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1 **Demographic patterns of human antibody levels to *Simulium damnosum* s.l.**

2 **saliva in onchocerciasis-endemic areas: an indicator of exposure to vector**

3 **bites**

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21

Abstract

Background: In onchocerciasis endemic areas in Africa, heterogenous biting rates by blackfly vectors on humans are assumed to partially explain age- and sex-dependent infection patterns with *Onchocerca volvulus*. To underpin these assumptions and further improve predictions made by onchocerciasis transmission models, demographic patterns in antibody responses to salivary antigens of *Simulium damnosum* s.l. are evaluated as a measure of blackfly exposure.

Methodology/Principal Findings: Recently developed IgG and IgM anti-saliva immunoassays for *S. damnosum* s.l. were applied to blood samples collected from residents in four onchocerciasis endemic villages in Ghana. Demographic patterns in antibody levels according to village, sex and age were explored by fitting generalized linear models. Antibody levels varied between villages but showed consistent patterns with age and sex. Both IgG and IgM responses declined with increasing age. IgG responses were generally lower in males than in females and exhibited a steeper decline in adult males than in adult females. No sex-specific difference was observed in IgM responses.

Conclusions/Significance: The decline in age-specific antibody patterns suggested development of immunotolerance or desensitization to blackfly saliva antigen in response to persistent exposure. The variation between sexes, and between adults and youngsters may reflect differences in behaviour influencing cumulative exposure. These measures of antibody acquisition and decay could be incorporated into onchocerciasis transmission models towards informing onchocerciasis control, elimination, and surveillance.

Author summary

Onchocerciasis, a disease caused by the helminth parasite *Onchocerca volvulus*, is transmitted by the bites of female *Simulium* blackflies. The disease is still endemic in many African countries, and the World Health Organization has proposed elimination of its transmission in

12 countries by 2030. Understanding the heterogeneity in human exposure to vector bites can help discern which portion of the population is at higher risk of acquiring/ transmitting infection and is fundamental to identifying target groups for serological monitoring and transmission control. Traditionally, blackfly biting rates are estimated by performing human landing catches, a method that is often considered unethical and which can be unreliable as a representative measure. Therefore, we used our recently developed immunoassays to measure human antibody responses to antigens contained in the saliva of blackflies and deposited into human skin when they bloodfeed. In onchocerciasis endemic communities in Ghana, we measured antibody responses to understand age- and/or sex-related demographic patterns in vector exposure. We observed lower antibody responses in males compared to in females, and a substantial decline with increasing age, suggesting that high blackfly biting pressure induces desensitization in the human host.

Introduction

Female blackflies of the *Simulium damnosum* sensu lato (s.l.) complex are the predominant vectors of *Onchocerca volvulus* in Africa. This filarial nematode causes human onchocerciasis, commonly known as river blindness and proposed for elimination of transmission (EOT) in 12 countries by 2030 [1]. Repeat exposure to bites of infective blackflies is a key driver of parasite acquisition, and high biting rates are important determinants of transmission intensity and resurgence following control interventions, particularly those based on mass drug administration (MDA) of ivermectin [2,3]. Therefore, 'stop-MDA surveys' and post-treatment surveillance protocols would be improved by monitoring exposure to vector bites in addition to exposure to the parasite and assessment of residual infection in informative age and sex groups depending on the epidemiological setting [4,5]. Annual biting rates (ABR, the number of bites/person/year) are predominantly estimated by performing human landing catches (HLCs) [6], but this is both labour-intensive

72 and often considered unethical. Furthermore, HLC methods likely lead to biased estimates of
73 ABRs as they are typically performed on a few adult males at the riverbank close to blackfly
74 breeding sites, where biting rates are likely to be higher and vector collectors are maximally
75 exposed [7]. Thus, HLCs may not capture the true exposure representative of the community,
76 age or sex groups or their activity patterns. Furthermore, when blackfly densities are high,
77 HLCs may be unable to capture all biting events thereby underestimating the biting rate [8,9].
78 Applying novel methods to measure individual and population exposure to blackfly vector
79 bites would greatly inform our understanding of exposure patterns to onchocerciasis and
80 inform mathematical transmission modelling of control and elimination [2].
81 Proteins in the saliva of blood-seeking arthropod vectors of human and veterinary diseases
82 provoke an immunomodulatory response in vertebrates following their exposure to vector
83 bites (e.g. by mosquitoes, sand flies, triatomine bugs, and tsetse flies) [10]. Measurement of
84 host anti-saliva antibodies has proven a useful surrogate marker to monitor individual host
85 biting rates [11–15], seasonal variations in exposure and infection [16,17], and to evaluate
86 vector control interventions [18–22]. Immunoassays to measure human anti-saliva antibody
87 responses to blackfly bites have not been available until recently developed by the authors,
88 specifically to measure human IgG and IgM antibody responses to saliva of *S. damnosum* s.l.
89 which is the main vector in Africa including the study sites in Ghana [23].
90 In the present study we report on the demographic patterns revealed by applying these assays
91 to community residents in the Bono East region of Ghana, a savannah setting. This area was
92 under vector control during the Onchocerciasis Control Programme in West Africa (OCP,
93 1974–2002) [24,25], and is where the first ivermectin community trials were conducted in the
94 late 1980's [26]. However, transmission persists despite many years of annual MDA, even
95 after adopting biannual MDA in 2010 [27,28]. With the risk of residual transmission leading
96 to possible resurgence of human infections if MDA were to stop [3], the area has been the

subject of comprehensive entomological [27,29–31], parasitological [32] and parasite genomic [33] research, and a source of valuable epidemiological and entomological data for parameterizing onchocerciasis transmission models [34–36]. Due to the lack of reliable tools to independently measure human exposure to vector bites, mathematical models have assumed age- and sex-dependent exposure rates based on patterns of human infection with *O. volvulus*. However, modelling outputs used to inform EOT and surveillance strategies are sensitive to such patterns [4,37]. The aim of this study is to apply our novel human IgG and IgM immunoassays as indicators of individual biting exposure, both between and within (by age and sex) onchocerciasis-endemic communities. The generated demographic patterns will help to scrutinize mathematical modelling assumptions.

Material and methods

Ethical statement

Ethical clearance was obtained from the Council for Scientific and Industrial Research (CSIR) Institutional Review Board (RPN008/CSIR-IRB/2019) in Accra, Ghana. Residents were informed of the objectives of the study and participants provided fully informed written consent; parents or guardians provided consent for <18-year-olds. At the time of the study, ivermectin treatment against onchocerciasis was administered as part of the on-going national biannual MDA programme [38]. Each participant was rewarded with a bar of washing soap, a commercial sachet of malt drink powder (Milo, Nestlé) and a can of condensed milk. Blackfly collections were carried out by local OCP vector collectors following standard HLC techniques [39]. Individual identifiers on data records were anonymized prior to analyses and data storage.

Study area

The study was conducted during the wet season in August 2019 in rural villages in an onchocerciasis-endemic area of the Pru river basin, Pru District, in the Bono East region of

122 Ghana. Fig 1 provides a map of the study area and the location of the study communities,
123 created using the 'ggplot2' and the 'rworldmap' package in R software [40,41].
124
125 **Fig 1. Map of Ghana indicating study communities in the Pru District, Bono East region.**
126
127 ## Recruitment, blood sampling and demographic information
128 Villages were selected on the criteria that (a) there was evidence of exposure to
129 *S. damnosum* s.l. vector bites [30,42], (b) they were under current ivermectin treatment as part
130 of the national onchocerciasis control programme [43], (c) the villages comprised >200
131 residents, and (d) the villages represented variable ABRs based on previous records [30,42].
132 Five villages were selected; due to the proximity of two of the villages, these were grouped
133 into a single cluster (Asubende and Senyase (ASU/SEN) resulting in four village clusters.
134 These included ASU/SEN, with high biting rates (400–850 flies/person/month), Beposo
135 [BEP] with moderate values (100–350), and Fawoman [FAW] and Ohiampe [OHI] with
136 lower biting rates (25–175 flies/person/month) [30,42].
137 To obtain blood samples from the village residents, two recruitment approaches were
138 adopted: in villages with <300 residents (ASU/SEN, FAW, and OHI) all residents were
139 invited to participate. In the larger village (BEP) an age/sex stratified sample was identified
140 based on assigning random numbers generated using R software [44], to the pages of the
141 paper-based census records compiled by the MDA programme. The households contained on
142 randomly selected pages were noted and invited to participate until reaching an estimated
143 sample of houses containing 250 individuals as required from statistical calculations (i.e.
144 ~1000 individuals in total across the four village clusters). Children younger than 4 years old
145 were not recruited.

146 A total of 958 participants were finally recruited, all tested for IgG antibodies, and a subset of
147 500 individuals tested for IgM antibodies (Table 1). For age stratification purposes, the
148 identified populations in all villages were divided into eight age categories (in years): 5–10,
149 11–20, 21–30, 31–40, 41–50, 51–60, 61–70, > 71, aiming to recruit 32 residents per age class
150 in each village, split equally between the sexes. In the case of IgM, samples were selected by
151 random number generation assigned to the ordered age-stratified full list of samples and
152 aimed to test around 40-60% of the samples tested by IgG per age class, with some variability
153 in percentage tested in the oldest age group. The numbers tested per age and sex strata per
154 village cluster are shown in Figs A and B and Table C in S1 File. The geographical
155 coordinates, elevation and number of individuals sampled and tested for each
156 immunoglobulin per village cluster are shown in Table 1.

157 **Table 1. Study communities, their geographical coordinates, elevation and numbers of**
158 **people sampled and tested for IgG and IgM per community in the Pru District, Ghana**

Community/ Cluster	Coordinates (degrees, minutes, seconds)		Elevation (masl)	No. sampled Tested for each immunoglobulin (%)	
	Long	Lat		IgG	IgM
Asubende/ Senyase [ASU/SEN]	08°01'08.8"N	00°58'52.4"W	153.3	186 100%	97 (52.2%)
Beposo [BEP]	08°00'26.7"N	000°57'40.2"W	118.0	253 100%	139 (54.9%)
Fawoman [FAW]	08°01'11.4"N	001°01'29.3"W	102.4	263 100%	124 (47.2%)
Ohiampe [OHI]	08°00'26.2"N	001°03'49.5"W	114.3	256 100%	140 (54.7%)
Total				958 100%	500 (52.2%)

159 Long: Longitude; Lat: Latitude; masl: metres above sea-level

160

161 Participants in each village/cluster were invited to the local school and assigned a
162 subject/sample identification number written on a personalized card. Two to four ml of blood
163 were collected into Ethylene Diamine Tetra Acetic acid (EDTA) tubes by venipuncture and
164 kept cool for 2–3 hours in an insulated cool box until centrifugation at 2500 rpm for 15 min to
165 separate the plasma. Samples were stored at 7° C until ELISA testing. Metadata on name, age,
166 sex, number of years of residence, house number, name of household head, and history of
167 clinical onchocerciasis were collected.

168 **Immunoassays to measure human exposure to blackfly bites**

169 Enzyme-linked immunosorbent assays (ELISA) previously developed were performed to
170 measure anti-*S. damnosum* s.l. saliva IgG and IgM human responses [23]. Briefly, host-
171 seeking *S. damnosum* s.l. females were collected following standard OCP vector collector
172 techniques in one study location (ASU), near the Pru river [39]. All collected flies were stored
173 in a cool box until dissected on the same day. The collected flies were anesthetized in a –20°
174 C freezer for 10 min after which their salivary glands were removed, and aliquots stored in
175 Tris-buffered saline (TBS) (one gland per µl TBS, pH 7.5) at –20° C until further use. Flat-
176 bottom 96-well microtiter plates (ThermoFisher Scientific) were coated with blackfly salivary
177 gland homogenate (SGH) with 0.2 µg SGH/well (for IgG ELISA) or 0.025 µg SGH/well (for
178 IgM ELISA) and incubated overnight at 4° C. The plates were blocked with 6% non-fat dried
179 milk (Bio-Rad) in phosphate-buffered saline (PBS) with 0.05% Tween 20 (PBS-Tw) and
180 incubated with sera diluted 1/100 (IgG ELISA) or 1/50 (IgM ELISA) in 2% non-fat dried
181 milk. The plates were washed and incubated with peroxidase-conjugated anti-human IgG
182 (1/1,000) or IgM antibody (1/70,000) (Sigma-Aldrich; Bethyl Laboratories, Inc). The ELISA
183 was developed using an orthophenylendiamine (OPD) solution in a phosphate-citrate buffer
184 (pH 5.5) with 0.1% hydrogen peroxide. The reaction was stopped after 5 min with 10%
185 sulfuric acid and the absorbance, optical density (OD) value was measured at 492 nm using a

186 Tecan Infinite M200 microplate reader (Schoeller). Further details of blackfly collection,
187 dissection, and the immunoassays were previously reported [23].

188 **Data standardization**

189 All plasma samples were tested in duplicate. Samples with a coefficient of variation (CoV) of
190 more than 20% were retested. Each plate included a blank control, the OD value of which was
191 subtracted from the sample OD values. A set of two positive (PC) and two negative control
192 (NC) samples were included in each plate to correct for inter-plate variability according to the
193 following formula: Standardized Optical Density (SOD) = OD_{sample}/(average OD_{PC} – average
194 OD_{NC}). Furthermore, a PC sample was titrated in duplicate on three separate plates at seven
195 serial dilutions from 1/50 to 1/3,200. The average of the three log-logit transformed standard
196 curves was used to convert and standardize sample SOD values which are reported below as
197 anti-saliva antibody arbitrary units/ml.

198 **Sample size calculation**

199 Sample sizes were calculated to achieve 90% statistical power (with type I error, α=0.05) to
200 detect a difference in mean IgG SOD antibody levels between males and females, for an
201 unpaired two-sample effect size D_{Cohen} of 0.23 and a variance in SOD of 0.16 [45]. By adding
202 15% to the calculated sample size as a correction factor for subsequent non-parametric
203 statistical testing, 460 people per sex group were required [46]. This estimate is also based on
204 the reasonable assumption that the differences in the mean antibody responses of the sexes
205 between clusters would be minimal as confirmed by subsequent analyses (test of village
206 cluster × sex interaction term: P>0.243). The equivalent statistical power to detect a
207 difference in IgM responses between sexes with an effect size D_{Cohen} of 0.3 and a variance in
208 SOD of 0.16 was 90%. Calculations were made in the R package 'power' using a two-sided t-
209 test.

210 **Statistical analyses**

211 Differences between clusters or sex categories were statistically tested by Wilcoxon rank sum
212 or Kruskal-Wallis tests with post-hoc Holm adjustment for multiple comparisons. Changes in
213 immune responses with age and/or sex were tested by fitting generalized linear models
214 (GLM) to the IgG and IgM anti-blackfly saliva antibody values, where a gamma distribution
215 and log-link function gave the best fit by log-likelihood goodness-of-fit statistics. Age, sex
216 and cluster interaction terms were tested, treating age as a continuous variable. Residence
217 duration (years) and age showed high multicollinearity; hence only age was retained in the
218 models. Correlation coefficients (r_s) between median IgG and IgM responses by age and sex
219 were estimated by the Spearman's rank method. All statistical analyses were performed in R
220 software [44], and graphical representations created using the 'ggplot2' package in R [40].

221 **Results**

222 **Univariate analyses of population antibody response distributions**

223 A total of 958 participants (186–263 per village cluster) between the ages of ≥ 5 to < 96 years
224 old were recruited and sampled for anti-saliva IgG antibodies; demographic characteristics are
225 summarized in Table 2 and in Figs A and B in S1 File. No differences were observed in the
226 age compositions between village clusters or between sexes, nor in the frequencies of
227 participants per age group (Figs A and B in S1 File). Thus, it is unlikely that the variations in
228 age-sex compositions of village clusters were a source of statistical bias.

229 Univariate analyses of demographic variables detected significant differences between the
230 median IgG responses among village clusters ($P<0.001$) (Fig 2A), and the median IgG
231 responses of males and females ($P<0.05$) (Fig 2B). Equivalent differences were not observed
232 in IgM responses (Fig 3); however, both IgG and IgM median antibody responses declined
233 with age ($P<0.001$) (Fig 2D and 3D). A breakdown of the IgG and IgM antibody distribution
234 with age per individual cluster is visualized in Figs D and E in S1 File.

235

236 **Fig 2. Human anti-blackfly saliva IgG antibody responses according to cluster, sex, and**
237 **age.** Boxplots showing the distributions of IgG responses (A) by cluster, (B) by sex, (C) by
238 age, and scatterplot (D) by age and sex (points) showing the best-fit lines (solid lines). IgG
239 levels are shown as units/ml. F: female; M: male. Village clusters: ASU/SEN: Asubende/
240 Senyase; BEP: Beposo; FAW: Fawoman; OHI: Ohiampe.

241

242 **Fig 3. Human anti-blackfly saliva IgM antibody responses according to cluster, sex and**
243 **age.** Boxplots showing the distributions of IgM responses (A) by cluster, (B) by sex, (C) by
244 age, and scatterplot (D) by age and sex (points) showing the best-fit lines (solid lines). IgM
245 levels are shown as units/ml. F: female; M: male. Village clusters: ASU/SEN: Asubende/
246 Senyase; BEP: Beposo; FAW: Fawoman; OHI: Ohiampe.

247

248 **Table 2. Summary statistics of participants and median antibody titres by immunoglobulin and village/cluster.**

Assay	Village	Participants (n)	Median age (Q1 – Q3) (in years)	Sex ratio (M:F)	Median duration of residency (Q1 – Q3) (in years)	Median antibody titre (units/ml)	Reported biting rate
IgG	**All**	958	21.0 (11.0 – 42.0)	0.84:1 (437:521)	16.0 (8.0 – 32.0)	2478.13 (1550.05 – 3413.36)	NA
	ASU/SEN	186	24.0 (14.0 – 39.0)	1:1 (93:93)	20.0 (10.0 – 35.0)	2507.34 (1699.94 - 3125.95)	High
	BEP	253	25.0 (11.0 – 46.0)	0.83:1 (115:138)	24.5 (11.0 – 46.0)	1951.63 (1269.37 – 2822.26)	Moderate
	FAW	263	22.0 (10.0 – 40.0)	0.71:1 (109:154)	13.0 (6.0 – 26.0)	2590.61 (1662.54 – 3535.01)	Low
	OHI	256	17.0 (11.0 – 40.5)	0.88:1 (120:136)	14.0 (7.0 – 24.0)	2956.20 (1703.47 – 4430.85)	Low
IgM	**All**	500	21.5 (10.0 – 42.0)	0.85:1 (230:270)	17.0 (7.0 – 35.0)	3628.43 (1687.59 – 9923.29)	NA
	ASU/SEN	97	23.0 (12.25 – 36.75)	1.37:1 (56:41)	21.0 (9.25 – 35.75)	2956.66 (1496.31 – 7690.82)	High
	BEP	137	24.0 (10.0 – 45.0)	0.72:1 (58:81)	24.0 (10.0 – 45.0)	4021.58 (1462.01 – 10396.13)	Moderate
	FAW	124	23.0 (9.0 – 40.0)	0.77:1 (54:70)	11.0 (6.0 – 29.0)	4102.16 (2148.38 – 10473.40)	Low
	OHI	140	17.5 (9.0 – 42.0)	0.79:1 (62:78)	15.0 (7.0 – 25.0)	3569.30 (1561.18 – 10754.69)	Low

249

250 The interquartile range (Q1 and Q3) of age, duration of residency, and the antibody titres are shown in brackets. Reported biting rates are
251 according to [30,42]. All: All clusters together; village clusters: ASU/SEN: Asubende/Senyase; BEP: Beposo; FAW: Fawoman; OHI: Ohiampe.
252 n: number; M: males; F: females; NA: not applicable

253 **Multivariate analyses of IgG responses according to village cluster, sex and age**

254 Accounting for cluster ID, sex, age, and the age x sex interaction term, multivariate models
255 revealed that IgG responses were significantly lower in BEP than in ASU/SEN (P<0.01),
256 similar between ASU/SEN and FAW (P=0.406), and higher in OHI than in ASU/SEN
257 (P<0.001) (Figs 4A and 5). Generally, males exhibited lower IgG responses than females
258 (P<0.001) (Figs 4B and 5). Fig 5 illustrates the (exponentiated) regression coefficient
259 estimates of the final model (summarized in Table F in S1 File).

260

261 **Fig 4. Regression effect plots for all explanatory variables contained in the final model**
262 **for the IgG responses.** Each plot visualizes the effect of a specific explanatory variable while
263 fixing the others to their reference level. The panels show the variations in responses between
264 village clusters (A), sex (B), age (C), and an interaction between age and sex (D). The vertical
265 lines in panel A and B and the shaded regions around the lines in panel C and D represent the
266 95% confidence intervals. M = Male; F = Female. Village clusters: ASU/SEN:
267 Asubende/Senyase; BEP: Beposo; FAW: Fawoman; OHI: Ohiampe.

268

269 **Fig 5. Exponentiated regression coefficient estimates for the final model per assay.** The
270 regression coefficient estimates for the final model are shown for IgG responses (A), and IgM
271 responses (B). The circles represent the exponentiated mean coefficient estimate, the value
272 shown above the corresponding circle, and the horizontal lines represent the 95% confidence
273 intervals of the estimate. Significance levels *** P<0.001; ** P<0.01; * P<0.05. The
274 referents were Asubende/Senyase for village clusters, and females for both sex and the age ×
275 sex interaction term. Village clusters: BEP: Beposo; FAW: Fawoman, OHI: Ohiampe.

276

277 IgG anti-saliva antibody responses decreased with increasing age in both sexes ($P<0.001$)
278 (Figs 4C and 5), but the response in males declined at a faster rate than in females (test of age
279 × sex interaction term; $P<0.001$) (Figs 4D and 5). Both adult male and female participants
280 ≥18 years old exhibited lower IgG antibody levels than those aged <18 years
281 ($P_{females}<0.001$; $P_{males}<0.001$) (Fig 5). Males and females in pre-teenage years showed a
282 similar antibody decline with increasing age (age × sex interaction: $P = 0.328$), whereas in
283 adults the decline was more rapid in males than in females (age × sex interaction: $P<0.01$)
284 (Fig 4D).
285
286 **Multivariate analyses of IgM responses according to village cluster, sex or age**
287 Equivalent multivariate analyses of anti-saliva IgM antibody responses for 500 recruits (Table
288 1) indicated no significant variation between study clusters ($P_{BEP} = 0.403$; $P_{FAW} = 0.301$; P_{OHI}
289 $= 0.632$) or sex ($P = 0.405$) (Figs 5 and 6A-B). However, there was a general decline in
290 response magnitude with increasing age ($P<0.01$) (Figs 5 and 6C), but in contrast to IgG, the
291 decline with age was not significantly different between sexes (test of age × sex interaction
292 term: $P=0.726$) (Fig 6D). Fig 5 illustrates the (exponentiated) regression coefficient estimates
293 of the final model (summarized in Table F in S1 File).
294
295 **Fig 6. Regression effect plots for all explanatory variables contained in the final model**
296 **for the IgM responses.** Each plot visualizes the effect of a specific explanatory variable
297 while fixing the others. The panels show the variations in responses between village clusters
298 (A), sex (B), age (C), and an interaction between age and sex (D). The vertical lines in panel
299 A and B and the shaded regions around the lines in panel C and D represent the 95%
300 confidence intervals. M = Male; F = Female. Village clusters: ASU/SEN: Asubende/Senyase;
301 BEP: Beposo; FAW: Fawoman; OHI: Ohiampe.

302

303 **Correlation between IgG and IgM responses**

304 The correlation between IgG and IgM antibody responses was generally low (r_s = 0.10,
305 P<0.05), and not dissimilar considering females or males alone (females: r_s = 0.12, P<0.1;
306 males: r_s = 0.08, P=0.21) (Fig F in S1 File).

307

308 ## Discussion

309 This study measured human antibody responses against blackfly saliva as an indicator of
310 individual bite exposure; an approach that has been validated for several vector–host systems
311 with the exception of blackflies [47,48]. Simuliids are important disease vectors of human and
312 bovine onchocerciasis [49], mansonelliasis caused by *Mansonella ozzardi* [50], and vesicular
313 stomatitis virus [51], yet they have not received as much attention as sand fly, mosquito or
314 tick vectors. Only recently, we successfully developed novel immunoassays against blackfly
315 saliva and this is the first study to apply these tools to an epidemiological setting. The
316 appropriate use of these assays will also improve our understanding of *O. volvulus*
317 transmission dynamics and will be used to monitor changes in biting rates and the success of
318 vector control interventions [23]. Heterogenous exposure to blackfly bites is an important
319 determinant of the observed age- and sex-dependent profiles of *O. volvulus* infection [5].
320 Evaluating age- and sex-associated exposure patterns using empirically collected data can
321 help inform transmission dynamics models, as these currently use assumed age- and sex-
322 dependent exposure patterns that have, at best, been derived from fitting the models to age-
323 and sex-specific infection profiles [5]. Such assumptions influence the choice of informative
324 age groups for serological monitoring in stop-MDA surveys [4], and have implications for
325 understanding the contribution of currently untreated groups to transmission and morbidity
326 [37], as well as for the design and evaluation of potential prophylactic strategies [52]. Testing

327 such assumptions with independently collected data is crucial for better parameterization of
328 transmission models and improved design of epidemiological surveys and surveillance
329 strategies. Therefore, we explored age-and sex-related patterns of IgG and IgM antibody titres
330 against *S. damnosum* s.l. saliva across well characterized endemic communities.

331 Both IgG and IgM antibody levels were high in children and gradually declined with
332 increasing age. Similar trends have been observed in human antibody responses to the saliva
333 of several mosquito species [53–58], posing the question of whether these patterns indicate
334 decreasing exposure to vector bites with age, or increasing immune tolerance and
335 desensitization with persistent or cumulative saliva exposure [59–62]. Investigation of human
336 IgG responses to sand fly saliva supports the latter proposition, with higher anti-saliva
337 antibody responses observed in new compared to long-term residents of a sand fly-endemic
338 region [63]. Similarly, desensitization to salivary antigens was detected in an area colonized
339 for more than 25 years by *Aedes* mosquitoes, compared to an area where individuals had been
340 exposed for no longer than 5 years [54]. Following that pattern, median IgG antibody
341 responses also tended to be lower in the current study villages where higher *S. damnosum* s.l.
342 biting rates were previously reported [30,42]. Antibody responses to *Anopheles* mosquito
343 bites measured after the summer season of high vector abundance were considerably higher
344 than those before the summer season; notwithstanding they consistently declined with
345 increasing host age [54,55]. Interestingly, in that study the decline appeared to be antigen-
346 dependent, as the trend was not detected in antibody responses to a specific recombinantly-
347 expressed protein as opposed to the whole salivary gland homogenate [64–66]. Several
348 immunogenic proteins were recently detected in *S. damnosum* s.l. saliva of which most were
349 well-known salivary antigens [23]. Future expression of these in recombinant forms may be
350 instructive and increase assay sensitivity. This is especially interesting for IgM as these
351 antibody responses were shown to be less specific than the IgG responses [23].

352 The median IgG response was lower in males than females, which can be attributed to the
353 greater rate of decline of the IgG response with increasing age in males. This may reflect sex
354 differences in behaviour such as daily habits, occupation, education, or clothing, that
355 influence physical exposure to blood-seeking blackflies. Male occupants of most ages are
356 responsible for agriculture, farming and fishing in the Pru region and may be less well
357 covered by protective clothing, whereas women are more covered, spend more time at home
358 performing domestic duties and/or engage in long-distance trading activities [67,68]. The
359 current study was limited in not recording the daily activities of the participants, though biting
360 blackflies appeared to be ubiquitous throughout the day within villages. That females show
361 greater levels of non-specific innate and adaptive immune responsiveness than males,
362 particularly post-puberty, suggests that hormonal involvement (reviewed in [69]) may
363 contribute to the sex differences observed in this study.

364 A non-mutually exclusive alternative driving factor behind the observed decline in antibody
365 levels may also be a lower exposure to blackfly bites with increasing age. Such patterns of
366 exposure were predicted by fitting age- and sex-structured onchocerciasis transmission
367 mathematical models to age- and sex-specific profiles of *O. volvulus* skin microfilariae in
368 another African savannah setting [5]. However, the model fits also indicated that vector
369 exposure of females should increase (rather than decrease) with age. If this is correct, and if
370 women indeed are more intensely exposed as they age, it further supports the case for
371 desensitization with increased long-term biting exposure. Therefore, one next step to better
372 understand exposure patterns with age and sex is to fit dynamic models of antibody
373 acquisition and decay to the (IgG) data obtained here [70,71]. Future studies of the molecular
374 and cellular mechanisms that underly immune tolerance and progressive desensitization to
375 blackfly saliva would also be most informative. Variations in IgG subclass responsiveness are

376 also possible as shown against *Anopheles* or *Aedes* saliva [55,72]. Interestingly, human IgG4
377 amongst bee-keepers was found to be associated with immunotolerance to bee venom [73,74].
378 Less clear in this study were the age and sex-related trends in IgM responses.
379 Although they also declined with age supporting the immunological desensitization
380 hypothesis, IgM responses are generally shorter lived than IgG responses, hence, likely to be
381 more indicative of recent exposure. A shorter half-life together with a lack of cumulative
382 increase after repeat exposure may partially explain the large number of low IgM responses
383 observed in the village residents, and the lack of correlations between individual host IgG and
384 IgM responses, particularly as the current study was limited to cross-sectional sampling
385 during the high biting season.
386 Future studies would benefit from quantifying short- and long-term kinetics in
387 individual anti-saliva Ig responses in the context of seasonal fluctuations in vector abundance
388 and distance to vector breeding habitats, to refine our understanding of the link between biting
389 rates and the Ig responses [16,53,54]. In fact, it would be very informative if antibody data
390 generated using our immunoassays could be used in spatial analyses to better understand
391 patterns of vector–human contact with increasing distance from breeding sites. At present,
392 mapping exercises such as those used for Onchocerciasis Elimination Mapping (OEM) collect
393 information on the distribution of breeding sites and vector presence. Our novel tool could
394 complement OEM to identify high-risk locations where exposure to vector bites would
395 provide additional information to seroprevalence surveys to guide start-MDA decisions and
396 identify informative age/sex groups for sampling [75]. Most of the information about the
397 relationship between vector density and distance from breeding sites pertains to African
398 savannah settings (such as those explored here), with less data available to characterize such a
399 relationship in forest and forest-savannah mosaic settings. Therefore, if our anti-vector saliva
400 assays could be combined with (seroprevalence) parasite exposure assays for a range of

401 epidemiological settings, it would be possible to obtain valuable information to help
402 elimination efforts. However, this necessitates the testing and validation of our assays for
403 other species/cytoforms of the *S. damnosum* complex.

404 The predominant vector species in the Bono East region are the savannah members of
405 the complex, *S. damnosum* sensu stricto/*S. sirbanum* [27,30]. It remains to be established if
406 the anti-*S. damnosum* s.l. IgG and IgM antibody responses represent a *damnosum* complex-
407 specific marker, or if member-specific markers of exposure would be more sensitive. We
408 acknowledge that more data need to be collected to ascertain the validity of our
409 immunoassays to reliably measure exposure to vector bites. Regarding empirical approaches,
410 there are no experimental or observational data yet to precisely quantify the relationship
411 between anti-salivary antibody levels and the number of vector bites. As colonizing blackflies
412 in the laboratory is notoriously difficult, collecting field data to understand the kinetics of the
413 antibody responses over a specified time frame would be helpful, particularly in settings with
414 strong seasonality, in which vector biting ceases or greatly decreases for several months
415 during the year. Regarding theoretical approaches, and as mentioned earlier, the exposure
416 profiles predicted by age- and sex-structured onchocerciasis transmission models are broadly
417 consistent with the proposed desensitization hypothesis. If this proves to be the case, we
418 expect vector saliva-naïve children still to respond well to salivary antigens as an indication of
419 continued exposure to vector bites pre- or post-MDA campaigns in areas with no vector
420 control or without major ecological changes affecting vector density. Our assay could then be
421 used to understand potential secular trends in vector biting rates due to anthropogenic change.
422 Ideally, multiplex assays could be developed to test simultaneously for both exposure to
423 vector bites and to parasite antigens.

424 ## Conclusion

425 Serological anti-saliva assays are useful tools to complement information collected by HLCs
426 by measuring human-vector contact and revealing heterogeneities in exposure at the
427 individual and community level that cannot be unravelled by HLCs alone. By novel
428 application to four onchocerciasis-endemic communities, this study successfully evaluated
429 age- and sex-related demographic patterns in blackfly bite exposure. The analyses uncovered
430 the possibility of age- and sex-specific immunotolerance or desensitization to blackfly saliva,
431 likely resulting from cumulative blackfly exposure with age. Concomitant studies of infection
432 levels in humans and flies, vector abundance, and immune responses to blackfly saliva and
433 parasite antigens would greatly help to better understand transmission risk and intensity, and
434 improve parameterization of transmission models with which to inform optimal interventions
435 and surveillance strategies to achieve and protect onchocerciasis elimination.

436

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445 ## References

446 1. World Health Organization. Department of Control of Neglected Tropical Diseases.
447 Ending the neglect to attain the Sustainable Development Goals – A road map for
448 neglected tropical diseases 2021–2030. Geneva World Health Organization. 2021
449 (Editor: Malecele MN). Available:
450 https://www.who.int/publications/i/item/9789240010352 (accessed 11 December
451 2021).

452 2. Hamley JID, Milton P, Walker M, Basáñez MG. Modelling exposure heterogeneity and
453 density dependence in onchocerciasis using a novel individual-based transmission
454 model, EPIONCHO-IBM: Implications for elimination and data needs. PLoS Negl
455 Trop Dis. 2019;13: 1–21. doi:10.1371/journal.pntd.0007557

456 3. Walker M, Stolk WA, Dixon MA, Bottomley C, Diawara L, Traoré MO, et al.
457 Modelling the elimination of river blindness using long-term epidemiological and
458 programmatic data from Mali and Senegal. Epidemics. 2017;18: 4–15.
459 doi:10.1016/j.epidem.2017.02.005

460 4. Hamley JID, Walker M, Coffeng LE, Milton P, de Vlas SJ, Stolk WA, et al. Structural
461 uncertainty in onchocerciasis transmission models influences the estimation of
462 elimination thresholds and selection of age groups for seromonitoring. J Infect Dis.
463 2020;221: S510–S518. doi:10.1093/infdis/jiz674

464 5. Filipe JAN, Boussinesq M, Renz A, Collins RC, Vivas-Martinez S, Grillet ME, et al.
465 Human infection patterns and heterogeneous exposure in river blindness. Proc Natl
466 Acad Sci USA. 2005;102: 15265–15270. doi:10.1073/pnas.0502659102

467 6. Otabil KB, Gyasi SF, Awuah E, Obeng-Ofori D, Rodríguez-Pérez MA, Katholi CR, et
468 al. The search for an efficient black fly trap for xenomonitoring of onchocerciasis. J
469 Parasitol Res. 2018;2018: 1–10. doi:10.1155/2018/5902367

470 7. Renz A, Wenk P. Studies on the dynamics of transmission of onchocerciasis in a
471 Sudan-savanna area of North Cameroon IV. The different exposure to *Simulium* bites
472 and transmission of boys and girls and men and women, and the resulting
473 manifestations of onchocerciasis. Ann Trop Med Parasitol. 1987;81: 215–228.
474 doi:10.1080/00034983.1987.11812115

475 8. Talom BAD, Enyong P, Cheke RA, Djouaka R, Hawkes FM. Capture of high numbers
476 of *Simulium* vectors can be achieved with Host Decoy Traps to support data acquisition
477 in the onchocerciasis elimination endgame. Acta Trop. 2021;221: 106020.
478 doi:10.1016/j.actatropica.2021.106020

479 9. Jacobi CA, Enyong P, Renz A. Individual exposure to *Simulium* bites and intensity of
480 *Onchocerca volvulus* infection. Parasit Vectors. 2010;3: 1–6.

481 10. Arcà B, Ribeiro JM. Saliva of hematophagous insects: a multifaceted toolkit. Curr

482 Opin Insect Sci. 2018;29: 102–109. doi:10.1016/j.cois.2018.07.012

483 11. Fontaine A, Pascual A, Orlandi-Pradines E, Diouf I, Remoué F, Pagès F, et al.
484 Relationship between exposure to vector bites and antibody responses to mosquito
485 salivary gland extracts. PLoS One. 2011;6: e29107. doi:10.1371/journal.pone.0029107

486 12. Doucoure S, Mouchet F, Cornelie S, DeHecq JS, Rutee AH, Roca Y, et al. Evaluation
487 of the human IgG antibody response to *Aedes albopictus* saliva as a new specific
488 biomarker of exposure to vector bites. PLoS Negl Trop Dis. 2012;6: e1487.
489 doi:10.1371/journal.pntd.0001487

490 13. Vlkova M, Rohousova I, Hostomska J, Pohankova L, Zidkova L, Drahota J, et al.
491 Kinetics of antibody response in BALB/c and C57BL/6 mice bitten by *Phlebotomus*
492 *papatasi*. PLoS Negl Trop Dis. 2012;6: e1719. doi:10.1371/journal.pntd.0001719

493 14. Caljon G, Van Den Abbeele J, Sternberg JM, Coosemans M, De Baetselier P, Magez S.
494 Tsetse fly saliva biases the immune response to Th2 and induces anti-vector antibodies
495 that are a useful tool for exposure assessment. Int J Parasitol. 2006;36: 1025–1035.
496 doi:10.1016/j.ijpara.2006.05.002

497 15. Schwarz A, Sternberg JM, Johnston V, Medrano-Mercado N, Anderson JM, Hume
498 JCC, et al. Antibody responses of domestic animals to salivary antigens of *Triatoma*
499 *infestans* as biomarkers for low-level infestation of triatomines. Int J Parasitol.
500 2009;39: 1021–1029. doi:10.1016/j.ijpara.2009.01.010

501 16. Kostalova T, Lestinova T, Sumova P, Vlkova M, Rohousova I, Berriatua E, et al.
502 Canine antibodies against salivary recombinant proteins of *Phlebotomus perniciosus*: A
503 longitudinal study in an endemic focus of canine leishmaniasis. PLoS Negl Trop Dis.
504 2015;9: e0003855. doi:10.1371/journal.pntd.0003855

505 17. Quinnell RJ, Soremekun S, Bates PA, Rogers ME, Garcez LM, Courtenay O. Antibody
506 response to sand fly saliva is a marker of transmission intensity but not disease
507 progression in dogs naturally infected with *Leishmania infantum*. Parasit Vectors.
508 2018;11: 1–12. doi:10.1186/s13071-017-2587-5

509 18. Brosseau L, Drame PM, Besnard P, Toto JC, Foumane V, Le Mire J, et al. Human
510 antibody response to *Anopheles* Saliva for comparing the efficacy of three malaria
511 vector control methods in Balombo, Angola. PLoS One. 2012;7: e44189.
512 doi:10.1371/journal.pone.0044189

513 19. Drame PM, Poinsignon A, Besnard P, Le Mire J, Dos-Santos MA, Sow CS, et al.
514 Human antibody response to *Anopheles gambiae* saliva: An immuno-epidemiological
515 biomarker to evaluate the efficacy of insecticide-treated nets in malaria vector control.
516 Am J Trop Med Hyg. 2010;83: 115–121. doi:10.4269/ajtmh.2010.09-0684

517 20. Gidwani K, Picado A, Rijal S, Singh SP, Roy L, Volfova V, et al. Serological markers
518 of sand fly exposure to evaluate insecticidal nets against visceral leishmaniasis in India
519 and Nepal: A cluster-randomized trial. PLoS Negl Trop Dis. 2011;5: e1296.

520 doi:10.1371/journal.pntd.0001296

521 21. Dinesh DS, Das P, Picado A, Davies C, Speybroeck N, Ostyn B, et al. Long-lasting
522 insecticidal nets fail at household level to reduce abundance of sandfly vector
523 *Phlebotomus argentipes* in treated houses in Bihar (India). Trop Med Int Health.
524 2008;13: 953–958. doi:10.1111/j.1365-3156.2008.02096.x

525 22. Schwarz A, Juarez JA, Richards J, Rath B, Machaca VQ, Castro YE, et al. Anti-
526 triatomine saliva immunoassays for the evaluation of impregnated netting trials against
527 Chagas disease transmission. Int J Parasitol. 2011;41: 591–594.
528 doi:10.1016/j.ijpara.2011.02.001

529 23. Willen L, Basáñez MG, Dvorak V, Veriegh FBD, Aboagye FT, Idun B, et al. Human
530 immune response against salivary antigens of *Simulium damnosum* s.l.: A new
531 epidemiological marker for exposure to blackfly bites in onchocerciasis endemic areas.
532 PLoS Negl Trop Dis. 2021;15: e0009512. doi:10.1371/journal.pntd.0009512

533 24. Hougard J-M, Alley ES, Yaméogo L, Dadzie KY, Boatin BA. Eliminating
534 onchocerciasis after 14 years of vector control: A proved strategy. J Infect Dis.
535 2001;184: 497–503. doi:10.4000/books.irdeditions.29100

536 25. O'Hanlon SJ, Slater HC, Cheke RA, Boatin BA, Coffeng LE, Pion SDS, et al. Model-
537 based geostatistical mapping of the prevalence of *Onchocerca volvulus* in West Africa.
538 PLoS Negl Trop Dis. 2016;10: e0004328. doi:10.1371/journal.pntd.0004328

539 26. Alley ES, Plaisier AP, Boatin BA, Dadzie KY, Remme J, Zerbo G, et al. The impact of
540 five years of annual ivermectin treatment on skin microfilarial loads in the
541 onchocerciasis focus of Asubende, Ghana. Trans R Soc Trop Med Hyg. 1994;88: 581–
542 584. doi:10.1016/0035-9203(94)90172-4

543 27. Lamberton PHL, Cheke RA, Winskill P, Tirados I, Walker M, Osei-Atweneboana MY,
544 et al. Onchocerciasis transmission in Ghana: Persistence under different control
545 strategies and the role of the simuliid vectors. PLoS Negl Trop Dis. 2015;9: e0003688.
546 doi:10.1371/journal.pntd.0003688

547 28. Osei-Atweneboana MY, Eng JKL, Boakye DA, Gyapong JO, Prichard RK. Prevalence
548 and intensity of *Onchocerca volvulus* infection and efficacy of ivermectin in endemic
549 communities in Ghana: a two-phase epidemiological study. Lancet. 2007;369: 2021–
550 2029. doi: 10.1016/S0140-6736(07)60942-8. .

551 29. Post RJ, Cheke RA, Boakye DA, Wilson MD, Osei-Atweneboana MY, Tetteh-Kumah
552 A, et al. Stability and change in the distribution of cytospecies of the *Simulium*
553 *damnosum* complex (Diptera: Simuliidae) in southern Ghana from 1971 to 2011.
554 Parasit Vectors. 2013;6: 1–11. doi:10.1186/1756-3305-6-205

555 30. Lamberton PHL, Cheke RA, Walker M, Winskill P, Osei-Atweneboana MY, Tirados I,
556 et al. Onchocerciasis transmission in Ghana: Biting and parous rates of host-seeking
557 sibling species of the *Simulium damnosum* complex. Parasit Vectors. 2014;7: 511.

558 doi:10.1186/s13071-014-0511-9

559 31. Cheke RA, Basáñez M, Perry M, White M, Garms R, Obuobie E, et al. Potential effects
560 of warmer worms and vectors on onchocerciasis transmission in West Africa. Philos
561 Trans R Soc B Biol Sci. 2015;370: 20130559. doi: 10.1098/rstb.2013.0559.

562 32. Frempong KK, Walker M, Cheke RA, Tetevi EJ, Gyan ET, Owusu EO, et al. Does
563 increasing treatment frequency address suboptimal responses to ivermectin for the
564 control and elimination of river blindness? Clin Infect Dis. 2016;62: 1338–1347.
565 doi:10.1093/cid/ciw144

566 33. Doyle SR, Bourguinat C, Nana-Djeunga HC, Kengne-Ouafo JA, Pion SDS, Bopda J, et
567 al. Genome-wide analysis of ivermectin response by *Onchocerca volvulus* reveals that
568 genetic drift and soft selective sweeps contribute to loss of drug sensitivity. PLoS Negl
569 Trop Dis. 2017;11: e0005816. doi:10.1371/journal.pntd.0005816

570 34. Plaisier AP, van Oortmarssen GJ, Habbema JDF, Remme J, Alley ES. ONCHOSIM: a
571 model and computer simulation program for the transmission and control of
572 onchocerciasis. Comput Methods Programs Biomed. 1990;31: 43–56.
573 doi:10.1016/0169-2607(90)90030-d.

574 35. Basáñez MG, Churcher TS, Grillet ME. *Onchocerca-Simulium* interactions and the
575 population and evolutionary biology of *Onchocerca volvulus*. Adv Parasitol. 2009;68:
576 263–313. doi:10.1016/S0065-308X(08)00611-8

577 36. Basáñez MG, Walker M, Turner HC, Coffeng LE, de Vlas SJ, Stolk WA. River
578 blindness: Mathematical models for control and elimination. Adv Parasitol. 2016;94:
579 247–341. doi:10.1016/bs.apar.2016.08.003

580 37. Hamley JID, Blok DJ, Walker M, Milton P, Hopkins AD, Hamill LC, et al. What does
581 the COVID-19 pandemic mean for the next decade of onchocerciasis control and
582 elimination? Trans R Soc Trop Med Hyg. 2021;115: 269–280.
583 doi:10.1093/trstmh/traa193

584 38. Turner HC, Osei-Atweneboana MY, Walker M, Tettevi EJ, Churcher TS, Asiedu O, et
585 al. The cost of annual versus biannual community-directed treatment of onchocerciasis
586 with ivermectin: Ghana as a case study. PLoS Negl Trop Dis. 2013;7: e2452.
587 doi:10.1371/journal.pntd.0002452

588 39. Walsh JF, Davies JB, Le Berre R, Garms R. Standardization of criteria for assessing
589 the effect of *Simulium* control in onchocerciasis control programmes. Trans R Soc Trop
590 Med Hyg. 1978;72: 675–676. doi:10.1016/0035-9203(78)90039-1

591 40. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York,
592 2009.

593 41. South A. rworldmap: A New R package for Mapping Global Data. R J. 2011;3: 35–43.

594 42. Veriegh FBD. Impacts of semi-annual ivermectin treatment and the spatio-temporal

595 distribution and abundance of *Simulium damnosum* sensu lato vectors on the
596 transmission of *Onchocerca volvulus*. PhD Thesis. Kwame Nkrumah University of
597 Science and Technology (KNUST), Ghana. 2020.

598 43. Biritwum NK, de Souza DK, Asiedu O, Marfo B, Amazigo UV, Gyapong JO.
599 Onchocerciasis control in Ghana (1974–2016). Parasit Vectors. 2021;14: 1–9.
600 doi:10.1186/s13071-020-04507-2

601 44. R Core Team (2015). R: A language and environment for statistical computing. R
602 Foundation for Statistical Computing, Vienna, Austria. Available: [https://www.r-
603 project.org/](https://www.r-project.org/) (accessed 11 December 2021).

604 45. Cohen J. Statistical Power Analysis for the Behavioral Sciences. Second edition.
605 Lawrence Erlbaum Associates; 1988.

606 46. Ryan TP. Sample Size Determination and Power. John Wiley & Sons, Inc.; 2013.
607 doi:10.1002/9781118439241

608 47. Andrade BB, Teixeira CR. Biomarkers for exposure to sand flies bites as tools to aid
609 control of leishmaniasis. Front Immunol. 2012;3: 121. doi:10.3389/fimmu.2012.00121

610 48. Lestinova T, Rohousova I, Sima M, de Oliveira CI, Volf P. Insights into the sand fly
611 saliva: Blood-feeding and immune interactions between sand flies, hosts, and
612 *Leishmania*. PLoS Negl Trop Dis. 2017;11: e0005600.
613 doi:10.1371/journal.pntd.0005600

614 49. Doyle SR, Armoo S, Renz A, Taylor MJ, Osei-Atweneboana MY, Grant WN.
615 Discrimination between *Onchocerca volvulus* and *O. ochengi* filarial larvae in
616 *Simulium damnosum* (s.l.) and their distribution throughout central Ghana using a
617 versatile high-resolution speciation assay. Parasit Vectors. 2016;9: 536.
618 doi:10.1186/s13071-016-1832-7

619 50. Yarzábal L, Basáñez MG, Ramírez-Pérez J, Ramírez A, Botto C, et al. Experimental
620 and natural infection of *Simulium sanchezi* by *Mansonella ozzardi* in the Middle
621 Orinoco region of Venezuela. Trans R Soc Trop Med Hyg. 1985;79: 29–33.
622 doi:10.1016/0035-9203(85)90226-3

623 51. Cupp EW, Maré CJ, Cupp MS, Ramberg FB. Biological transmission of vesicular
624 stomatitis virus (New Jersey) by *Simulium vittatum* (Diptera: Simuliidae). J Med
625 Entomol. 1992;29: 137–140. doi:10.1093/jmedent/29.2.137

626 52. Turner HC, Walker M, Lustigman S, Taylor DW, Basáñez MG. Human
627 onchocerciasis: Modelling the potential long-term consequences of a vaccination
628 programme. PLoS Negl Trop Dis. 2015;9: e0003938.
629 doi:10.1371/journal.pntd.0003938

630 53. Rizzo C, Ronca R, Fiorentino G, Verra F, Mangano V, Poinsignon A, et al. Humoral
631 response to the *Anopheles gambiae* salivary protein gSG6: A serological indicator of

632 exposure to Afrotropical malaria vectors. PLoS One. 2011;6: e17980.
633 doi:10.1371/journal.pone.0017980

634 54. Buezo Montero S, Gabrieli P, Montarsi F, Borean A, Capelli S, De Silvestro G, et al.
635 IgG antibody responses to the *Aedes albopictus* 34k2 salivary protein as novel
636 candidate marker of human exposure to the tiger mosquito. Front Cell Infect Microbiol.
637 2020;10: 377. doi:10.3389/fcimb.2020.00377

638 55. Rizzo C, Ronca R, Lombardo F, Mangano V, Sirima SB, Nèbiè I, et al. IgG1 and IgG4
639 antibody responses to the *Anopheles gambiae* salivary protein gSG6 in the sympatric
640 ethnic groups Mossi and Fulani in a malaria hyperendemic area of Burkina Faso. PLoS
641 One. 2014;9: e96130. doi:10.1371/journal.pone.0096130

642 56. Londono-Renteria B, Drame PM, Montiel J, Vasquez AM, Tobón-Castaño A, Taylor
643 M, et al. Identification and pilot evaluation of salivary peptides from *Anopheles*
644 *albimanus* as biomarkers for bite exposure and malaria infection in Colombia. Int J
645 Mol Sci. 2020;21: 691. doi:10.3390/ijms21030691

646 57. Montiel J, Carbal LF, Tobón-Castaño A, Vásquez GM, Fisher ML, Londono-Rentería
647 B. IgG antibody response against *Anopheles* salivary gland proteins in asymptomatic
648 *Plasmodium* infections in Nariño, Colombia. Malar J. 2020;19: 42.
649 doi:10.1186/s12936-020-3128-9

650 58. Poinsignon A, Cornelie S, Ba F, Boulanger D, Sow C, Rossignol M, et al. Human IgG
651 response to a salivary peptide, gSG6-P1, as a new immuno-epidemiological tool for
652 evaluating low-level exposure to *Anopheles* bites. Malar J. 2009;8: 198.
653 doi:10.1186/1475-2875-8-198

654 59. Peng Z, Simons FER. Mosquito allergy: Immune mechanisms and recombinant
655 salivary allergens. Int Arch Allergy Immunol. 2004;133: 198–209.
656 doi:10.1159/000076787

657 60. Feingold BF, Benjamini E, Michaeli D. The allergic responses to insect bites. Annu
658 Rev Entomol. 1968;13: 137–158. doi:10.1146/annurev.en.13.010168.001033

659 61. Peng Z, Simons FER. A prospective study of naturally acquired sensitization and
660 subsequent desensitization to mosquito bites and concurrent antibody responses. J
661 Allergy Clin Immunol. 1998;101: 284–286. doi:10.1016/S0091-6749(98)70395-1

662 62. Mellanby K. Man's reaction to mosquito bites. Nature. 1946;158: 554.

663 63. Mondragon-Shem K, Al-Salem WS, Kelly-Hope L, Abdeladhim M, Al-Zahrani MH,
664 Valenzuela JG, et al. Severity of Old World cutaneous leishmaniasis is influenced by
665 previous exposure to sandfly bites in Saudi Arabia. PLoS Negl Trop Dis. 2015;9:
666 e0003449. doi:10.1371/journal.pntd.0003449

667 64. Rizzo C, Lombardo F, Ronca R, Mangano V, Sirima S, Nèbiè I, et al. Differential
668 antibody response to the *Anopheles gambiae* gSG6 and cE5 salivary proteins in

669 individuals naturally exposed to bites of malaria vectors. Parasit Vectors. 2014;7: 549.
670 doi:10.1186/s13071-014-0549-8

671 65. Ribeiro JMC, Arcà B, Lombardo F, Calvo E, Phan VM, Chandra PK, et al. An
672 annotated catalogue of salivary gland transcripts in the adult female mosquito, *Aedes*
673 *aegypti*. BMC Genom. 2007;8: 6. doi:10.1186/1471-2164-8-6

674 66. Londono-Renteria BL, Shakeri H, Rozo-Lopez P, Conway MJ, Duggan N, Jaberi-
675 Douraki M, et al. Serosurvey of human antibodies recognizing *Aedes aegypti* D7
676 salivary proteins in Colombia. Front Public Health. 2018;6: 11.
677 doi:10.3389/fpubh.2018.00111

678 67. World Health Organization & Onchocerciasis Control Programme in West Africa.
679 Socio-demographic study in the Pru Basin. Ouagadougou 1l–15 March 2002.
680 Available: https://apps.who.int/iris/handle/10665/311605 (accessed 11 December
681 2021).

682 68. Kim R, van Moorsel T, Kapstein EB. The National and Regional Socio-Economic
683 Impact of Newmont Ghana's Ahafo Mine. Steward Redqueen Rep. 2013. Available:
684 https://s24.q4cdn.com/382246808/files/doc_downloads/operations_projects/africa/quic
685 k_links/Socio-economic-impact-of-Ahafo-Operations.pdf (accessed 11 December
686 2021).

687 69. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol.
688 2016;16: 626–638. doi:10.1038/nri.2016.90

689 70. White MT, Griffin JT, Akpogheneta O, Conway DJ, Koram KA, Riley EM, et al.
690 Dynamics of the antibody response to *Plasmodium falciparum* infection in African
691 children. J Infect Dis. 2014;210: 1115–1122. doi:10.1093/infdis/jiu219

692 71. Yman V, White MT, Rono J, Arcà B, Osier FH, Troye-Blomberg M, et al. Antibody
693 acquisition models: A new tool for serological surveillance of malaria transmission
694 intensity. Sci Rep. 2016;6: 1–10. doi:10.1038/srep19472

695 72. Remoue F, Alix E, Cornelie S, Sokhna C, Cisse B, Doucoure S, et al. IgE and IgG4
696 antibody responses to *Aedes* saliva in African children. Acta Trop. 2007;104: 108–115.
697 doi:10.1016/j.actatropica.2007.07.011

698 73. Varga EM, Kausar F, Aberer W, Zach M, Eber E, Durham SR, et al. Tolerant
699 beekeepers display venom-specific functional IgG4 antibodies in the absence of
700 specific IgE. J Allergy Clin Immunol. 2013;131: 1419–1421.
701 doi:10.1016/j.jaci.2012.08.037

702 74. García-Robaina JC, De La Torre-Morín F, Vazquez-Moncholi C, Fierro J, Bonnet-
703 Moreno C. The natural history of *Apis*-specific IgG and IgG4 in beekeepers. Clin Exp
704 Allergy. 1997;27: 418–423. doi:10.1111/j.1365-2222.1997.tb00727.x

705 75. World Health Organization. Department of Control of Neglected Tropical Diseases.

706 Report of the Third Meeting of the WHO Onchocerciasis Technical Advisory
707 Subgroup Geneva, Switzerland, 26–28 February 2019 (Editor: Cantey P). Available:
708 https://www.who.int/publications/i/item/9789240006638 (accessed 11 December
709 2021).