB,
599 Poinsignon A, Sokhna C, Puget K, Trape JF, Pascual A, Druilhe P, Fusai T, Rogier C. A multiplex assay for the
600 simultaneous detection of antibodies against 15 *Plasmodium falciparum* and *Anopheles gambiae* saliva antigens.
601 Malar J. 2010;9:317. doi: 10.1186/1475-2875-9-317.
602 43. Perraut R, Varela ML, Loucoubar C, Niass O, Sidibé A, Tall A, Trape JF, Wotodjo AN, Mbengue B,
603 Sokhna C, Vigan-Womas I, Touré A, Richard V, Mercereau-Puijalon O. Serological signatures of declining
604 exposure following intensification of integrated malaria control in two rural Senegalese communities. PLoS
605 One. 2017;12(6):e0179146. doi: 10.1371/journal.pone.0179146.
606 44. Poinsignon A, Cornelie S, Mestres-Simon M, Lanfrancotti A, Rossignol M, Boulanger D, Cisse B,
607 Sokhna C, Arcà B, Simondon F, Remoue F. Novel peptide marker corresponding to salivary protein gSG6
608 potentially identifies exposure to *Anopheles* bites. PLoS One. 2008;3(6):e2472. doi:
609 10.1371/journal.pone.0002472.
610 45. Poinsignon A, Cornelie S, Ba F, Boulanger D, Sow C, Rossignol M, Sokhna C, Cisse B, Simondon F,
611 Remoue F. Human IgG response to a salivary peptide, gSG6-PI, as a new immuno-epidemiological tool for
612 evaluating low-level exposure to *Anopheles* bites. Malar J. 2009;8. doi: 10.1186/1475-2875-8-198. PubMed
613 PMID: WOS:000269875000001.
614 46. Remoue F, Cisse B, Ba F, Sokhna C, Herve JP, Boulanger D, Simondon F. Evaluation of the antibody
615 response to *Anopheles* salivary antigens as a potential marker of risk of malaria. Trans R Soc Trop Med Hyg.
616 2006;100(4):363-70. doi: 10.1016/j.trstmh.2005.06.032.
617 47. Sagna AB, Kassié D, Couvray A, Adja AM, Hermann E, Riveau G, Salem G, Fournet F, Remoue F.
618 Spatial assessment of contact between humans and *Anopheles* and *Aedes* mosquitoes in a medium-sized African
619 urban setting, using salivary antibody-based biomarkers. J Infect Dis. 2019;220(7):1199-208. doi:
620 10.1093/infdis/jiz289.
621 48. Yman V, White MT, Rono J, Arca B, Osier FH, Troye-Blomberg M, Bostrom S, Ronca R, Rooth I,
622 Farnert A. Antibody acquisition models: A new tool for serological surveillance of malaria transmission
623 intensity. Sci Rep. 2016;6. doi: 10.1038/srep19472. PubMed PMID: WOS:000369380100001.
624 49. Proietti C, Verra F, Bretscher MT, Stone W, Kanoi BN, Balikagala B, Egwang TG, Corran P, Ronca R,
625 Arcà B, Riley EM, Crisanti A, Drakeley C, Bousema T. Influence of infection on malaria-specific antibody
626 dynamics in a cohort exposed to intense malaria transmission in northern Uganda. Parasite Immunol. 2013;35(5-
627 6):164-73. doi: 10.1111/pim.12031.

50. Andrade BB, Rocha BC, Reis-Filho A, Camargo LM, Tadei WP, Moreira LA, Barral A, Barral-Netto M. Anti-*Anopheles darlingi* saliva antibodies as marker of *Plasmodium vivax* infection and clinical immunity in the Brazilian Amazon. Malar J. 2009;8:121. doi: 10.1186/1475-2875-8-121.
51. Londono-Renteria B, Drame PM, Montiel J, Vasquez AM, Tobón-Castaño A, Taylor M, Vizcaino L, Lenhart AAE. Identification and pilot evaluation of salivary peptides from *Anopheles albimanus* as biomarkers for bite exposure and malaria infection in Colombia. Int J Mol Sci. 2020;21(3). doi: 10.3390/ijms21030691.
52. Montiel J, Carbal LF, Tobón-Castaño A, Vásquez GM, Fisher ML, Londono-Rentería B. IgG antibody response against *Anopheles* salivary gland proteins in asymptomatic *Plasmodium* infections in Narino, Colombia. Malar J. 2020;19(1):42. doi: 10.1186/s12936-020-3128-9.
53. Kerkhof K, Sluydts V, Willen L, Kim S, Canier L, Heng S, Tsuboi T, Sochantha T, Sovannaroth S, Ménard D, Coosemans M, Durnez L. Serological markers to measure recent changes in malaria at population level in Cambodia. Malar J. 2016;15(1):1-18. doi: 10.1186/s12936-016-1576-z.
54. Charlwood JD, Hall T, Nenhep S, Rippon E, Branca-Lopes A, Steen K, Arca B, Drakeley C. Spatial repellents and malaria transmission in an endemic area of Cambodia with high mosquito net usage. MalariaWorld J. 2017;8(11).
55. Pollard EJM, Patterson C, Russell TL, Apairamo A, Oscar J, Arcà B, Drakeley C, Burkot TR. Human exposure to *Anopheles farauti* bites in the Solomon Islands is not associated with IgG antibody response to the gSG6 salivary protein of *Anopheles gambiae*. Malar J. 2019;18(1):334. doi: 10.1186/s12936-019-2975-8.
56. *Plasmodium falciparum* Paratsite Rate 2-10 year olds [Internet]. MAP. 2020. Available from: https://malariaatlas.org/.
57. Arcà B, Lombardo F, Struchiner CJ, Ribeiro JM. Anopheline salivary protein genes and gene families: an evolutionary overview after the whole genome sequence of sixteen *Anopheles* species. BMC Genomics. 2017;18(1):153. doi: 10.1186/s12864-017-3579-8.
58. Londono-Renteria B, Montiel J, Calvo E, Tobón-Castaño A, Valdivia HO, Escobedo-Vargas K, Romero L, Bosantes M, Fisher ML, Conway MJ, Vásquez GM, Lenhart AE. Antibody responses against *Anopheles darlingi* immunogenic peptides in *Plasmodium* infected humans. Front Cell Infect Microbiol. 2020;10(455). doi: 10.3389/fcimb.2020.00455.
59. Trung HD, Bortel WV, Sochantha T, Keokenchanh K, Briët OJ, Coosemans M. Behavioural heterogeneity of Anopheles species in ecologically different localities in Southeast Asia: a challenge for vector control. Trop Med Int Health. 2005;10(3):251-62.
60. Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Chareonviriyaphap T, Coetzee M, Mbogo CM, Hemingway J, Patil AP, Temperley WH. A global map of dominant malaria vectors. Parasit Vectors. 2012;5(1):69.
61. Sinka ME, Rubio-Palis Y, Manguin S, Patil AP, Temperley WH, Gething PW, Van Boeckel T, Kabaria CW, Harbach RE, Hay SI. The dominant *Anopheles* vectors of human malaria in the Americas: occurrence data, distribution maps and bionomic précis. Parasit Vectors. 2010;3(1):72. doi: 10.1186/1756-3305-3-72.
62. Herrera S, Ochoa-Orozco SA, González IJ, Peinado L, Quiñones ML, Arévalo-Herrera M. Prospects for malaria elimination in Mesoamerica and Hispaniola. PLoS Negl Trop Dis. 2015;9(5):e0003700. Epub 2015/05/15. doi: 10.1371/journal.pntd.0003700. PubMed PMID: 25973753; PubMed Central PMCID: PMCPMC4431857.
63. Chaumeau V, Fustec B, Nay Hsel S, Montazeau C, Naw Nyo S, Metaane S, Sawasdichai S, Kittiphanakun P, Phatharakokordbun P, Kwansomboon N, Andolina C, Cerqueira D, Chareonviriyaphap T, Nosten FH, Corbel V. Entomological determinants of malaria transmission in Kayin state, Eastern Myanmar: A 24-month longitudinal study in four villages. Wellcome Open Res. 2018;3:109. Epub 2019/06/27. doi: 10.12688/wellcomeopenres.14761.4. PubMed PMID: 31206035; PubMed Central PMCID: PMCPMC6544137.
64. Takken W, Lindsay S. Increased threat of urban malaria from *Anopheles stephensi* mosquitoes, Africa. Emerg Infect Dis. 2019;25(7):1431-3. Epub 2019/07/17. doi: 10.3201/eid2507.190301. PubMed PMID: 31063455.
65. Sinka ME, Pironon S, Massey NC, Longbottom J, Hemingway J, Moyes CL, Willis KJ. A new malaria vector in Africa: Predicting the expansion range of *Anopheles stephensi* and identifying the urban populations at risk. Proc Natl Acad Sci USA. 2020;117(40):24900. doi: 10.1073/pnas.2003976117.
66. Sandfort M, Vantaux A, Kim S, Obadia T, Pepey A, Gardais S, Khim N, Lek D, White M, Robinson LJ, Witkowski B, Mueller I. Forest malaria in Cambodia: the occupational and spatial clustering of *Plasmodium vivax* and *Plasmodium falciparum* infection risk in a cross-sectional survey in Mondulkiri province, Cambodia. Malar J. 2020;19(1):413. doi: 10.1186/s12936-020-03482-4.
67. World Health Organization. Global vector control response 2017–2030. Geneva: WHO, 2017.
68. Cutts JC, Agius PA, Zaw L, Powell R, Moore K, Draper B, Simpson JA, Fowkes FJI. Pregnancy-specific malarial immunity and risk of malaria in pregnancy and adverse birth outcomes: a systematic review. BMC Med. 2020;18(1):14. Epub 2020/01/17. doi: 10.1186/s12916-019-1467-6. PubMed PMID: 31941488; PubMed Central PMCID: PMCPMC6964062.

69. Cutts JC, Powell R, Agius PA, Beeson JG, Simpson JA, Fowkes FJ. Immunological markers of *Plasmodium vivax* exposure and immunity: a systematic review and meta-analysis. BMC Med. 2014;12:150. Epub 2014/09/10. doi: 10.1186/s12916-014-0150-1. PubMed PMID: 25199532; PubMed Central PMCID: PMCPMC4172944.
70. Fowkes FJI, Richards JS, Simpson JA, Beeson JG. The relationship between anti-merozoite antibodies and incidence of *Plasmodium falciparum* malaria: A systematic review and meta-analysis. PLoS Med. 2010;7(1):e1000218-e. doi: 10.1371/journal.pmed.1000218. PubMed PMID: 20098724.
71. Alvarenga PH, Francischetti IMB, Calvo E, Sa-Nunes A, Ribeiro JMC, Andersen JF. The Function and Three-Dimensional Structure of a Thromboxane A(2)/Cysteinyl Leukotriene-Binding Protein from the Saliva of a Mosquito Vector of the Malaria Parasite. PLoS Biol. 2010;8(11). doi: 10.1371/journal.pbio.1000547. PubMed PMID: WOS:000284762300019.
72. Arcà B, Lombardo F, Valenzuela JG, Francischetti IMB, Marinotti O, Coluzzi M, Ribeiro JMC. An updated catalogue of salivary gland transcripts in the adult female mosquito, Anopheles gambiae. J Exp Biol. 2005;208(20):3971-86. doi: 10.1242/jeb.01849.
73. Calvo E, Dao A, Pham VM, Ribeiro JM. An insight into the sialome of Anopheles funestus reveals an emerging pattern in anopheline salivary protein families. Insect Biochem Mol Biol. 2007;37(2):164-75. doi: 10.1016/j.ibmb.2006.11.005.
74. Calvo E, Mans BJ, Andersen JF, Ribeiro JMC. Function and evolution of a mosquito salivary protein family. J Biol Chem. 2006;281(4):1935-42. doi: 10.1074/jbc.M510359200.
75. Choumet V, Carmi-Leroy A, Laurent C, Lenormand P, Rousselle JC, Namane A, Roth C, Brey PT. The salivary glands and saliva of Anopheles gambiae as an essential step in the Plasmodium life cycle: A global proteomic study. Proteomics. 2007;7(18):3384-94. doi: 10.1002/pmic.200700334.
76. Das S, Radtke A, Choi Y, Mendes AM, Valenzuela JG, Dimopoulos G. Transcriptomic and functional analysis of the Anopheles gambiae salivary gland in relation to blood feeding. BMC Genomics. 2010;11(1). doi: 10.1186/1471-2164-11-566.
77. Di Gaetano S, Del Gatto A, Pirone L, Comegna D, Zaccaro L, Saviano M, Arca B, Capasso D, Pedone E. A selective alpha(v)beta(5) integrin antagonist hidden into the anophelin family protein cE5 from the malaria vector Anopheles gambiae. Peptide Science. 2018;110(5). doi: 10.1002/pep2.24054. PubMed PMID: WOS:000450664700016.
78. Dixit R, Sharma A, Mourya DT, Kamaraju R, Patole MS, Shouche YS. Salivary gland transcriptome analysis during Plasmodium infection in malaria vector Anopheles stephensi. Int J Infect Dis. 2009;13(5):636-46. doi: 10.1016/j.ijid.2008.07.027.
79. Francischetti IM, Ma D, Andersen JF, Ribeiro JM. Evidence for a lectin specific for sulfated glycans in the salivary gland of the malaria vector, Anopheles gambiae. PLoS One. 2014;9(9):e107295. doi: 10.1371/journal.pone.0107295.
80. Francischetti IM, Valenzuela JG, Pham VM, Garfield MK, Ribeiro JM. Toward a catalog for the transcripts and proteins (sialome) from the salivary gland of the malaria vector Anopheles gambiae. J Exp Biol. 2002;205(Pt 16):2429-51.
81. Ghosh AK, Devenport M, Jethwaney D, Kalume DE, Pandey A, Anderson VE, Sultan AA, Kumar N, Jacobs-Lorena M. Malaria parasite invasion of the mosquito salivary gland requires interaction between the Plasmodium TRAP and the Anopheles saglin proteins. PLoS Pathog. 2009;5(1):e1000265. doi: 10.1371/journal.ppat.1000265.
82. Isaacs AT, Mawejje HD, Tomlinson S, Rigden DJ, Donnelly MJ. Genome-wide transcriptional analyses in Anopheles mosquitoes reveal an unexpected association between salivary gland gene expression and insecticide resistance. BMC Genomics. 2018;19(1). doi: 10.1186/s12864-018-4605-1.
83. Jariyapan N, Baimai V, Poovorawan Y, Roytrakul S, Saeung A, Thongsahuan S, Suwannamit S, Otsuka Y, Choochote W. Analysis of female salivary gland proteins of the Anopheles barbirostris complex (Diptera: Culicidae) in Thailand. Parasitol Res. 2010;107(3):509-16. doi: 10.1007/s00436-010-1883-1.
84. Jariyapan N, Choochote W, Jitpakdi A, Harnnoi T, Siriyasatein P, Wilkinson MC, Bates PA. A glycine- and glutamate-rich protein is female salivary gland-specific and abundant in the malaria vector Anopheles dirus B (Diptera: Culicidae). J Med Entomol. 2006;43(5):867-74. doi: 10.1603/0022-2585(2006)43[867:AGAGPI]2.0.CO;2.
85. Jariyapan N, Roytrakul S, Paemanee A, Junkum A, Saeung A, Thongsahuan S, Sor-suwan S, Phattanawiboon B, Poovorawan Y, Choochote W. Proteomic analysis of salivary glands of female Anopheles barbirostris species A2 (Diptera: Culicidae) by two-dimensional gel electrophoresis and mass spectrometry. Parasitol Res. 2012;111(3):1239-49. doi: 10.1007/s00436-012-2958-y.
86. Kamiya T, Greischar MA, Mideo N. Epidemiological consequences of immune sensitisation by pre-exposure to vector saliva. PLoS Negl Trop Dis. 2017;11(10). doi: 10.1371/journal.pntd.0005956.
87. Khaireh BA, Briolant S, Pascual A, Mokrane M, MacHault V, Travaillé C, Khaireh MA, Farah IH, Ali HM, Abdi AIA, Ayeh SN, Darar HY, Ollivier L, Waiss MK, Bogreau H, Rogier C, Pradines B. Plasmodium

748 vivax and Plasmodium falciparum infections in the Republic of Djibouti: Evaluation of their prevalence and
749 potential determinants. Malar J. 2012;11. doi: 10.1186/1475-2875-11-395.
750 88. Korochkina S, Barreau C, Pradel G, Jeffery E, Li J, Natarajan R, Shabanowitz J, Hunt D, Frevert U,
751 Vernick KD. A mosquito-specific protein family includes candidate receptors for malaria sporozoite invasion of
752 salivary glands. Cell Microbiol. 2006;8(1):163-75. doi: 10.1111/j.1462-5822.2005.00611.x.
753 89. Lombardo F, Ronca R, Rizzo C, Mestres-Simòn M, Lanfrancotti A, Currà C, Fiorentino G, Bourgouin
754 C, Ribeiro JM, Petrarca V, Ponzi M, Coluzzi M, Arcà B. The Anopheles gambiae salivary protein gSG6: an
755 anopheline-specific protein with a blood-feeding role. Insect Biochem Mol Biol. 2009;39(7):457-66. doi:
756 10.1016/j.ibmb.2009.04.006.
757 90. Pandey RK, Bhatt TK, Prajapati VK. Novel Immunoinformatics Approaches to Design Multi-epitope
758 Subunit Vaccine for Malaria by Investigating Anopheles Salivary Protein. Sci Rep. 2018;8(1):1125. doi:
759 10.1038/s41598-018-19456-1.
760 91. Pedro PM, Sallum MAM. Spatial expansion and population structure of the neotropical malaria vector,
761 Anopheles darlingi (Diptera: Culicidae). Biol J Linn Soc. 2009;97(4):854-66. doi: 10.1111/j.1095-
762 8312.2009.01226.x.
763 92. Phattanawiboon B, Jariyapan N, Mano C, Roytrakul S, Paemanee A, Sor-Suwan S, Sriwichai P,
764 Saeung A, Bates PA. Salivary Gland Proteome during Adult Development and after Blood Feeding of Female
765 Anopheles dissidens Mosquitoes (Diptera: Culicidae). PLoS One. 11(9):e0163810. doi:
766 10.1371/journal.pone.0163810.
767 93. Pirone L, Ripoll-Rozada J, Leone M, Ronca R, Lombardo F, Fiorentino G, Andersen JF, Pereira PJB,
768 Arcà B, Pedone E. Functional analyses yield detailed insight into the mechanism of thrombin inhibition by the
769 antihemostatic salivary protein cE5 from Anopheles gambiae. J Biol Chem. 2017;292(30):12632-42. doi:
770 10.1074/jbc.M117.788042. PubMed PMID: PDB/3U69PDB/4E05PDB/5NHU.
771 94. Rawal R, Vijay S, Kadian K, Singh J, Pande V, Sharma A. Towards a Proteomic Catalogue and
772 Differential Annotation of Salivary Gland Proteins in Blood Fed Malaria Vector Anopheles culicifacies by Mass
773 Spectrometry. PLoS One. 2016;11(9):e0161870. doi: 10.1371/journal.pone.0161870.
774 95. Ronca R, Kotsyfakis M, Lombardo F, Rizzo C, Currà C, Ponzi M, Fiorentino G, Ribeiro JM, Arcà B.
775 The Anopheles gambiae cE5, a tight- and fast-binding thrombin inhibitor with post-transcriptionally regulated
776 salivary-restricted expression. Insect Biochem Mol Biol. 2012;42(9):610-20. doi: 10.1016/j.ibmb.2012.04.008.
777 96. Sarr JB, Remoue F, Samb B, Dia I, Guindo S, Sow C, Maiga S, Tine S, Thiam C, Schacht AM,
778 Simondon F, Konate L, Riveau G. Evaluation of antibody response to Plasmodium falciparum in children
779 according to exposure of Anopheles gambiae s.l or Anopheles funestus vectors. Malar J. 2007;6. doi:
780 10.1186/1475-2875-6-117.
781 97. Scarpassa VM, Debat HJ, Alencar RB, Saraiva JF, Calvo E, Arcà B, Ribeiro JMC. An insight into the
782 sialotranscriptome and virome of Amazonian anophelines. BMC Genomics. 2019;20(1):166. doi:
783 10.1186/s12864-019-5545-0.
784 98. Wells MB, Andrew DJ. Salivary gland cellular architecture in the Asian malaria vector mosquito
785 *Anopheles stephensi*. Parasit Vectors. 2015;8:617-.
786 99. Zocevic A, Carmi-Leroy A, Sautereau J, d'Alayer J, Lenormand P, Rousselle JC, Namane A, Choumet
787 V. New markers in Anopheles gambiae salivary glands after Plasmodium berghei infection. Vector Borne
788 Zoonotic Dis. 2013;13(2):119-27. doi: 10.1089/vbz.2012.0964.
789 100. The mal ERARCPoTfME. malERA: An updated research agenda for diagnostics, drugs, vaccines, and
790 vector control in malaria elimination and eradication. PLoS Med. 2017;14(11). doi:
791 10.1371/journal.pmed.1002455.
792 101. The mal ERARCPoBS, Enabling T. malERA: An updated research agenda for basic science and
793 enabling technologies in malaria elimination and eradication. PLoS Med. 2017;14(11). doi:
794 10.1371/journal.pmed.1002451.
795 102. The mal ERARCPoCtR, Measuring T. malERA: An updated research agenda for characterising the
796 reservoir and measuring transmission in malaria elimination and eradication. PLoS Med. 2017;14(11). doi:
797 10.1371/journal.pmed.1002452.
798 103. Andrade BB, Barral-Netto M. Biomarkers for susceptibility to infection and disease severity in human
799 malaria. Mem Inst Oswaldo Cruz. 2011;106:70-8. doi: 10.1590/s0074-02762011000900009. PubMed PMID:
800 WOS:000294440600009.
801 104. Andrade BB, Teixeira CR, Barral A, Barral-Netto M. Haematophagous arthropod saliva and host
802 defense system: A tale of tear and blood. An Acad Bras Cienc. 2005;77(4):665-93. doi: 10.1590/S0001-
803 37652005000400008.
804 105. Billingsley PF, Baird J, Mitchell JA, Drakeley C. Immune interactions between mosquitoes and their
805 hosts. Parasite Immunol. 2006;28(4):143-53. doi: 10.1111/j.1365-3024.2006.00805.x. PubMed PMID:
806 WOS:000236064500004.

106. Cantillo JF, Fernández-Caldas E, Puerta L. Immunological aspects of the immune response induced by mosquito allergens. Int Arch Allergy Immunol. 2014;165(4):271-82. doi: 10.1159/000371349.
107. Coutinho-Abreu IV, Guimaraes-Costa AB, Valenzuela JG. Impact of insect salivary proteins in blood feeding, host immunity, disease, and in the development of biomarkers for vector exposure. Curr Opin Insect. 2015;10:98-103. doi: 10.1016/j.cois.2015.04.014. PubMed PMID: WOS:000369017500015.
108. Domingos A, Pinheiro-Silva R, Couto J, do Rosário V, de la Fuente J. The Anopheles gambiae transcriptome – a turning point for malaria control. Insect Mol Biol. 2017;26(2):140-51. doi: 10.1111/imb.12289.
109. Doucoure S, Cornelie S, Drame PM, Marie A, Ndille EE, Mathieu-Daudé F, Mouchet F, Poinsignon A, Remoue F. Biomarkers of vector bites: Arthropod immunogenic salivary proteins in vector-borne diseases control. In: Preedy VR, Patel VB, editors. General Methods in Biomarker Research and their Applications. 2-22015. p. 1177-205.
110. Doucoure S, Drame PM. Salivary Biomarkers in the Control of Mosquito-Borne Diseases. Insects. 2015;6(4):961-76. doi: 10.3390/insects6040961.
111. Drame PM, Poinsignon A, Marie A, Noukpo H, Doucoure S, Cornelie S, Remoue F, Manguin S. New Salivary Biomarkers of Human Exposure to Malaria Vector Bites. In: Manguin S, editor. Anopheles mosquitoes: New insights into malaria vectors2013. p. 755-95.
112. Fontaine A, Diouf I, Bakkali N, Missé D, Pagès F, Fusai T, Rogier C, Almeras L. Implication of haematophagous arthropod salivary proteins in host-vector interactions. Parasit Vectors. 2011;4(1). doi: 10.1186/1756-3305-4-187.
113. Foy BD, Killeen GF, Magalhaes T, Beier JC. Immunological targeting of critical insect antigens. Am Entomol. 2002;48(3):150-62. doi: 10.1093/ae/48.3.150.
114. Gillespie RD, Mbow ML, Titus RG. The immunomodulatory factors of bloodfeeding arthropod saliva. Parasite Immunol. 2000;22(7):319-31. doi: 10.1046/j.1365-3024.2000.00309.x.
115. Hopp CS, Sinnis P. The innate and adaptive response to mosquito saliva and Plasmodium sporozoites in the skin. Ann N Y Acad Sci. 2015;1342:37-43. doi: 10.1111/nyas.12661. PubMed PMID: WOS:000353102900005.
116. Hugo RLE, Birrell GW. Proteomics of Anopheles Vectors of Malaria. Trends Parasitol. 2018;34(11):961-81. doi: 10.1016/j.pt.2018.08.009.
117. Leitner WW, Denis ACS, Wali T. Immunological consequences of arthropod vector-derived salivary factors. Eur J Immunol. 2011;41(12):3396-400. doi: 10.1002/eji.201190075.
118. Lombardo F, Lanfrancotti A, Mestres-Simón M, Rizzo C, Coluzzi M, Arcà B. At the interface between parasite and host: The salivary glands of the African malaria vector Anopheles gambiae. Parassitologia. 2006;48(4):573-80.
119. Mathema VB, Na-Bangchang K. A brief review on biomarkers and proteomic approach for malaria research. Asian Pac J Trop Med. 2015;8(4):253-62. doi: 10.1016/S1995-7645(14)60327-8.
120. Peng Z, Estelle F, Simons R. Mosquito allergy and mosquito salivary allergens. Protein Peptide Lett. 2007;14(10):975-81. doi: 10.2174/092986607782541088. PubMed PMID: WOS:000253577700007.
121. Ribeiro JMC, Francischetti IMB. Role of Arthropod Saliva in Blood Feeding: Sialome and Post-Sialome Perspectives. Annu Rev Entomol. 2003;48:73-88. doi: 10.1146/annurev.ento.48.060402.102812.
122. Sagna A, Poinsignon A, Remoue F, Wikel SK, Aksoy S, Dimopoulos G. Epidemiological Applications of Assessing Mosquito Exposure in a Malaria-Endemic Area. In: Wikel SK, Dimopoulos G, Aksoy S, editors. Arthropod Vector: Controller of Disease Transmission, Volume 2: Vector Saliva-Host Pathogen Interactions2017. p. 209-29.
123. Sá-Nunes A, De Oliveira CJF. Sialogenins and immunomodulators derived from blood feeding parasites. In: Kini R, Clemetson K, Markland F, McLane M, Morita T, editors. Toxins and Hemostasis2011. p. 131-52.
124. Miot HA, Lima HC. Allergy to Hematophagous Arthropods Bites. Current Dermatology Reports. 2014;3(1):6-12.
125. Peng Z, Simons FER. Mosquito allergy: immune mechanisms and recombinant salivary allergens. Int Arch Allergy Appl Immunol. 2004;133(2):198-209.
126. Pingen M, Schmid MA, Harris E, McKimmie CS. Mosquito biting modulates skin response to virus infection. Trends Parasitol. 2017;33(8):645-57.
127. Sinden RE, Blagborough AM, Churcher T, Ramakrishnan C, Biswas S, Delves MJ. The design and interpretation of laboratory assays measuring mosquito transmission of Plasmodium. Trends Parasitol. 2012;28(11):457-65.
128. Abonuusum A, Owusu-Daako K, Tannich E, May J, Garms R, Kruppa T. Malaria transmission in two rural communities in the forest zone of Ghana. Parasitol Res. 2011;108(6):1465-71. doi: 10.1007/s00436-010-2195-1.

129. Badu K, Afrane YA, Larbi J, Stewart VA, Waitumbi J, Angov E, Ong'echa JM, Perkins DJ, Zhou G, Githeko A, Yan G. Marked variation in MSP-1 19antibody responses to malaria in western Kenyan highlands. BMC Infect Dis. 2012;12. doi: 10.1186/1471-2334-12-50.
130. Chaccour CJ, Kobylinski KC, Bassat Q, Bousema T, Drakeley C, Alonso P, Foy BD. Ivermectin to reduce malaria transmission: A research agenda for a promising new tool for elimination. Malar J. 2013;12(1). doi: 10.1186/1475-2875-12-153.
131. Coulibaly D, Travassos MA, Tolo Y, Laurens MB, Kone AK, Traore K, Sissoko M, Niangaly A, Diarra I, Daou M, Guindo B, Rebaudet S, Kouriba B, Dessay N, Piarroux R, Plowe CV, Doumbo OK, Thera MA, Gaudart J. Spatio-temporal dynamics of asymptomatic malaria: Bridging the gap between annual Malaria resurgences in a Sahelian environment. Am J Trop Med Hyg. 2017;97(6):1761-9. doi: 10.4269/ajtmh.17-0074.
132. Dhawan R, Kumar M, Mohanty AK, Dey G, Advani J, Prasad TSK, Kumar A. Mosquito-borne diseases and omics: Salivary gland proteome of the female aedes aegypti mosquito. OMICS: J Integrative Biol. 2017;21(1):45-54. doi: 10.1089/omi.2016.0160.
133. Fontaine A, Pascual A, Diouf I, Bakkali N, Bourdon S, Fusai T, Rogier C, Almeras L. Mosquito salivary gland protein preservation in the field for immunological and biochemical analysis. Parasit Vectors. 2011;4:33. doi: 10.1186/1756-3305-4-33.
134. Fontaine A, Pascual A, Orlandi-Pradines E, Diouf I, Remoue F, Pages F, Fusai T, Rogier C, Almeras L. Relationship between Exposure to Vector Bites and Antibody Responses to Mosquito Salivary Gland Extracts. PLoS One. 2011;6(12). doi: 10.1371/journal.pone.0029107. PubMed PMID: WOS:000298369100156.
135. Jeon SH, Park JW, Lee BH. Characterization of human IgE and mouse IgG1 responses to allergens in three mosquito species by immunoblotting and ELISA. Int Arch Allergy Immunol. 2001;126(3):206-12. doi: 10.1159/000049515.
136. Kelly-Hope LA, McKenzie FE. The multiplicity of malaria transmission: A review of entomological inoculation rate measurements and methods across sub-Saharan Africa. Malar J. 2009;8(1). doi: 10.1186/1475-2875-8-19.
137. Kusi KA, Bosomprah S, Dodoo D, Kyei-Baafour E, Dickson EK, Mensah D, Angov E, Dutta S, Sedegah M, Koram KA. Anti-sporozoite antibodies as alternative markers for malaria transmission intensity estimation. Malar J. 2014;13(1). doi: 10.1186/1475-2875-13-103.
138. Li B, Calvo E, Marinotti O, James AA, Paskewitz SM. Characterization of the c-type lysozyme gene family in Anopheles gambiae. Gene. 2005;360(2):131-9. doi: 10.1016/j.gene.2005.07.001.
139. Londono-Renteria B, Patel JC, Vaughn M, Funkhauser S, Ponnusamy L, Grippin C, Jameson SB, Apperson C, Mores CN, Wesson DM, Colpitts TM, Meshnick SR. Long-lasting permethrin-impregnated clothing protects against mosquito bites in outdoor workers. Am J Trop Med Hyg. 2015;93(4):869-74. doi: 10.4269/ajtmh.15-0130.
140. Mwanziva C, Manjurano A, Mbugi E, Mweya C, Mkali H, Kivuyo MP, Sanga A, Ndaro A, Chambo W, Mkwizu A, Kitau J, Kavishe R, Dolmans W, Chilongola J, Mosha FW. Defining malaria burden from morbidity and mortality records, self treatment practices and serological data in Magugu, Babati district, northern Tanzania. Tanzan J Health Res. 2011;13(2). doi: 10.4314/thrb.v13i2.62980.
141. Sarr J, Orlandi-Pradines E, Fortin S, Sow C, Cornelie S, Rogerie F, Guindo S, Konate L, Fusa T, Riveau G, Rogier C, Remoue F. Assessment of exposure to Plasmodium falciparum transmission in a low endemicity area by using multiplex fluorescent microsphere-based serological assays. Parasit Vectors. 2011;4(1). doi: 10.1186/1756-3305-4-212.
142. Satoguina J, Walther B, Drakeley C, Nwakanma D, Oriero EC, Correa S, Corran P, Conway DJ, Walther M. Comparison of surveillance methods applied to a situation of low malaria prevalence at rural sites in the Gambia and Guinea Bissau. Malar J. 2009;8(1). doi: 10.1186/1475-2875-8-274.
143. Smithuis FM, Kyaw MK, Phe UO, Van Der Broek I, Katterman N, Rogers C, Almeida P, Kager PA, Stepniewska K, Lubell Y, Simpson JA, White NJ. Entomological determinants of insecticide-treated bed net effectiveness in Western Myanmar. Malar J. 2013;12(1). doi: 10.1186/1475-2875-12-364.
144. Ubillos I, Campo JJ, Jiménez A, Dobaño C. Development of a high-throughput flexible quantitative suspension array assay for IgG against multiple Plasmodium falciparum antigens. Malar J. 2018;17(1). doi: 10.1186/s12936-018-2365-7.
145. van den Hoogen LL, Présumé J, Romilus I, Mondélus G, Elismé T, Sepúlveda N, Stresman G, Druetz T, Ashton RA, Joseph V, Eisele TP, Hamre KES, Chang MA, Lemoine JF, Tetteh KKA, Boncy J, Existe A, Drakeley C, Rogier E. Quality control of multiplex antibody detection in samples from large-scale surveys: the example of malaria in Haiti. Sci Rep. 2020;10(1). doi: 10.1038/s41598-020-57876-0.
146. Varela ML, Koffi D, White M, Niang M, Mbengue B, Diene Sarr F, Touré AO, Perraut R. Practical example of multiple antibody screening for evaluation of malaria control strategies. Malar J. 2020;19(1). doi: 10.1186/s12936-020-03186-9.
147. Wanjala CL, Kweka EJ. Impact of highland topography changes on exposure to malaria vectors and immunity in Western Kenya. Front Public Health. 2016;4(OCT). doi: 10.3389/FPUBH.2016.00227.

148. Armiyanti Y, Arifianto RP, Riana EN, Senjarini K, Widodo W, Fitri LE, Sardjono TW. Identification of antigenic proteins from salivary glands of female Anopheles maculatus by proteomic analysis. Asian Pac J Trop Biomed. 2016;6(11):924-30. doi: 10.1016/j.apjtb.2016.08.012.
149. Brummer-Korvenkontio H, Palosuo T, François G, Reunala T. Characterization of Aedes communis, Aedes aegypti and Anopheles stephensi mosquito saliva antigens by immunoblotting. Int Arch Allergy Immunol. 1997;112(2):169-74. doi: 10.1159/000237450.
150. Cornelie S, Remoue F, Doucoure S, Ndiaye T, Sauvage FX, Boulanger D, Simondon F. An insight into immunogenic salivary proteins of Anopheles gambiae in African children. Malar J. 2007;6:75. doi: 10.1186/1475-2875-6-75.
151. Fontaine A, Fusaï T, Briolant S, Buffet S, Villard C, Baudelet E, Pophillat M, Granjeaud S, Rogier C, Almeras L. Anopheles salivary gland proteomes from major malaria vectors. BMC Genomics. 2012;13:614. doi: 10.1186/1471-2164-13-614.
152. Marie A, Holzmuller P, Tchioffo MT, Rossignol M, Demettre E, Seveno M, Corbel V, Awono-Ambéné P, Morlais I, Remoue F, Cornelie S. Anopheles gambiae salivary protein expression modulated by wild Plasmodium falciparum infection: highlighting of new antigenic peptides as candidates of An. gambiae bites. Parasit Vectors. 2014;7:599. doi: 10.1186/s13071-014-0599-y.
153. Owhashi M, Harada M, Suguri S, Omae H, Ishii A. Identification of an eosinophil chemotactic factor from anopheline mosquitoes as a chitinase family protein. Parasitol Res. 2008;102(3):357-63. doi: 10.1007/s00436-007-0769-3.
154. Penneys NS, Nayar JK, Bernstein H, Knight JW, Leonardi C. Mosquito Salivary Gland Antigens Identified by Circulating Human Antibodies. Arch Dermatol. 1989;125(2):219-22. doi: 10.1001/archderm.1989.01670140071012.
155. Sor-suwan S, Jariyapan N, Roytrakul S, Paemanee A, Phumee A, Phattanawiboon B, Intakhan N, Chanmol W, Bates PA, Saeung A, Choochote W. Identification of salivary gland proteins depleted after blood feeding in the malaria vector Anopheles campestris-like mosquitoes (Diptera: Culicidae). PLoS One. 2014;9(3):e90809. doi: 10.1371/journal.pone.0090809.
156. Sor-Suwan S, Jariyapan N, Roytrakul S, Paemanee A, Saeung A, Thongsahuan S, Phattanawiboon B, Bates PA, Poovorawan Y, Choochote W. Salivary gland proteome of the human malaria vector, Anopheles campestris-like (Diptera: Culicidae). Parasitol Res. 2013;112(3):1065-75. doi: 10.1007/s00436-012-3233-y.
157. Peng ZK, Li HB, Simons FER. Immunoblot analysis of salivary allergens in 10 mosquito species with worldwide distribution and the human IgE responses as these allergens. J Allergy Clin Immunol. 1998;101(4):498-505. doi: 10.1016/s0091-6749(98)70357-4. PubMed PMID: WOS:000073113500012.
158. Cornelie S, Senglat M, Doucoure S, Demettre E, Remoue F. Characterization of immunogenic proteins in *Anopheles gambiae* salivary glands and their potential use as a marker of exposure to malaria. Am J Trop Med Hyg. 2008;79(6):320-. PubMed PMID: WOS:000261644601456.
159. Drame PM, Poinsignon A, Besnard P, Cornelie S, Foumane V, Sow CS, Le Mire J, Fortes F, Boulanger D, Carnevale P, Simondon F, Remoue F. Human antibody response to *Anopheles gambiae* saliva: A new immuno-epidemiological marker to evaluate the effectiveness of insecticides treated nets (ITNs)? Am J Trop Med Hyg. 2008;79(6):358-. PubMed PMID: WOS:000261644601582.
160. Drame PM, Poinsignon A, Besnard P, Cornelie S, Le Mire J, Fortes F, Toto JC, Sembene M, Simondon F, Carnevale P, Remoue F. Human antibody response to *Anopheles* salivary gSG6-P1 peptide: New immuno-epidemiological tool for evaluating the efficacy of insecticides treated nets (ITNs) in malaria vector control. Am J Trop Med Hyg. 2010;83(5):260-. PubMed PMID: WOS:000295819701240.
161. Poinsignon A, Cornelie S, Mestres-Simon M, Lanfrancotti A, Rossignol M, Boulanger D, Cisse B, Sokhna C, Arca B, Simondon F, Remoue F. Human IgG response to *Anopheles gambiae* salivary proteins as an immuno-epidemiological marker of exposure to malaria vector bites. Am J Trop Med Hyg. 2008;79(6):218-. PubMed PMID: WOS:000261644601102.
162. Poinsignon A, Drame PM, Marie A, Noukpo H, Cornelie S, Remoue F. Development of a biomarker of exposure to *Anopheles* bites based on human antibody response to salivary proteins: From concept to application in the field. Pathog Glob Health. 2013;107(8):455-. PubMed PMID: WOS:000335056200147.
163. Poinsignon A, Drame PM, Samb B, Cornelie S, Sow C, Dia I, Riveau G, Konate L, Sokhna C, Remoue FJ. Development of a new biomarker of exposure to *Anopheles* bites based on human antibody responses to salivary proteins: From the concept to the applications. Am J Trop Med Hyg. 2010;83(5):184-. PubMed PMID: WOS:000295819700617.
164. Sagna AB, Kassie D, Couvray A, Hermann E, Riveau G, Salem G, Fournet F, Remoue F. Spatial risk of urban exposure to *Anopheles* and *Aedes* mosquito bites in africa using salivary antibody-based biomarkers. Am J Trop Med Hyg. 2018;99(4):47-. PubMed PMID: WOS:000461386602150.
165. Dragovic SM, Agunbiade TA, Freudzon M, Yang J, Hastings AK, Schleicher TR, Zhou X, Craft S, Chuang YM, Gonzalez F, Li Y, Hrebikova G, Tripathi A, Mlambo G, Almeras L, Ploss A, Dimopoulos G,

985 Fikrig E. Immunization with AgTRIO, a Protein in Anopheles Saliva, Contributes to Protection against
986 Plasmodium Infection in Mice. Cell Host Microbe. 2018;23(4):523-35.e5. doi: 10.1016/j.chom.2018.03.008.
987 166. King JG, Vernicks KD, Hillyer JF. Members of the salivary gland surface protein (SGS) family are
988 major immunogenic components of mosquito saliva. J Biol Chem. 2011;286(47):40824-34. doi:
989 10.1074/jbc.M111.280552.
990 167. Vogt MB, Lahon A, Arya RP, Kneubehl AR, Spencer Clinton JL, Paust S, Rico-Hesse R. Mosquito
991 saliva alone has profound effects on the human immune system. PLoS Negl Trop Dis. 2018;12(5). doi:
992 10.1371/journal.pntd.0006439.
993 168. Wang J, Zhang Y, Zhao YO, Li MW, Zhang L, Dragovic S, Abraham NM, Fikrig E. Anopheles
994 gambiae circumsporozoite protein-binding protein facilitates plasmodium infection of mosquito salivary glands.
995 J Infect Dis. 2013;208(7):1161-9. doi: 10.1093/infdis/jit284.
996 169. Almeida AP, Billingsley PF. Induced immunity against the mosquito Anopheles stephensi: reactivity
997 characteristics of immune sera. Med Vet Entomol. 1999;13(1):53-64. doi: 10.1046/j.1365-2915.1999.00143.x.
998 170. Boulanger D, Doucoure S, Grout L, Ngom A, Rogerie F, Cornelie S, Sokhna C, Mouchet F, Riveau G,
999 Simondon F, Remoue FJ. Immunoglobulin G antibody profiles against Anopheles salivary proteins in domestic
1000 animals in Senegal. J Med Entomol. 2011;48(3):691-3. doi: 10.1603/me10183.
1001 171. Kerkhof K, Canier L, Kim S, Heng S, Sochantha T, Sovannaroth S, Vigan-Womas I, Coosemans M,
1002 Sluydts V, Ménard D, Durnez L. Implementation and application of a multiplex assay to detect malaria-specific
1003 antibodies: a promising tool for assessing malaria transmission in Southeast Asian pre-elimination areas. Malar
1004 J. 2015;14:338. doi: 10.1186/s12936-015-0868-z.
1005 172. Sagna AB, Gaayeb L, Sarr JB, Senghor S, Poinsignon A, Boutouaba-Combe S, Schacht AM, Hermann
1006 E, Faye N, Remoue F, Riveau G. Plasmodium falciparum infection during dry season: IgG responses to
1007 Anopheles gambiae salivary gSG6-P1 peptide as sensitive biomarker for malaria risk in Northern Senegal.
1008 Malar J. 2013;12:301. doi: 10.1186/1475-2875-12-301.
1009 173. Ya-Umphan P, Cerqueira D, Cottrell G, Parker DM, Fowkes FJI, Nosten F, Corbel V. *Anopheles*
1010 salivary biomarker as a proxy for estimating *Plasmodium falciparum* malaria exposure on the Thailand-
1011 Myanmar Border. Am J Trop Med Hyg. 2018;99(2):350-6.
1012 174. Aka KG, Traoré DF, Sagna AB, Zoh DD, Assi SB, Tchiekoi BN, Adja AM, Remoue F, Poinsignon A.
1013 Pattern of antibody responses to Plasmodium falciparum antigens in individuals differentially exposed to
1014 Anopheles bites. Malar J. 2020;19(1):83. doi: 10.1186/s12936-020-03160-5.
1015 175. Londono-Renteria BL, Eisele TP, Keating J, James MA, Wesson DM. Antibody response against
1016 Anopheles albimanus (Diptera: Culicidae) salivary protein as a measure of mosquito bite exposure in Haiti. J
1017 Med Entomol. 2010;47(6):1156-63. doi: 10.1603/me09240.
1018 176. Manning JE, Oliveira F, Coutinho-Abreu IV, Herbert S, Meneses C, Kamhawi S, Baus HA, Han A,
1019 Czajkowski L, Rosas LA, Cervantes-Medina A, Athota R, Reed S, Mateja A, Hunsberger S, James E,
1020 Pleguezuelos O, Stoloff G, Valenzuela JG, Memoli MJ. Safety and immunogenicity of a mosquito saliva
1021 peptide-based vaccine: a randomised, placebo-controlled, double-blind, phase 1 trial. Lancet.
1022 2020;395(10242):1998-2007. doi: 10.1016/S0140-6736(20)31048-5. PubMed PMID:
1023 ClinicalTrials.gov/NCT03055000.
1024 177. Mendes-Sousa AF, Vale VF, Queiroz DC, Pereira-Filho AA, da Silva NCS, Koerich LB, Moreira LA,
1025 Pereira MH, Sant'Anna MR, Araújo RN, Andersen J, Valenzuela JG, Gontijo NF. Inhibition of the complement
1026 system by saliva of Anopheles (Nyssorhynchus) aquasalis. Insect Biochem Mol Biol. 2018;92:12-20. doi:
1027 10.1016/j.ibmb.2017.11.004.
1028 178. Peng Z, Beckett AN, Engler RJ, Hoffman DR, Ott NL, Simons FE. Immune responses to mosquito
1029 saliva in 14 individuals with acute systemic allergic reactions to mosquito bites. J Allergy Clin Immunol.
1030 2004;114(5):1189-94. doi: 10.1016/j.jaci.2004.08.014.
1031 179. Londono-Renteria B, Cardenas JC, Troupin A, Colpitts TM. Natural mosquito-pathogen hybrid IgG4
1032 antibodies in vector-borne diseases: A hypothesis. Front Immunol. 2016;7(SEP). doi:
1033 10.3389/fimmu.2016.00380.
1034 180. Owhashi M, Harada M, Suguri S, Ohmae H, Ishii A. The role of saliva of Anopheles stephensi in
1035 inflammatory response: identification of a high molecular weight neutrophil chemotactic factor. Parasitol Res.
1036 2001;87(5):376-82. doi: 10.1007/s004360000355.
1037 181. Armiyanti Y, Nuryady MM, Arifianto RP, Nurmariana E, Senjarini K, Fitri LE, Sardjono TW.
1038 Detection of immunogenic proteins from Anopheles sundaicus salivary glands. Revista da Sociedade Brasileira
1039 de Medicina Tropical. 2015;48(4):410-6. doi: 10.1590/0037-8682-0185-2015.
1040 182. Opasawatchai A, Yolwong W, Thuncharoen W, Inrueangsri N, Itsaradisaikul S, Sasisakulporn C,
1041 Jotikasthira W, Matangkasombut O, Reamtong O, Manuyakorn W, Songnuan W, Matangkasombut P. Novel
1042 salivary gland allergens from tropical mosquito species and IgE reactivity in allergic patients. World Allergy
1043 Organ J. 2020;13(2). doi: 10.1016/j.waojou.2020.100099.

183. Orlandi-Pradines E, Almeras L, Denis de Senneville L, Barbe S, Remoué F, Villard C, Cornelie S, Penhoat K, Pascual A, Bourgouin C, Fontenille D, Bonnet J, Corre-Catelin N, Reiter P, Pagés F, Laffite D, Boulanger D, Simondon F, Pradines B, Fusaï T, Rogier C. Antibody response against saliva antigens of Anopheles gambiae and Aedes aegypti in travellers in tropical Africa. Microbes Infect. 2007;9(12-13):1454-62. doi: 10.1016/j.micinf.2007.07.012.

Supplementary Files

Appendix 1. Supplementary Methodology.

Search strategy

We performed a systematic review with multilevel modelling of the published literature according to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines [17] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) specifications [16]. The protocol was registered with PROSPERO (CRD42020185449).

The electronic databases PubMed, Scopus, Web of Science, African Index Medicus, and the Latin American and Caribbean Health Sciences Literature (LILACS) were searched for studies published before June 30, 2020 investigating *Anopheles* salivary antigens as a biomarker for mosquito exposure or malaria transmission. Search terms were as follows: *Anophel** AND saliva* AND (antibod* OR sero* OR antigen OR marker* OR biomarker* OR gSG6* OR gSG* OR SG* OR cE5). The reference lists of included studies were screened for additional studies, and Google Scholar was used to identify additional works by key authors. No formal attempt was made to identify unpublished population studies as it would have required significant description of the design, methods and analysis used in these studies, and a review of ethical issues.

Selection criteria

The primary criteria for inclusion in this systematic review was the reporting of observations of seroprevalence or total levels of Immunoglobulin (Ig) antibodies (including all isotypes and subclasses) in human sera against recombinant or synthetic peptide *Anopheles* salivary antigens. We considered for inclusion: cross-sectional studies, cohort studies, intervention studies and case-control studies of individuals or populations (including sub-populations) living in all geographies with natural exposure to *Anopheles* mosquitoes. Studies that were solely performed in participants not representative of the wider population (i.e. mosquito allergic patients, soldiers, returned travellers) were excluded. The minimum quality criteria for inclusion in this review were: antibody detection performed using enzyme-linked immunosorbent assay (ELISA), multiplex or Luminex assays.

The exposure variables of interest included entomological and malariometric parameters, including: (i) human biting rate (HBR), defined as the number of bites received per person per unit of time; (ii) entomological inoculation rate (EIR), defined as the number of infectious bites per person per unit of time, calculated as the HBR multiplied by the sporozoite index; (iii) estimates of malaria prevalence; (iv) population-level seroprevalence estimates against *Plasmodium* spp. malarial antigens. To ensure HBR estimates were given for

the same unit of time (bites per person per night), biting rates given per week were divided by 7, and biting rates given per month we multiplied by 12 and divided by 365. Similar approaches were employed to ensure consistent units for EIR (infectious bites per person per year). *Plasmodium* spp. infections had to be confirmed by either microscopy, rapid diagnostic test (RDT) or molecular methods (polymerase chain reaction (PCR)). *Plasmodium* spp. diagnosis was included for all *Plasmodium* spp. combined and the species-level if provided. Where exposure estimates were not provided, we attempted to source data from other publications by the authors, or using the site geolocation and year to obtain estimates of EIR from the Pangaea dataset [18]. *P. falciparum* rates in 2-10 year olds (globally, 2000–2017) and dominant vector species (DVS) from the Malaria Atlas Project (MAP) [19]. Studies of salivary antigens where exposure variables could not be sourced and data that could not be extracted were excluded.

Selection of studies

One author performed database searches and screened reference lists to identify possible studies. One author screened studies against inclusion criteria, with discussion and input from a second reviewer.

Approaches to include all available studies

The authors of any studies that did not contain relevant information on the study design, populations, eligibility criteria, or key study data, were contacted and relevant data requested. Authors were contacted via an initial email detailing the precise nature of the systematic review and the data required. If the authors did not reply to three email requests, or were unable to provide relevant data, the studies were deemed to insufficiently meet inclusion/quality criteria and were excluded. As measurement of antibody levels does not produce a common metric between studies, authors were asked to classify their participants as ‘responders’ or ‘no-responders’ according to seropositivity (antibody level relative to unexposed sera) within each study, to allow comparisons of seroprevalence between studies [68-70]. Studies that were only able to provide antibody levels or categorised seropositivity based upon arbitrary cut offs were excluded from multilevel modelling analyses and included in narrative terms. Where the salivary antibody response and exposure variable were measured in the same population and reported in multiple publications, the study with the largest sample size was included, otherwise the earliest study was included.

Data extraction

Data were extracted using a data collection form by one reviewer. Any data that was provided at the sub-population level was extracted at the lowest level i.e. if a study was performed across multiple sites, and an

estimate for both salivary antibody seroprevalence/levels and the exposure of interest is given for each site, it was included the site level, rather than an aggregated level.

Measures

Outcomes

The primary outcome of interest of our systematic review was the reported antibody response (both seroprevalence and levels of all Ig subclasses and isotypes) to *Anopheles* salivary antigens. Multilevel modelling analyses were performed where the seroprevalence of antibodies against the same antigen and the exposure of interest were reported in more than one study.

Exposures

The primary exposures of interest included in the multilevel modelling analyses were the HBR and EIR, a measure of the average number of bites received per person per night and infectious bites received per person per year, respectively. Secondary exposures assessed include the prevalence of any *Plasmodium* spp. infection (including *P. falciparum* only, *P. vivax* only, or untyped infections). Additional secondary exposures include the *P. falciparum* infection rate in 2-10 year olds extracted from MAP, as well as the seroprevalence of antimalarial antibodies against pre-erythrocytic and blood-stage antigens.

Clinical and methodological heterogeneity were explored using prespecified variables to minimize spurious findings. Variables considered for inclusion were study design (cohort, cross-sectional, repeated cross-sectional), DVS, study participants (adults only, children only, adults and children), preparation of salivary antigen (recombinant full-length protein, synthetic peptide), malaria detection methodology (light microscopy, RDT, PCR), and entomological vector collection methodology (human landing catch, light traps, and spray catches).

Statistical analysis

Where there were sufficient data to pool observations of the same exposure and outcome measures, generalised linear multilevel modelling was used to undertake analyses quantifying associations between the exposures of interest and salivary antibody seroprevalence measurements. Models were generalised through use of the logit link function and binomial distribution (statistical notation for HBR model shown below as equation one). Seroprevalence was modelled in binomial form as the number of individuals seropositive to the total sample size. A three-level random effects model with a nested framework was used to account for dependency in the

data, with random intercepts for country (level-3) and study (level-2) estimated. Hence level-1 units represented multiple salivary antibody observations within a study induced by the study design (*i.e.* multiple time points, sites, age categories). Additionally, study-level random slopes for entomological and malariometric exposures were estimated to permit the effects to vary across studies. Model structure was determined empirically through likelihood ratio tests ($p<0.05$), with the exception of country at the 3rd level which was included in HBR and EIR analyses to estimate country-specific seroprevalence estimates of anti-salivary antibodies. The associations between the various exposures and the different salivary antigens were analysed separately, however observations of IgG seroprevalence against the recombinant full-length protein (gSG6) and synthetic peptide (gSG6-P1, the one peptide determined in all studies utilising peptides) form of the gSG6 antigen were analysed together, with a fixed term for antigen construct considered for inclusion in the model. Of note, gSG6 peptide 2 (gSG6-P2) was excluded from being analysed with gSG6 and gSG6-P1, as the two studies that reported anti-gSG6-P2 IgG seroprevalence also reported the seroprevalence of anti-gSG6-P1 IgG, and only one could be included. Potential effect modification of the associations between the exposures of interest and the anti-*Anopheles* salivary antibody responses was explored was undertaken by estimating interaction terms for DVS (*An. gambiae sensu lato* (*s.l.*) only, *An. gambiae s.l.* and other DVS, or *An. gambiae s.l.* absent) and for vector collection method (human landing catch or other indirect measures *e.g.* light traps, spray catches, etc.). For the association between *Plasmodium* spp. prevalence and gSG6 IgG seropositivity interaction terms for malaria detection methodology (light microscopy or PCR), and malarial species type (*P. falciparum* only, or *P. falciparum* and *P. vivax*) were estimated. Other variables considered for inclusion in adjusted models were study design, participant, salivary antigen construct; however, these variables showed no association with anti-gSG6 IgG and were thus excluded.

Akaike's information criterion (AIC) and Bayesian information criterion (BIC) fit indices were used to determine the best fitting functional forms for the association between log odds of gSG6 IgG seropositivity and HBR, EIR and *Plasmodium* spp. prevalence - linear, log, quadratic and cubic functions were fitted, with a log transformation exhibiting superior model fit (Appendix 1 – Table 1). To aid interpretation, we present our results as a relative increase in the odds of the gSG6 IgG seropositivity for a 2-fold (100% relative) increase in the exposures. Additional relative per cent changes in HBR and EIR are also presented.

Appendix 1 – Table 1. Model selection process, showing the log likelihood, Akaike's information criterion (AIC) and Bayesian information criterion (BIC) fit indices for each model estimating different functional forms for the association between gSG6 IgG seropositivity and respective exposures.

Model	Log likelihood	AIC	BIC
HBR			
Linear	-1533.3	3076.6	3091.2
Log	**-1492.8**	**2995.7**	**3010.1**
Quadratic	-1523.7	3059.4	3077.0
Cubic	-1523.7	3061.3	3081.9
EIR			
Linear	-1003.40	2016.80	2027.27
Log	**-530.65**	**1071.30**	**1079.49**
Quadratic	-1002.65	2017.30	2029.87
Cubic	-976.36	1966.72	1981.38
Plasmodium spp. prevalence			
Linear	-2777.45	5564.91	5582.03
Log	**-2597.24**	**5202.47**	**5215.90**
Quadratic	-2775.47	5562.95	5583.50
Cubic	-2769.91	5553.82	5577.80

Empirical Bayes best linear unbiased predictions (BLUPs) were used to estimate the probability of gSG6 IgG seropositivity in the average study and country, which is equivalent to an estimated gSG6 IgG seroprevalence. In order to maximise the number of included studies in our modelling, we predicted anti-gSG6 seroprevalence according to endemicity class, derived by applying established endemicity cut-offs to *Pf*PR_{2-10} estimates [20] extracted from MAP using site year and geolocation (if MAP data unavailable endemicity as stated in study). Intraclass correlation coefficients (ICCs) and 95% reference ranges were estimated for country-, study- and slope-specific heterogeneity (where appropriate) using estimated model variance components.

Statistical notation for the generalised linear multilevel model (mixed-effects, logistic) used to estimate the association between Anopheles gambiae gSG6 IgG seropositivity and human biting rate (HBR).

The model can be formally written as:

$$\text{logit}\{\Pr(y_{ij}=1)\,|\,x_{ij},\zeta_{1j},\zeta_{2i},\zeta_{3j}\log(HBR)_{ij}\} = \beta_1 + \beta_2\log(HBR)_{ij} + \zeta_{1j} + \zeta_{2i} + \zeta_{3i}\log(HBR)_{ij}\,, \quad (1)$$

where

$$\zeta_{1j} \sim N(0,\psi_1),\ \zeta_{2i} \sim N(0,\psi_2) \text{ and } \zeta_{3j}\log(HBR)_{ij} \sim N(0,\psi_3)\,, \quad (2)$$

Where x_{ij} is the vector of model covariates, β_1 is the model constant and represents the log odds (probability) of gSG6 IgG seropositivity for a log HBR of zero, β_2 the fixed effect for log HBR for country *j* and study *i*, ζ_{1j} the random-effect (i.e. intercept) for between-country heterogeneity in probability of gSG6 IgG seropositivity, $\zeta_{2i,}$ the random-effect (i.e. intercept) for between-study heterogeneity in probability of gSG6 IgG seropositivity, and ζ_{3i} the random-effect (i.e. coefficient) for between-study heterogeneity in the effect of log HBR.

Risk of bias in individual studies

For cross-sectional, cohort or intervention studies, selection bias was assessed by reviewing the studies' inclusion and exclusion criteria. Any case-control studies, or studies that presented salivary antibody data stratified by malaria infection status were included in narrative terms only. Risk of bias was assessed by one reviewer using the Risk of Bias in Prevalence Studies tool [24]. The risk of bias pertains to the reported observations of anti-*Anopheles* salivary antibody seroprevalence included in the multilevel modelling.

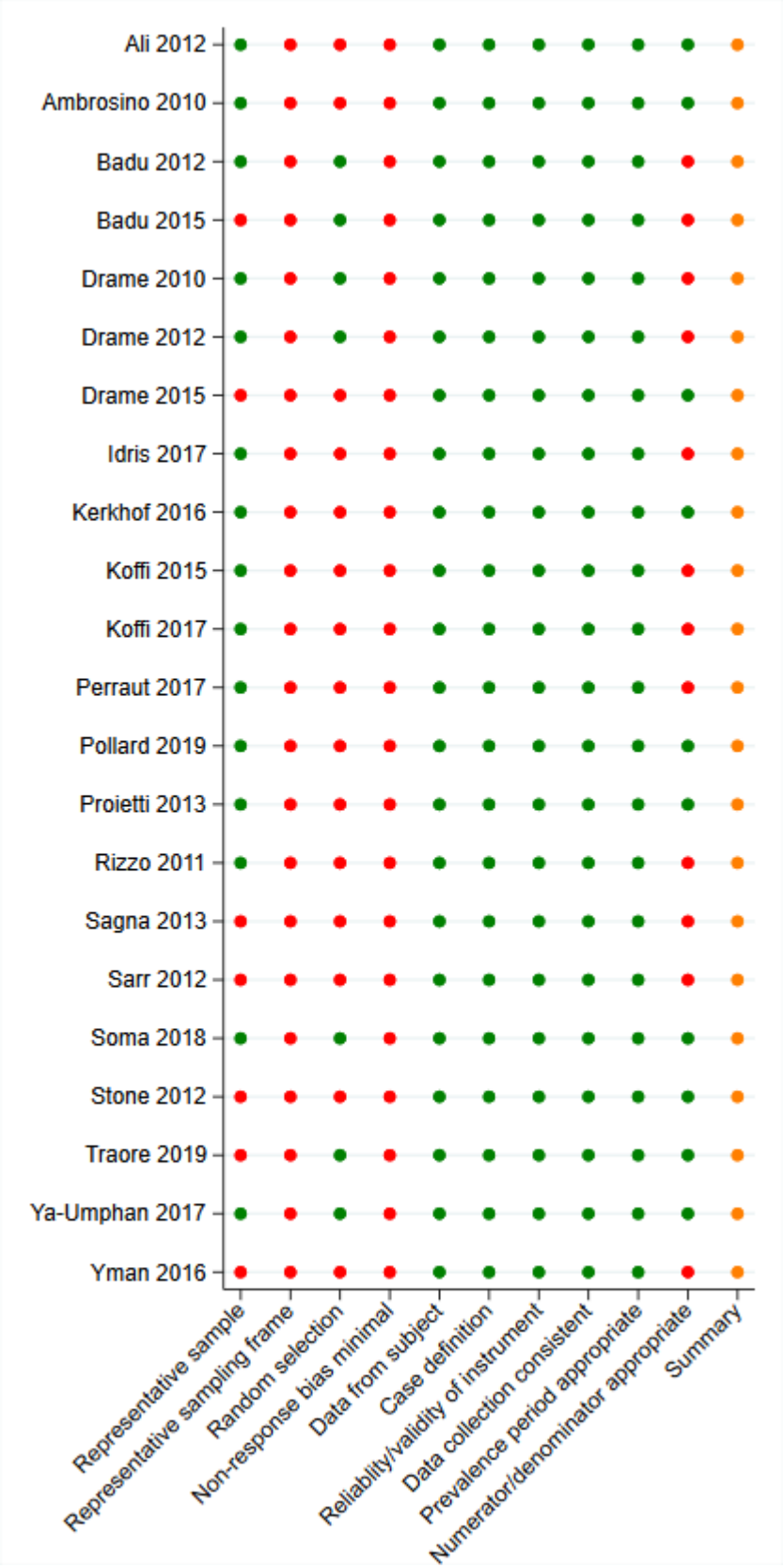
Appendix 2. Risk of Bias assessment.

Risk of bias was assessed for each study by one independent reviewer using the *Risk of Bias in Prevalence Studies* tool [24]. This tool comprises 10 items and a summary assessment to assess the external validity (selection and non-response bias) and internal validity (measurement bias) of the study's seroprevalence observations. The risk of bias pertains to the reported observations of anti-*Anopheles* salivary antibody seroprevalence included in the multilevel modelling.

With regard to external validity, seven of the studies included in the review were performed in specific populations (*i.e.* children only) that were not representative of the national population and were deemed to be at high risk of selection bias. Only 7 studies included some form of random sampling, and frequently insufficient detail was provided on the sampling frame; as such most studies were included as high risk of selection bias. Furthermore, no studies reported participant response-rate, and as such were indicated as high risk of nonresponse bias.

In terms of internal validity, all studies had an acceptable case definition, with the same mode of data collection, a valid instrument and an acceptable prevalence period, so were all deemed to be of low risk. However, 12 studies did not include a denominator, instead only reporting the study sample size and prevalence estimate, and were included as high risk.

Overall, due to the specific nature of some of the sample populations for which these prevalence observations are given (*i.e.* children only) and as participant non-response rate is not given, we conclude that there is a moderate risk of study bias. According to the *Risk of Bias in Prevalence Studies* tool [24], this implies that future research is likely to have an impact on our confidence in the prevalence observations.



Appendix 2 - Figure 1: Risk of Bias assessment. Red – high risk, orange – moderate risk, green – low risk.

Appendix 3. Reasons for study exclusion.

Appendix 3 - Table 1: Reasons for study exclusion

Studies	Reason	References
30	Does not measure anti-salivary antibody responses in individuals/populations	[57, 71-99]
28	Review article	[100-127]
20	*Anopheles* salivary antigens not assessed	[128-147]
10	Wrong antibody detection methodologies	[148-157]
7	Grey literature	[158-164]
6	Not performed in humans	[165-170]
4	Data already captured by our review from another publication	[171-174]
3	Unable to determine appropriate exposure estimate	[4, 14, 175]
3	Not in population with natural exposure	[176-178]
1	Hypothesis study	[179]
1	Pooled sera	[180]
1	Does not provide estimate of seroprevalence/total levels of antibodies against salivary proteins	[181]
1	Study population not representative: Mosquito allergic patients	[182]
1	Study population not representative: Soldiers with transient exposure	[183]

Appendix 4. Association between gSG6 IgG seropositivity and human biting rate

Appendix 4 – Table 1: Unadjusted association between gSG6 IgG seropositivity and log Human Biting Rate (HBR).

Variable	*Log Odds Ratio (SE)*	*95% CI*	*p-value*	*RE*
Fixed part				
log HBR	0.29 (0.08)	0.14, 0.45	<0.001	
Random part				
ψ_1[c]				1.29
ψ_2				1.55
ψ_3				0.06
ρ_1[d]				0.21
ρ_2[e]				0.47
ℓ				-1492.8
Model fit indices				
AIC				2995.7
BIC				3010.1

Human biting rate (HBR) association: log odds ratio and standard error (SE), 95% confidence interval (95%CI), p-value, random-effect components (RE): variances (ψ), conditional intraclass correlation coefficient (ρ)[a] and model log likelihood (ℓ) from generalised linear multilevel modelling (mixed-effects, logistic).[b] This analysis is based upon n=132 study-specific observations from 12 studies. Of note, 5 studies that measured HBR and IgG antibodies to gSG6 were excluded from this analysis as they only reported gSG6 IgG levels.

[a] $\rho = \frac{\psi_k + \ldots + \psi_{nk}}{\psi_k + \ldots + \psi_{nk} + \pi^2/3}$, where ψ_k through ψ_{nk} are random-effect variance estimates pertaining to each of the respective variance components (see table notes [c-e]) from the generalised linear multilevel modelling (mixed-effects, logistic) for a specific ICC estimate.

[b] Generalised linear multilevel modelling (mixed-effects, logistic) estimating the association between log transformed HBR and anti-gSG6 IgG seropositivity with random-effects for country-specific and study-specific heterogeneity in gSG6 IgG seroprevalence and study-specific heterogeneity in effect of HBR.

[c] ψ_1, ψ_2 and ψ_3 represent variances of the random-effects for country, study and effect of HBR respectively.

[d] ρ_1 represents conditional ICC for salivary antibody observations from the same country but different study.

[e] ρ_2 represents conditional ICC for salivary antibody observations from the same country and study with the median HBR

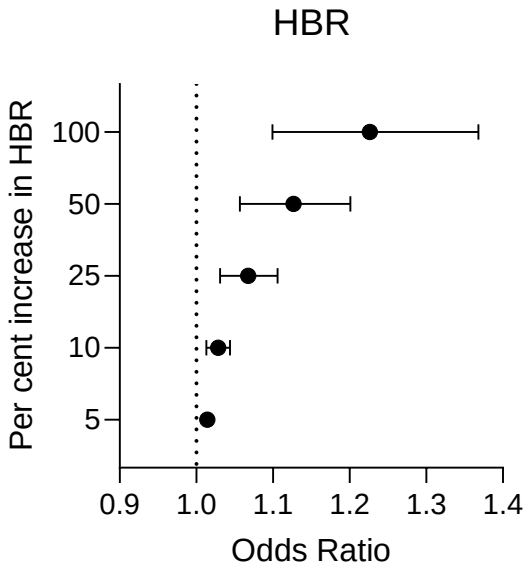


Figure 2 – Supplement 1. Estimated relative change in odds of anti-gSG6 IgG seropositivity (95% confidence interval) for given relative per cent increases in HBR (bites/person/night).

HBR has been log transformed to account for the non-linear relationship between HBR and log odds of gSG6 IgG seropositivity, where a 100% relative increase in HBR corresponds to a 2-fold increase in HBR. Estimated using generalised linear multilevel modelling (mixed-effects, logistic) of the association between anti-gSG6 IgG seropositivity and log HBR, with random-effects for country-specific and study-specific heterogeneity in gSG6 IgG seroprevalence and study-specific heterogeneity in effect of HBR (see Appendix 4).

Appendix 6. Association between gSG6 IgG seropositivity and entomological inoculation rate

Appendix 6 – Table 1: Unadjusted association between gSG6 IgG seropositivity log Entomological Inoculation Rate (EIR).

Variable	*Log Odds Ratio (SE)*	*95% CI*	*p-value*	*RE*
Fixed part				
log EIR	0.15 (0.04)	0.07, 0.23	<0.001	
Random part				
ψ_1 [c]				1.02
ψ_2				2.15
ψ_3				0.01
ρ_1 [d]				0.16
ρ_2 [e]				0.49
ℓ				-530.7
Model fit indices				
AIC				1071.3
BIC				1079.5

Entomological inoculation rate (EIR) association: log odds ratio and standard error (SE), 95% confidence interval (95%CI), p-value, random-effect components (RE): variances (ψ), conditional intraclass correlation coefficient (ρ)[a] and model log likelihood (ℓ) from generalised linear multilevel modelling (mixed-effects, logistic).[b] This analysis is based upon n=38 study-specific observations from 8 studies.

[a] $\rho = \frac{\psi_k + \ldots + \psi_{nk}}{\psi_k + \ldots + \psi_{nk} + \pi^2/3}$, where ψ_k through ψ_{nk} are random-effect variance estimates pertaining to each of the respective variance components (see table notes [c-e]) from the generalised linear multilevel (mixed-effects, logistic) modelling for a specific ICC estimate.

[b] Generalised linear multilevel modelling (mixed-effects, logistic) estimating the association between log transformed EIR and anti-gSG6 IgG seropositivity with random-effects for country-specific and study-specific heterogeneity in gSG6 IgG seroprevalence and study-specific heterogeneity in effect of EIR.

[c] ψ_1, ψ_2 and ψ_3 represent variances of the random-effects for country, study and effect of EIR respectively.

[d] ρ_1 represents the conditional ICC for salivary antibody observations from the same country but different study .

[e] ρ_2 represents the conditional ICC for salivary antibody observations from the same country and study with the median EIR

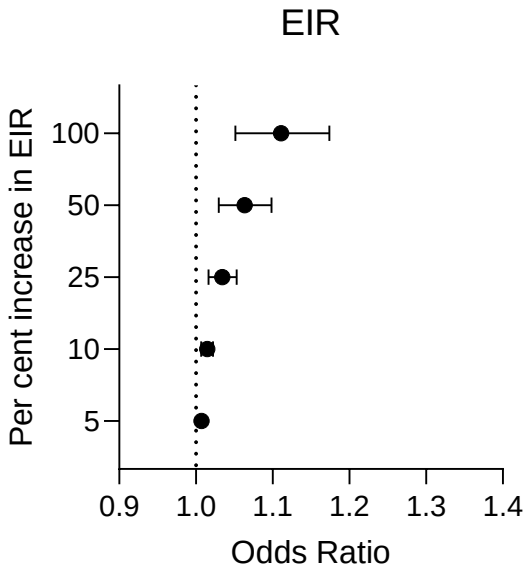


Figure 4 – Supplement 1. Estimated change in odds of anti-gSG6 IgG seropositivity (95% confidence interval) for given relative per cent increases in EIR (infective bites/person/night). EIR has been log transformed to account for the non-linear relationship between EIR and log odds of gSG6 IgG seropositivity, where a 100% relative increase in EIR corresponds to a 2-fold increase in EIR. Estimated using generalised linear multilevel modelling (mixed-effects, logistic) of the association between anti-gSG6 IgG seropositivity and log EIR, with random-effects for country-specific and study-specific heterogeneity in gSG6 IgG seroprevalence and study-specific heterogeneity in effect of EIR (see Appendix 6).

Appendix 5. Association between gSG6 IgG seropositivity and Human Biting Rate (HBR), moderated by dominant vector species

Appendix 5 – Table 1: Association between gSG6 IgG seropositivity and log Human Biting Rate (HBR), moderated by dominant vector species

Variable	*Log Odds Ratio (SE)*	*95% CI*	*p-value*	*RE*
Fixed part				
log HBR	0.46 (0.11)	0.25, 0.66	<0.001	
DVS			<0.001*	
An. gambiae s.l. only	Ref.			
An. gambiae s.l. & other DVS	1.00 (0.18)	0.65, 1.25	<0.001	
Non-*An. gambiae s.l.*	1.09 (0.68)	-0.24, 2.42	0.109	
log HBR by DVS			<0.001*	
An. gambiae s.l. only	Ref.			
An. gambiae s.l. & other DVS	-0.26 (0.08)	-0.41, -0.11	0.001	
Non-*An. gambiae s.l.*	-0.38 (0.11)	-0.59, -0.17	<0.001	
Random part				
ψ_1 [c]				0.96
ψ_2				2.32
ψ_3				0.08
ρ_1 [d]				0.14
ρ_2 [e]				0.51
ℓ				-1488.8
Model fit indices				
AIC				2995.5
BIC				3021.5

Human biting rate (HBR) X dominant vector species (DVS) association: log odds ratio and standard error (SE), 95% confidence interval (95%CI), p-value, random-effect components (RE): variances (ψ), conditional intraclass correlation coefficient (ρ)[a] and model log likelihood (ℓ) from generalised linear multilevel modelling (mixed-effects, logistic).[b] *indicates p-value from joint Wald test for polytomous variables. This analysis is based upon n=132 study-specific observations from 12 studies. Of note, 5 studies that measured HBR and IgG antibodies to gSG6 were excluded from this analyses as they only reported gSG6 IgG levels.

[a] $\rho = \frac{\psi_k + \ldots + \psi_{nk}}{\psi_k + \ldots + \psi_{nk} + \pi^2/3}$, where ψ_k through ψ_{nk} are random-effect variance estimates pertaining to each of the respective variance components (see table notes [c-e]) from the generalised linear multilevel modelling (mixed-effects, logistic) for a specific ICC estimate.

[b] Generalised linear multilevel modelling (mixed-effects, logistic) estimating the association between log transformed HBR and anti-gSG6 IgG seropositivity including an interaction term between DVS and log HBR with random-effects for country-specific and study-specific heterogeneity in gSG6 IgG seroprevalence and study-specific heterogeneity in effect of HBR.

[c] ψ_1, ψ_2 and ψ_3 represent variances of the random-effects for country, study and effect of HBR respectively.

[d] ρ_1 represents the conditional ICC for salivary antibody observations from the same country but different study .

[e] ρ_2 represents the conditional ICC for salivary antibody observations from the same country and study with the median HBR

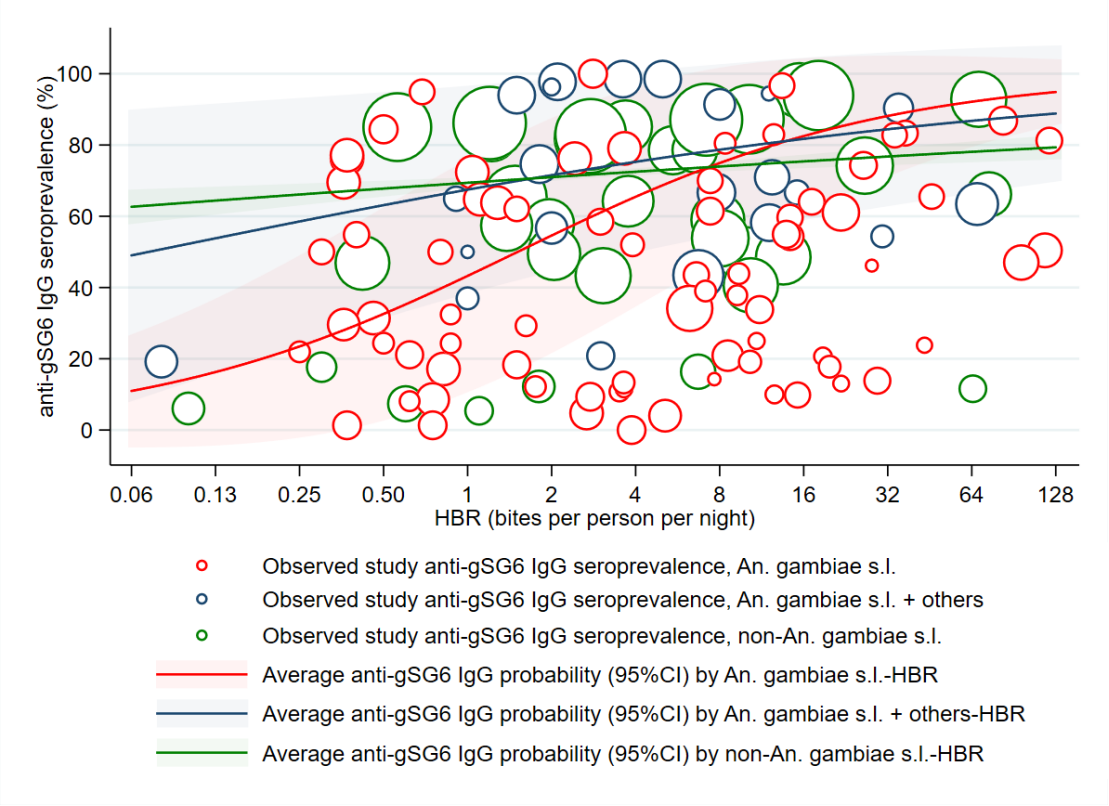


Figure 3 – Supplement 1. Association between anti-gSG6 IgG seroprevalence and *Anopheles* species-specific log_2 human biting rate (HBR).

Figure shows the observed anti-gSG6 IgG (either recombinant or peptide form) seroprevalence (%) and HBR for each study-specific observation coloured by dominant vector species (DVS), as well as the predicted average anti-gSG6 IgG seroprevalence (predicted probability for the average study and country) with 95% confidence intervals (95%CI). Coloured circles and lines denote DVS, with red indicating where *An. gambiae s.l.* is the only DVS, navy where other DVS were present in addition to *An. gambiae s.l.* and green where *An. gambiae s.l.* was absent. Circles are proportional to the size of the sample for each study-specific estimate. Association estimated using generalised linear multilevel modelling (mixed-effects, logistic) to account for the hierarchical nature of the data, where study-specific anti-gSG6 IgG observations, are nested within study, and study is nested within country.

Appendix 7. Association between gSG6 IgG seropositivity and malaria prevalence

Appendix 7 – Table 1: Unadjusted association between gSG6 IgG seropositivity and log *Plasmodium* spp. prevalence.

Variable	*Log Odds Ratio (SE)*	*95% CI*	*p-value*	*RE*
Fixed part				
log *Plasmodium* spp. prevalence	0.46 (0.32)	-0.16, 1.08	0.148	
Random part				
ψ_1[c]				17.21
ψ_2				1.25
ρ_1[d]				0.85
ℓ				-2597.2
Model fit indices				
AIC				5202.5
BIC				5215.9

Any *Plasmodium* species infections (including prevalence estimates of *P. falciparum* only, *P. vivax* only, both *P. falciparum* and *P. vivax* and un-typed infections): log odds ratio and standard error (SE), 95% confidence interval (95%CI), p-value, random-effect components (RE): variances (ψ), conditional intraclass correlation coefficient (ρ)[a] and model log likelihood (ℓ) from generalised linear multilevel modelling (mixed-effects, logistic).[b] This analysis is based upon n=212 study-specific observations from 14 studies. Of note, 6 studies that measured *Plasmodium* spp. prevalence and IgG antibodies to gSG6 were excluded from this analysis as 5 only reported gSG6 IgG levels and 1 was a case control study.

[a] $\rho = \frac{\psi_k + \ldots + \psi_{nk}}{\psi_k + \ldots + \psi_{nk} + \pi^2/3}$, where ψ_k through ψ_{nk} are random-effect variance estimates pertaining to each of the respective variance components (see table notes [c-d]) from the generalised linear multilevel modelling (mixed-effects, logistic)for a specific ICC estimate.

[b] Generalised linear multilevel modelling (mixed-effects, logistic) estimating the association between the log prevalence of any *Plasmodium* spp. infection and anti-gSG6 IgG seropositivity with random-effects for study-specific heterogeneity in gSG6 IgG seroprevalence and study-specific heterogeneity in effect of *Plasmodium* spp. prevalence.

[c] ψ_1 and ψ_2 represent variances of the random-effects for study and effect of *Plasmodium* spp. prevalence respectively.

[d] ρ_1 represents the conditional ICC for salivary antibody observations from the same study and with the median *Plasmodium* spp. prevalence.

Appendix 8. Association between gSG6 IgG seropositivity and antimalarial antibody seroprevalence

Antibodies against *P. falciparum* pre-erythrocytic stage antigens

The pooled analysis of 159 study-specific observations from eight studies showed that a 2-fold increase in PfCSP IgG seropositivity was associated with a 2.19-fold (OR: 2.19; 95%CI: 1.18-4.04, p=0.013) increase in odds of anti-gSG6 IgG seropositivity [10, 13, 32, 33, 42, 43, 49, 53]. Furthermore we observed that gSG6 IgG levels increased with increasing PfCSP IgG seroprevalence in four studies [13, 32, 33, 53], with another study contributing only a single estimate [10].

Antibodies against *P. falciparum* blood stage antigens

Furthermore, we observed a 2-fold increase PfAMA1 IgG seroprevalence was associated with a 2.47-fold (OR: 2.47; 95%CI: 2.25-2.71; p<0.001) increase in odds of gSG6 IgG seropositivity, based upon 62 study-specific observations from eight studies [10, 13, 15, 32, 33, 43, 48, 49]. A similar association was observed for $PfMSP1_{19}$ IgG, with 2-fold increase in seroprevalence associated with 2.49-fold (OR: 2.49; 95%CI: 1.21-5.12; p=0.014) increase in odds of gSG6 IgG seropositivity. This association was derived from 163 study-specific observations from ten studies [10, 13, 15, 32, 33, 37, 43, 48, 53]. Analysis of 47 study-specific observations from three studies indicated that a 2-fold increase in PfMSP2 IgG seroprevalence was associated with a 41% (OR: 1.41; 95%CI: 1.21-1.65; p<0.001) increase in odds of gSG6 IgG seropositivity [13, 43, 48]. While 17 study-specific observations from two studies showed a 2-fold increase in PfMSP3 IgG seroprevalence was associated with a 2.66-fold (OR: 2.66; 95%CI: 2.36-3.00; p<0.001) increase in odds of gSG6 IgG seropositivity [10, 48].

The pooled analysis of 128 study-specific observations from five studies showed that a 2-fold increase in PfGLURP IgG seroprevalence was associated with a 3.05-fold (OR: 3.05; 95%CI: 2.58-3.61; p<0.001) increase in odds of gSG6 IgG seropositivity [32, 33, 42, 43, 53]. And 18 study-specific observations from five studies indicated that 2-fold increase in *P. falciparum* schizont extract IgG seropositivity was associated with a 5.69-fold (OR: 5.69; 95%CI: 0.03-1188.69; p=0.523) increase in odds of gSG6 IgG seropositivity [15, 32, 33, 40, 43].

We observed that increasing seroprevalence of IgG antibodies against PfAMA1 saw increased levels of anti-gSG6 IgG in three studies [15, 32, 33], but no association in another [13]. The levels of gSG6 IgG increased with increasing $PfMSP1_{19}$ IgG seroprevalence in three studies [15, 32, 37], but showed no association in three other studies [13, 33, 53]. No association between gSG6 IgG levels and MSP2 IgG seroprevalence was observed

in one study [13]. PfGLURP IgG seroprevalence and gSG6 IgG antibody levels were reported in three studies, with one study reporting increased levels [32] , one study reporting no association [53], and one study reporting decreased levels of anti-gSG6 IgG with increasing anti-PfGLURP seroprevalence [33]. One study showed increasing gSG6 IgG levels with increasing *P. falciparum* schizont extract IgG, while three other studies showed no association [32, 33, 40]. Of note, one study provided a single seroprevalence estimate of antibodies against PfAMA1, $PfMSP1_{19}$ and PfMSP3 so no relationships can be drawn [10].

Antibodies against *P. vivax* antigens

In pooled analyses of 115 study-specific observations from two studies [15, 53], we observed that 2-fold increase in the seroprevalence of PvAMA1 was associated with a 3.87-fold (OR: 3.87; 95%CI: 3.46-4.32; $p<0.001$) increase in the odds of anti-gSG6 IgG seropositivity. Furthermore, in 103 study-specific observations from two studies [15, 53], 2-fold increase in $PvMSP1_{19}$ IgG seroprevalence was associated with a 2.37-fold (OR: 2.37; 95%CI: 2.26-2.50; $p<0.001$) increase in the odds of anti-gSG6 IgG seropositivity. However, neither study showed an association between the levels of gSG6 IgG and the seroprevalence of PvAMA1 and $PvMSP1_{19}$ IgG [15, 53].

Appendix 8 – Table 1: Associations between anti-gSG6 IgG seropositivity and log of antimalarial antibody seroprevalence.

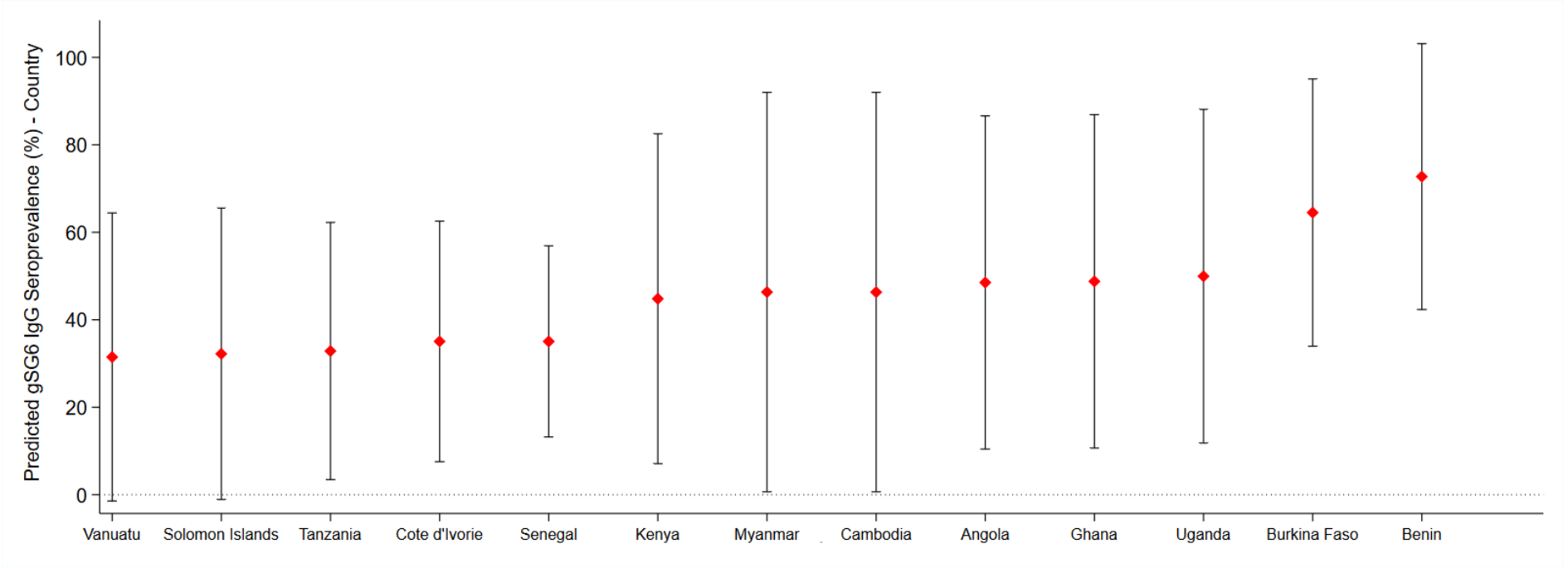
Exposure	*Log Odds Ratio (SE)*	*95%CI*	*p- value*	*Study-specific n*	*Studies*	*References*
Pre-erythrocytic antigens						
log PfCSP IgG Seroprevalence (%)	1.13 (0.45)	0.24, 2.01	0.013	159	8	[10, 13, 32, 33, 42, 43, 49, 53]
Blood stage antigens						
log PfAMA1 IgG Seroprevalence (%)[a]	1.30 (0.07)	1.17, 1.44	<0.001	62	8	[10, 13, 15, 32, 33, 43, 48, 49]
log PfMSP1$_{19}$ IgG Seroprevalence (%)	1.31 (0.53)	0.27, 2.36	0.014	163	10	[10, 13, 15, 32, 33, 37, 43, 48, 53]
log PfMSP2 IgG Seroprevalence (%)	0.50 (0.11)	0.27, 0.72	<0.001	47	3	[13, 43, 48]
log PfMSP3 IgG Seroprevalence (%)[a]	1.41 (0.09)	1.24, 1.58	<0.001	17	2	[10, 48]
log PfGLURP IgG Seroprevalence (%)	1.61 (0.12)	1.37, 1.85	<0.001	128	5	[32, 33, 42, 43, 53]
log PfSchizont Extract IgG Seroprevalence (%)	2.51 (3.93)	-5.20, 10.22	0.523	18	5	[15, 32, 33, 40, 43]
log PvAMA1 IgG Seroprevalence (%)	1.95 (0.08)	1.79, 2.11	<0.001	115	2	[15, 53]
log PvMSP1$_{19}$ IgG Seroprevalence (%)	1.25 (0.04)	1.17, 1.32	<0.001	103	2	[15, 53]

Effects for each exposure represent separate generalised linear multilevel modelling (mixed-effects, logistic) analyses estimating the association between the log of the seroprevalence of antimalarial antibodies and the seroprevalence of anti-gSG6 IgG, with the inclusion of a random intercept for study-specific heterogeneity and a random coefficient to allow the effect of the antimalarial antigen to vary across studies. Table shows log odds ratio and standard error (SE), 95% confidence interval (95%CI) and p-value, number of study-specific salivary antibody observations (Study-specific n) and studies, with associated references. Random effects not shown. Of note, one 1 study that measured antimalarial antibody seroprevalence and IgG antibodies to gSG6 could not be included in analyses as they only reported gSG6 IgG levels.

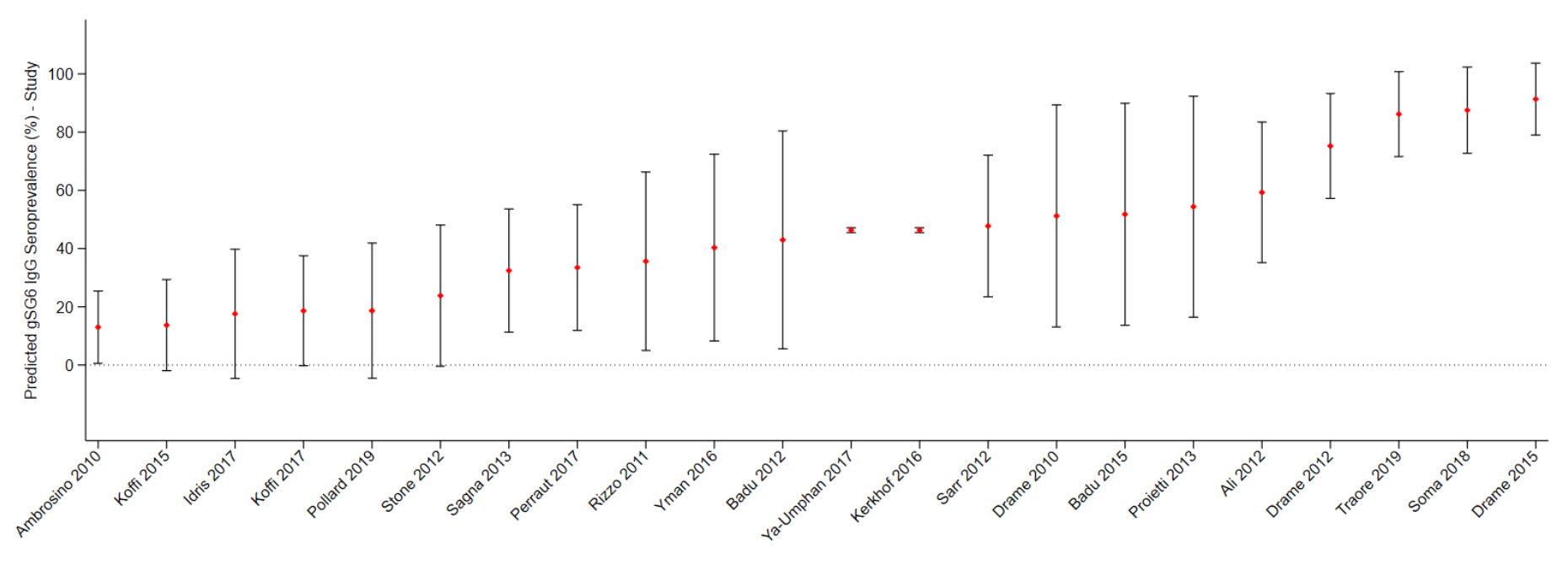
[a] Studies did not include a random coefficient (*i.e.* slope); as empirical support was not shown.

Appendix 9. Country and study-specific predicted probability of gSG6 IgG seropositivity.

In order to obtain estimates of gSG6 IgG seroprevalence for each country and study, an intercept only three-level random effects logistic regression was fitted to 301 study-specific observations from 22 studies. The predicted probability of gSG6 IgG seropositivity was calculated at the country-level (Appendix 9 – Figure 1), indicating that the seroprevalence was lowest in the Pacific Region (Vanuatu (31%) and Solomon Islands (32%)) and highest in Benin (72%) and Burkina Faso (65%). Furthermore, the predicted probability of gSG6 IgG seropositivity was calculated at the study-level (Appendix 9 – Figure 2) indicating that the seroprevalence was lowest in Ambrosino *et al.* [42] (13%) and highest in Drame *et al.* [7] (91%).



Appendix 9 – Figure 1: Predicted gSG6 IgG seroprevalence by country. Predicted probabilities of gSG6 IgG seropositivity including country-specific random effects with 95% confidence intervals. Estimated from intercept-only three-level random-effects logistic regression to account for the hierarchical nature of the data, with study-specific anti-gSG6 IgG observation nested within study nested within country. Based upon n=301 study-specific observations from 22 studies. Of note, 9 studies that measured IgG antibodies to gSG6 were excluded from this analysis as 8 only reported gSG6 IgG levels and 1 was a case control study.



Appendix 9 – Figure 2: Predicted gSG6 IgG seroprevalence by study. Predicted probabilities of gSG6 IgG seropositivity including study-specific random effects with 95% confidence intervals. Estimated from intercept-only three-level random-effects logistic regression to account for the hierarchical nature of the data, with study-specific anti-gSG6 IgG observation nested within study nested within country. Based upon n=301 study-specific observations from 22 studies. Of note, 9 studies that measured IgG antibodies to gSG6 were excluded from this analysis as 8 only reported gSG6 IgG levels and 1 was a case control study.

Appendix 10. Association between alternative salivary biomarkers and exposures of interest.

Our systematic review identified a paucity of studies that assessed the relationship between our exposures of interest and most alternate *Anopheles* salivary biomarkers (that is non-*An. gambiae* gSG6 IgG), thus preventing the estimation of a pooled association. The exceptions being that we observed that a 2-fold increase in HBR was associated with a 12% increase (OR: 1.12; 95%CI: 1.02-1.24; p=0.017) in odds of anti-*An. funestus* fSG6 IgG seropositivity (six study-specific observations from two studies [8, 41]; Appendix 10 – Table 1), as well as a 12.97-fold (OR: 12.97; 95%CI: 10.95-15.36; p<0.001) and 4.04-fold (OR: 4.04; 95%CI: 3.60-4.54; p<0.001) increase in odds of anti-gSG6-P2 IgG seropositivity associated with 2-fold increase in seroprevalence of PfCSP and PfGLURP IgG, respectively (115 and 116 study-specific observations from two studies respectively [42, 53], Table 2-3). The associations between exposures of interest and the additional salivary biomarkers are further discussed in narrative terms in below.

Human biting rate

In addition to the increased odds of *An. funestus* fSG6 seropositivity with increasing HBR, the majority of studies reported a positive association between HBR and the seroprevalence and levels of anti-gSG6-P1 IgM [7], the levels of gSG6-P2 IgG [44], the seroprevalence and levels of anti-cE5 IgG [26], the levels of anti-fSG6 IgG [8, 41], the seroprevalence and levels of anti-f5'nuc IgG [41] and the median levels of anti-*An. gambiae* salivary gland extracts (SGE) SGE IgG and IgG4 [6, 25, 46]. One study reported similar median levels of anti-gSG6 IgG1 across populations and time points, whilst reporting that anti-gSG6 IgG4 titre increased with increasing HBR in one of the populations, but not in the other [27]. Similarly, there was no consistent association between HBR and the levels of anti-cE5 IgG [30], levels of anti-*An. gambiae* SGE IgE [25] and the seroprevalence and levels of anti-g5'nuc IgG [41].

Entomological inoculation rate

Ali *et al*. [41] reported higher seroprevalence and levels anti-fSG6 IgG and anti-f5'nuc IgG with increasing EIR, while anti-g5'nuc IgG seroprevalence and levels were not associated with EIR. An additional study reported gSG6-P2 IgG seroprevalence estimates of 0% for three sites, irrespective of EIR [42].

Malaria prevalence

Two studies showed that increased *Plasmodium* spp. prevalence was associated with higher median levels of anti-*An. gambiae* SGE IgG [6, 29], while another study showed different anti-*An. gambiae* SGE IgG levels for

very similar prevalence of malaria and slightly lower levels of anti-*An. gambiae* SGE IgE and IgG4 for the time point with greater malaria prevalence [25]. Kerkhof *et al.* [53] showed increasing levels of anti-gSG6-P2 IgG for higher prevalence of any *Plasmodium* spp. infection, while Londono-Renteria *et al.* [51] showed lower levels of IgG antibodies against TRANS-P1, TRANS-P2, PEROX-P1, PEROX-P2 and PEROX-P3 in the site with higher PCR confirmed malaria prevalence. Additionally, several case-controlled studies, and two cross-sectional study, reported median antibody levels stratified by malaria infection status. These studies show higher levels of anti-*An. darlingi* SGE IgG [50], anti-*An. gambiae* SGE IgG [46], anti-*An. dirus* SGE IgG and IgM [28], and IgG antibodies against SGEs of two Colombian strains of *An. albimanus* in *Plasmodium* spp. infected individuals, compared to non-infected [52]. While Montiel *et al.* [52] observed no association between anti-*An. darlingi* SGE IgG levels and infection status.

Antimalarial antibody seroprevalence

Our multilevel modelling indicated that there were 12.97-fold (OR: 12.97; 95%CI: 10.95-15.36; $p<0.001$) and 4.04-fold (OR: 4.04; 95%CI: 3.60-4.54; $p<0.001$) increase in odds of anti-gSG6-P2 IgG seropositivity associated with a 2-fold increase in the seroprevalence of PfCSP and PfGLURP IgG, respectively [42, 53] (Appendix 10 – Tables 2 and 3). However, we observed weak positive associations between the levels of IgG antibodies against gSG6-P2 peptide and the seroprevalence of IgG antibodies against $PfMSP1_{19}$, PfGLURP and $PvMSP1_{19}$, but no association with PfCSP or PvAMA1 [53].

Appendix 10 –Table 1: Association between fSG6 IgG seropositivity and human biting rate

Variable	*Log Odds Ratio (SE)*	*95% CI*	*p-value*	*RE*
Fixed part				
log HBR	0.17 (0.07)	0.03, 0.31	0.017	
Random part				
ψ_1 [c]				0.47
ρ_1 [d]				0.13

Association between human biting rate (HBR) and fSG6 IgG: log odds ratio and standard error (SE), 95% confidence interval (95%CI), p-value, random-effect components (RE): variances (ψ), conditional intraclass correlation coefficient (ρ)[a] and model log likelihood (ℓ) from generalised linear multilevel modelling (mixed-effects, logistic).[b] This analysis is based upon n=6 study-specific observations.

[a] $\rho = \frac{\psi_k + \ldots + \psi_{nk}}{\psi_k + \ldots + \psi_{nk} + \pi^2/3}$, where ψ_k through ψ_{nk} are random-effect variance estimates pertaining to each of the respective variance components (see table notes [c-d]) from generalised linear multilevel model (mixed-effects, logistic) for a specific ICC estimate.

[b] Generalised linear multilevel modelling (mixed-effects, logistic) estimating the association between anti-*An. funestus* fSG6 IgG seropositivity and log transformed HBR with random-effects for study-specific heterogeneity in fSG6 IgG seropositivity.

[c] ψ_1 represents variance of the random-effect for study.

[d] ρ_1 represents conditional ICC for salivary antibody observations from the same study.

Appendix 10 – Table 2: Association between gSG6-P2 IgG seropositivity and log PfCSP IgG seroprevalence

Variable	*Log Odds Ratio (SE)*	*95% CI*	*p-value*	*RE*
Fixed part				
log PfCSP IgG Seroprevalence	3.70 (0.12)	3.45, 3.94	<0.001	
Random part				
ψ_1 [c]				25.2
ρ_1 [d]				0.88

Association between log PfCSP seroprevalence and gSG6-P2 IgG: log odds ratio and standard error (SE), 95% confidence interval (95%CI), p-value, random-effect variances (ψ), conditional intraclass correlation coefficient (ρ)[a] and model log likelihood (ℓ) from logistic mixed-effects modelling.[b] This analysis is based upon n=115 study-specific observations.

[a] $\rho = \frac{\psi_k + \ldots + \psi_{nk}}{\psi_k + \ldots + \psi_{nk} + \pi^2/3}$, where ψ_k through ψ_{nk} are random-effect (RE) variance estimates pertaining to each of the respective variance components (see table notes [c-d]) from generalised linear multilevel model (mixed-effects, logistic) for a specific ICC estimate.

[b] Generalised linear multilevel modelling (mixed-effects, logistic) estimating the association between log PfCSP seroprevalence and anti-gSG6-P2 IgG seropositivity with random-effects for study-specific heterogeneity in gSG6-P2 IgG seropositivity.

[c] ψ_1 represents variance of the random-effect for study.

[d] ρ_1 represents conditional ICC for salivary antibody observations from the same study.

Appendix 10 – Table 3: Association between gSG6-P2 IgG seropositivity and log PfGLURP IgG seroprevalence

Variable	*Log Odds Ratio (SE)*	*95% CI*	*p-value*	*RE*
Fixed part				
log PfGLURP IgG Seroprevalence	2.01 (0.09)	1.85, 2.18	<0.001	
Random part				
ψ_1 [c]				24.3
ρ_1 [d]				0.88

Association between log PfGLURP seroprevalence and gSG6-P2 IgG: log odds ratio and standard error (SE), 95% confidence interval (95%CI), p-value, random-effect variances (ψ), conditional intraclass correlation coefficient (ρ)[a] and model log likelihood (ℓ) from logistic mixed-effects modelling.[b] This analysis is based upon n=116 study-specific observations.

[a] $\rho = \frac{\psi_k + \ldots + \psi_{nk}}{\psi_k + \ldots + \psi_{nk} + \pi^2/3}$, where ψ_k through ψ_{nk} are random-effect (RE) variance estimates pertaining to each of the respective variance components (see table notes [c-d]) from generalised linear multilevel model (mixed-effects, logistic) for a specific ICC estimate.

[b] Generalised linear multilevel modelling (mixed-effects, logistic) estimating the association between log PfGLURP seroprevalence and anti-gSG6-P2 IgG seropositivity with random-effects for study-specific heterogeneity in gSG6-P2 IgG seropositivity.

[c] ψ_1 represents variance of the random-effect for study.

[d] ρ_1 represents conditional ICC for salivary antibody observations from the same study.