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Serological Markers of Exposure to *Plasmodium falciparum* and *Plasmodium vivax* Infection in Southwestern Ethiopia

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Abstract. As malaria control and elimination efforts ramp up in Ethiopia, more sensitive tools for assessing exposure to coendemic *Plasmodium falciparum* and *Plasmodium vivax* are needed to accurately characterize malaria risk and epidemiology. Serological markers have been increasingly explored as cost-effective tools for measuring transmission intensity and evaluating intervention effectiveness. The objectives of this study were to evaluate the efficacy of a panel of 10 serological markers as a proxy for malaria exposure and to determine underlying risk factors of seropositivity. We conducted cross-sectional surveys in two sites of contrasting malaria transmission intensities in southwestern Ethiopia: Arjo in Oromia Region (low transmission) and Gambella in Gambella Regional State (moderate transmission). We measured antibody reactivity against six *P. falciparum* (AMA-1, CSP, EBA175RIII-V, MSP-1_{42}, MSP-3, RH2ab) and four *P. vivax* (DBPII[Sal1], EBP2, MSP-1_{19}, RBP2b) targets. We used mixed effects logistic regressions to assess predictors of seropositivity. *Plasmodium* spp. infection prevalence by quantitative polymerase chain reaction was 1.36% in Arjo and 10.20% in Gambella. Seroprevalence and antibody levels against all 10 antigens were higher in Gambella than in Arjo. We observed spatial heterogeneities in seroprevalence across Arjo and smaller variations across Gambella. Seroprevalence in both sites was lowest against *Pf*CSP and highest against *Pf*AMA-1, *Pf*MSP-1_{42}, and *Pv*MSPS-1_{19}. Male sex, age, and agricultural occupation were positively associated with seropositivity in Arjo; associations were less pronounced in Gambella. Our findings demonstrate that seroprevalence and antibody levels to specific *Plasmodium* antigens can be used to identify high-risk groups and geographical areas where interventions to reduce malaria transmission should be implemented.

INTRODUCTION

Ethiopia is one of the few countries in Africa where *Plasmodium falciparum* and *Plasmodium vivax* are coendemic.[1] In 2018, Ethiopia accounted for 6% of malaria cases globally and approximately 12% of the global burden of disease as a result of *P. vivax*.[2,3] Approximately two-thirds of Ethiopia's population resides in malarious areas. Malaria transmission in Ethiopia is seasonal, unstable, and varies widely across agroecological zones (i.e., lowlands, midlands, and highlands).[4–6] Widespread epidemics historically have occurred every 5–8 years but are occurring with increasing magnitude and frequency, signaling the urgent need for continuous surveillance and interventions targeting both *P. falciparum* and *P. vivax* in Ethiopia.[6]

Accurate and cost-effective metrics are required not only for diagnosing *Plasmodium* spp. infections but also for monitoring changes in malaria transmission and evaluating the impact of malaria interventions.[7–9] Traditionally, direct indicators such as the entomological inoculation rate (EIR), defined as the number of infectious bites per person per unit time, and parasite prevalence (PrP), defined as the proportion of individuals who are found to be carrying parasites in their blood at a given point in time, have been used to measure transmission intensity. However, EIR and PrP become difficult to measure precisely as transmission declines.[7] Serological markers have been increasingly investigated as reliable, economical alternatives in settings where EIR and PrP have low discriminative value for measuring changes in transmission patterns and dynamics. *Plasmodium* spp. infection elicits antigen-specific antibody responses that may persist for months or years after last exposure, and can therefore serve as a proxy measure of malaria exposure and transmission intensity.[10] Antibody testing can be performed on peripheral blood samples or dried blood spots (DBSs) that are routinely collected in cross-sectional studies or other ongoing surveillance efforts that focus on direct parasite detection.[11] Conventional sampling methods and the timing of epidemiologic surveys may fail to capture malarial blood-stage infections. One key advantage that antibody detection methods confer over parasite detection methods (i.e., polymerase chain reaction [PCR] tests, rapid diagnostic tests, and microscopy) is their ability to identify recent and historic exposure, as opposed to identifying only individuals who are infected with blood-stage parasites at the time of sampling.[11] The persistence of antibodies after the clearance of infections effectively smooths out the effect of seasonality, such that trends in transmission can be identified.[10] Even in settings of unstable or low transmission, certain antibodies remain at sufficiently high levels from which estimates of recent transmission and thereby changes in transmission intensity can be inferred.[10,12,13]

Development of anti-*Plasmodium* antibody responses is influenced by the complex interplay of human (e.g., host genetic and immune factors), parasite (e.g., parasite density), and environmental factors (e.g., proximity to mosquito breeding sites).[14] Antibody responses against parasite antigens vary in longevity. Some responses are short lived, indicating recent exposure, whereas other antibody responses are long lived, reflecting cumulative malaria exposure.[12] The objectives of

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this study were to assess the suitability of antibodies against an array of *P. falciparum* and *P. vivax* antigens for determining differences in levels of transmission intensity and to identify individual-level factors associated with antibody seropositivity. We used a bead-based multiplexed assay to detect IgG antibodies against six *P. falciparum* (one preerythrocytic and five blood-stage) and four *P. vivax* (blood-stage) antigen targets. This panel included markers of recent and cumulative exposure to *P. falciparum* and *P. vivax* that were selected for their high immunogenicity or potential as vaccine candidates.[15,16] Our findings have implications for monitoring changes in transmission intensity following the implementation of malaria control interventions as Ethiopia works toward its goal of achieving nationwide malaria elimination by 2030.

MATERIALS AND METHODS

Ethics statement. Ethical approval was obtained from the institutional review boards of the University of California Irvine and Jimma University Institute of Health, Ethiopia. Verbal and written informed consent were obtained from all heads of households before participants were enrolled. Assent was obtained from every individual residing within consented households who was willing to participate in the study.

Description of the study area. This study was conducted in two areas of southwestern Ethiopia with contrasting malaria transmission intensities: the Arjo-Didessa sugarcane plantation area and its vicinity in Oromia Region, and the Saudi Star rice development irrigation project area and its vicinity in Gambella Region (Figure 1).

Arjo-Didessa. Arjo-Didessa sugarcane plantation (hereafter Arjo) is a low-transmission area.[17] It is located among Jimma-Arjo, Dabo Hana, and Buno-Bedele districts in Oromia Regional State, 395 km southwest of Ethiopia's capital, Addis Ababa. Development of the state-owned sugarcane plantation began in 2006 and currently spans about 5,000 ha. The estimated population in the plantation area is 50,000, and the total population in the surrounding nonirrigated districts is 215,000. Most of the population in Arjo depends on subsistence farming, and many residents are migrant workers who live onsite to harvest and plant sugarcane. The altitude in this study area ranges from 1,300 to 2,280 m above sea level, and the mean annual rainfall is 1,477 mm.[17]

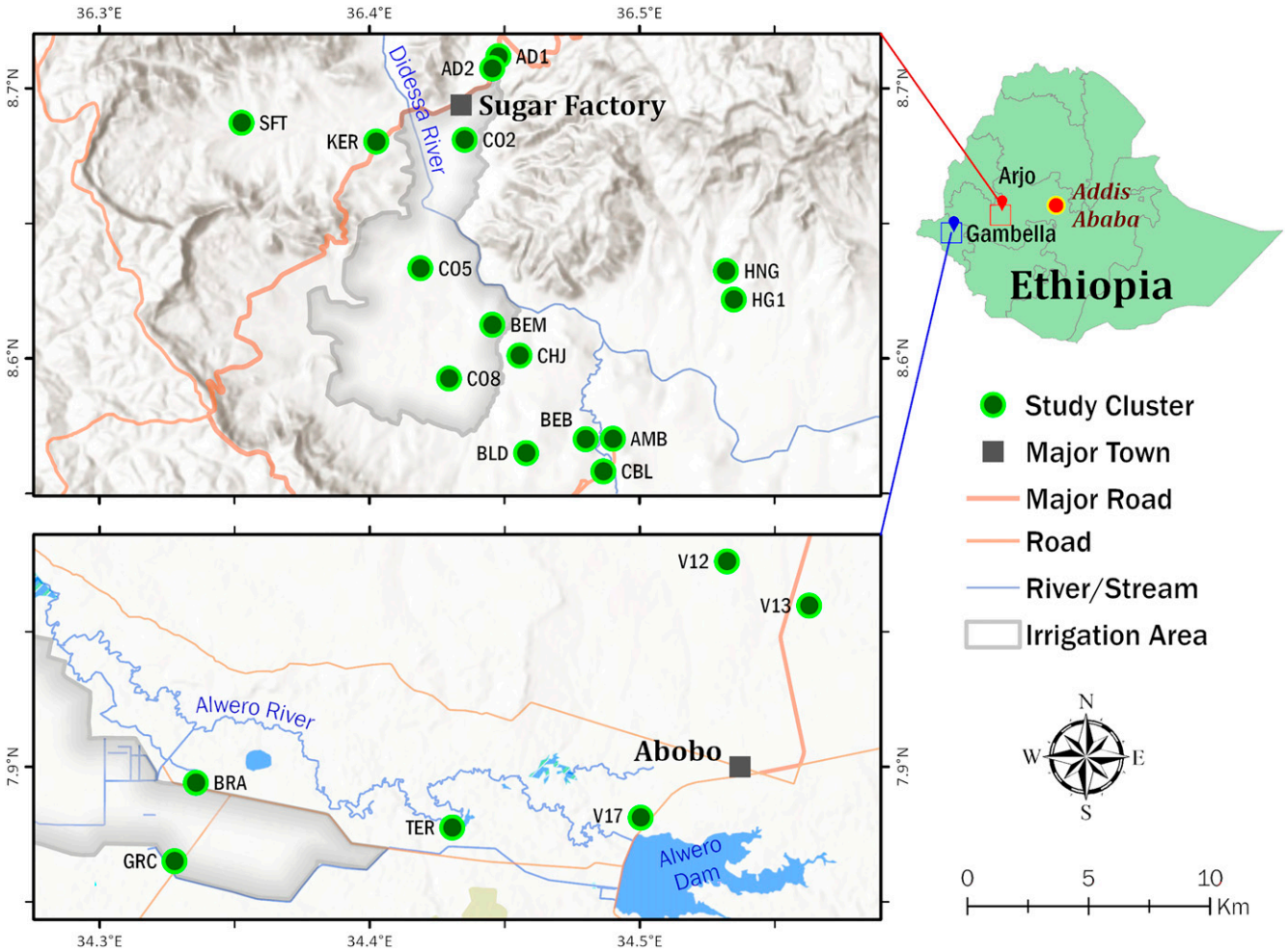

FIGURE 1. Map of study sites (top: Arjo; bottom: Gambella) with study clusters highlighted in green. The 15 study clusters in Arjo are Abote Didessa 1 (AD1), Abote Didessa 2 (AD2), Ambelta (AMB), Bekelcha Biftu (BEB), Beyima (BEM), Bildema Deru (BLD), Chilalo Bildema (CBL), Chefe Jallela (CHJ), Command-2 (CO2), Command-5 (CO5), Command-8 (CO8), Soyama (HG1), Hunda Gudina (HNG), Kerka (KER), and Sefera Tabiya (SFT). The 6 clusters in Gambella are SaudiStar Bravo Camp (BRA), SaudiStar GRC Camp (GRC), Terkodi (TER), Ville 12 (V12), Ville 13 (V13), and Ville 17 (V17).

Gambella. The Saudi Star rice development irrigation project area (hereafter Gambella) is located in Abobo woreda (district) of Anuak zone in Gambella Regional State, 811 km west of Addis Ababa. Gambella is an area of moderate-to-high malaria transmission intensity.[6,18] The irrigation project area currently spans 140,000 ha within and around the eastern portion of Gambella National Park in Gambella Regional State. Irrigation projects have been underway in the area since 2012. The estimated total population size in the district is 26,080. Most residents in Gambella make a living by farming and fishing. The altitude in this area ranges from 500 to 700 m above sea level. The mean annual rainfall ranges between 800 and 1,200 mm.[19]

Study design and sample collection. We conducted a cross-sectional blood survey in Arjo and Gambella in October 2018. Malaria incidence tends to peak between September and December, following the June–August rains. Trained field staff visited households in Arjo and Gambella to enroll participants, administer structured questionnaires, and collect blood samples. Residents were eligible if their axillary temperature was $\leq$ 37.5 °C and if they reported no symptoms of malaria at the time of collection. The study population comprised individuals $\geq$ 15 years old residing in 15 clusters in Arjo and in 6 clusters in Gambella. A cluster is defined as 100–250 households falling within a radius of 250–500 m. Handheld global positioning system units were used to record household coordinates. A structured questionnaire was used to collect information on sociodemographic factors (i.e., age, sex, ethnicity, level of education, and occupation) and usage of protective measures (i.e., bednet usage and household indoor residual spraying [IRS] status within the past year). Finger-prick sampling was used to collected whole blood and to create DBSs on Whatman 3MM filter paper.

Molecular parasite detection and serological assays. DNA was extracted from DBSs using the saponin/Chelex method.[20] Real-time quantitative PCR (qPCR) was performed on a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, Carlsbad, CA) to identify *P. falciparum*, *P. vivax*, *Plasmodium malariae*, and *Plasmodium ovale* infections, as previously described.[21] A cycle threshold value less than or equal to 39 was used to define infection positivity by qPCR.

Anti-*P. falciparum* and *P. vivax* IgG antibody levels in plasma. Antibodies specific for six *P. falciparum* and four *P. vivax* antigens were measured using a multiplexed bead-based immunoassay. Sources and quantities of conjugated antigens are listed in Supplemental Table 1 and have been used in previous studies.[15,22,23] Proteins were conjugated to magnetic microspheres with unique spectral addresses in accordance with the manufacturer's instructions (Luminex, Austin, TX) and as detailed in excellent methodology papers.[24,25] Beads were mixed by vortexing for 1 minute, and all spins were 2 minutes in length. Activated beads were incubated with proteins overnight at 4 °C. Proteins were tested at various coupling concentrations to obtain an optimal signal, which was determined by testing a standard pool of plasma made from 20 Kenyan adults from the Nyanza Province region in 2000. The Kenyan plasma pool was serially diluted, and the linear range was found from the standard curve for each bead. A final plasma dilution of 1:400 was found to be optimal for this multianalyte assay. Each plasma sample was plated with the master mix of conjugated microspheres at a 1:1 ratio for a final sample dilution of 1:400. The secondary antibody used was R-Phycoerythrin-AffiniPure F(ab′)$_2$ fragment goat anti-human IgG F(ab′)$_2$ (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:100 in phosphate-buffered saline with bovine serum albumin, Tween 20, and sodium azide (PBS-TBN). The serially diluted standard pooled Kenyan plasma was run on each plate to detect any plate-to-plate variations, which were negligible. Plates were run according to manufacturer protocols on the MAGPIX™ platform (EMD Millipore, Burlington, MA) to determine the mean fluorescence intensities (MFIs) corresponding to each antigen for each individual Ethiopian participant plasma sample. For each antigen, we determined the mean and standard deviation (SD) of the MFI among seven individual, malaria-naïve adult North American negative controls. Negative control mean MFI + 3 SD was used as the cutoff for seropositivity for each plate.

Data analysis. All statistical analyses were performed in R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria), and all maps were generated using ArcGIS Pro 3.0 (Esri, Redlands, CA). We conducted mixed effects logistic regressions to examine the associations between antibody seropositivity and explanatory factors. Multivariable regressions were used to identify independent risk factors of antibody seropositivity while controlling for potential confounders. Cluster of residence was included as a random effect to account for expected similarities in exposure among households within the same "village". Regressions were performed separately for Arjo and Gambella because seroprevalence for each of the 10 antibodies was significantly different between the two sites. Age, sex, occupation, and bednet ownership were included in models as individual-level covariates. Age was categorized into two groups, reflecting the age-based definition of youth that Ethiopia's National Youth Policy deems "most suitable for research and policy purposes".[26] Individuals were grouped by those

TABLE 1
Demographic characteristics of study participants and households in Arjo and Gambella, Ethiopia, October 2018

	Arjo (N = 473)		Gambella (N = 328)	
Characteristic	*N*	%	*n*	%
Individual-level characteristics				
Age				
< 30 years old	273	57.72	183	55.79
≥ 30 years old	200	42.28	145	44.21
Sex				
Female	286	60.47	131	39.94
Male	187	39.53	197	60.06
Occupation*				
Agricultural	261	55.18	108	32.93
Nonagricultural	212	44.82	220	67.07
Bednet ownership				
No	193	40.80	125	38.11
Yes	280	59.20	203	61.89
Household-level characteristics				
Indoor residual spraying within the past year				
No	302	63.85	187	56.40
Yes	171	36.15	154	43.60
Distance to irrigation scheme				
< 500 m	218	46.09	148	45.12
≥ 500 m	255	53.91	180	54.88

*Specific self-reported nonagricultural occupations included fisherman, herdsman, miner, guardsman, soldier, peace officer, office worker, teacher, salesperson, shop clerk, server, business owner, shopkeeper, and trader.

TABLE 2
Plasmodium parasites detected by quantitative polymerase chain reaction in Arjo and Gambella, Ethiopia, October 2018

Study site	Plasmodium species, *n* (%)				
	P. falciparum	*P. vivax*	*P. malariae*	*P. ovale*	*P. vivax* + *P. ovale*
Arjo ($N = 441$)	3 (0.68)	3 (0.69)	0	0	0
Gambella ($N = 304$)	17 (5.59)	9 (2.96)	1 (0.33)	3 (0.99)	1 (0.33)

who were between 15 and 29 years old ("youth") and those who were 30 years or older at the time of sampling. Occupations were categorized as agricultural or nonagricultural. Household-level characteristics that were adjusted for in the regressions included IRS status (whether a household had undergone IRS within the past year) and distance to irrigation scheme (< 500 or ≥ 500 m). Adjusted odds ratios (AORs) and 95% confidence intervals (CIs) are reported. Statistical significance was defined as $P < 0.05$. Site-level antibody seroprevalence was compared using the χ^2 test or Fisher's exact test. Antibody levels among seropositive participants were compared between sites using Wilcoxon rank-sum tests. *P* values were adjusted for multiple comparisons using the Benjamini–Hochberg approach when appropriate.

RESULTS

Characteristics of the study population. A total of 473 community members in Arjo and 328 community members in Gambella completed the structured questionnaire, which captured age, biological sex, occupation, bednet usage, and household IRS within the past year (Table 1). Two residents in Arjo consented to participating but did not complete the questionnaire or provide blood samples and were thus excluded from the study. The median age of participants was 27 years (interquartile range [IQR] 20–36) in Arjo and 28 years (IQR 23–36) in Gambella. The study population in Arjo was female biased (60.5%), whereas the inverse sex ratio was reported among participants in Gambella (60.1% male). About 55% of the participants in Arjo made a living as agricultural workers, compared with only 33% of participants in Gambella. Many study participants did not own bednets (41.7% in Arjo; 32.0% in Gambella). However, nearly all participants who owned bednets reported sleeping under bednets every night (94.6% in Arjo; 96.9% in Gambella). Within the year prior to the study, fewer than half of the households surveyed in both Arjo and Gambella reported having been sprayed with insecticides. Median distance from household to irrigation scheme was 734.3 m in Arjo and 2846.1 m in Gambella.

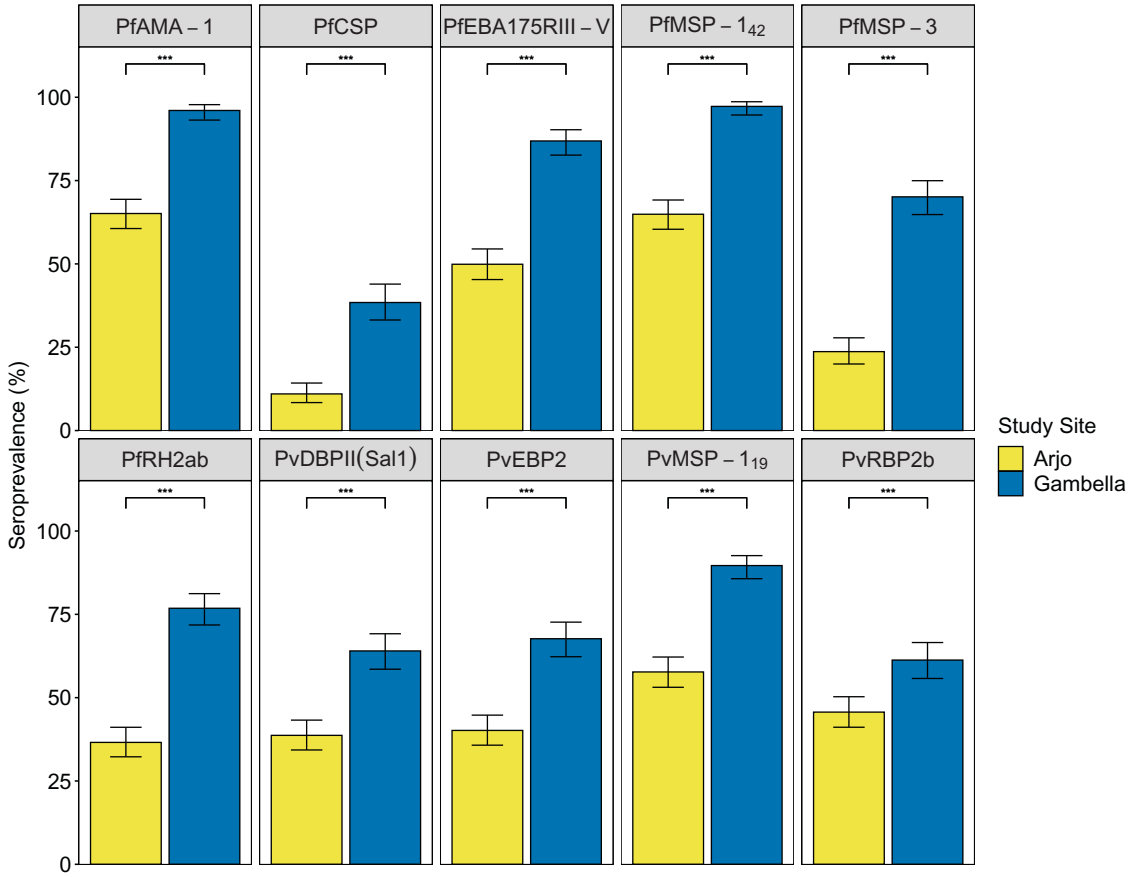

FIGURE 2. Site-level antibody seroprevalence against six *Plasmodium falciparum* and four *Plasmodium vivax* antigens in Arjo (yellow bars) vs. Gambella (blue bars) in October 2018. Asterisks indicate *P* values adjusted for multiple comparisons (***$P < 0.001$). Error bars represent 95% CIs.

qPCR-based parasite detection. DNA was extracted from DBSs that were successfully matched to 441 participants in Arjo and 304 participants in Gambella. *Plasmodium* spp. infection prevalence in Arjo was 1.36% compared with 10.20% prevalence in Gambella (Table 2). We detected no mixed infections and no monoinfections of *P. malariae* or *P. ovale* in Arjo. In Gambella, *P. falciparum* accounted for the greatest proportion of malaria-positive samples, followed by *P. vivax*. One mixed infection of *P. vivax* and *P. ovale* was detected. Because of the low prevalence observed in both Arjo and Gambella, infection status (as detected by qPCR) was not included in subsequent statistical analyses.

Variations in antibody seroprevalence. Antibody seroprevalence against all six *P. falciparum* and four *P. vivax* antigens was significantly higher in Gambella than in Arjo (adjusted P values < 0.001; Figure 2). Among the six *P. falciparum* antibodies, site-level seroprevalence was lowest for *Pf*CSP and highest for *Pf*AMA-1 and *Pf*MSP-1_{42} in both study sites. Among the four *P. vivax* antibodies, seroprevalence for *Pv*MSP-1_{19} was highest in both Arjo and Gambella. Spatial variations in *P. falciparum* (Figure 3A and B) and *P. vivax* (Figure 4A and B) antibody seroprevalence were observed at the cluster level in both Arjo and Gambella. Regarding the *P. falciparum* antibody panel, the widest variations in cluster-level seroprevalence in Arjo were observed for *Pf*MSP-1_{42} (mean ± SD, 65.58 ± 19.44%) and *Pf*AMA-1 (66.83 ± 17.78%; Supplemental Table 2). In comparison, mean *Pf*MSP-1_{42} and *Pf*AMA-1 seroprevalence in the Gambella study clusters were 97.42 ± 3.08% and 96.03 ± 2.63%, respectively (Supplemental Table 2). We observed no *Pf*CSP-seropositive individuals in cluster CBL in Arjo, and seroprevalence did not exceed 30% across the other 14 clusters of Arjo. *Pf*CSP seroprevalence was higher overall in Gambella (42.37 ± 19.29%). The range of seroprevalence for all four *P. vivax* antibodies was similar across study clusters in Arjo (Figure 4A and B). In Gambella, variations in seroprevalence at the cluster level varied depending on the antigen target. Small variations across clusters were observed in antibody seroprevalence to *Pv*DBPII(Sal1) and *Pv*MSP-1_{19} (64.68 ± 4.62% and 88.42 ± 6.61%, respectively), whereas greater cluster-level variations were observed in the seroprevalence of antibodies against *Pv*RBP2b (60.46 ± 25.30%) and *Pv*EBP2 (69.04 ± 16.24%).

Variations in antibody levels. Because seropositivity is a binary measure that is subject to bias, we also looked at antibody levels (expressed as MFIs). We found that MFI values against all 10 antigen targets were on average greater in Gambella than in Arjo (Supplemental Table 3). The distributions of MFI values were significantly different between Arjo and Gambella for all antibodies (Wilcoxon rank-sum test,

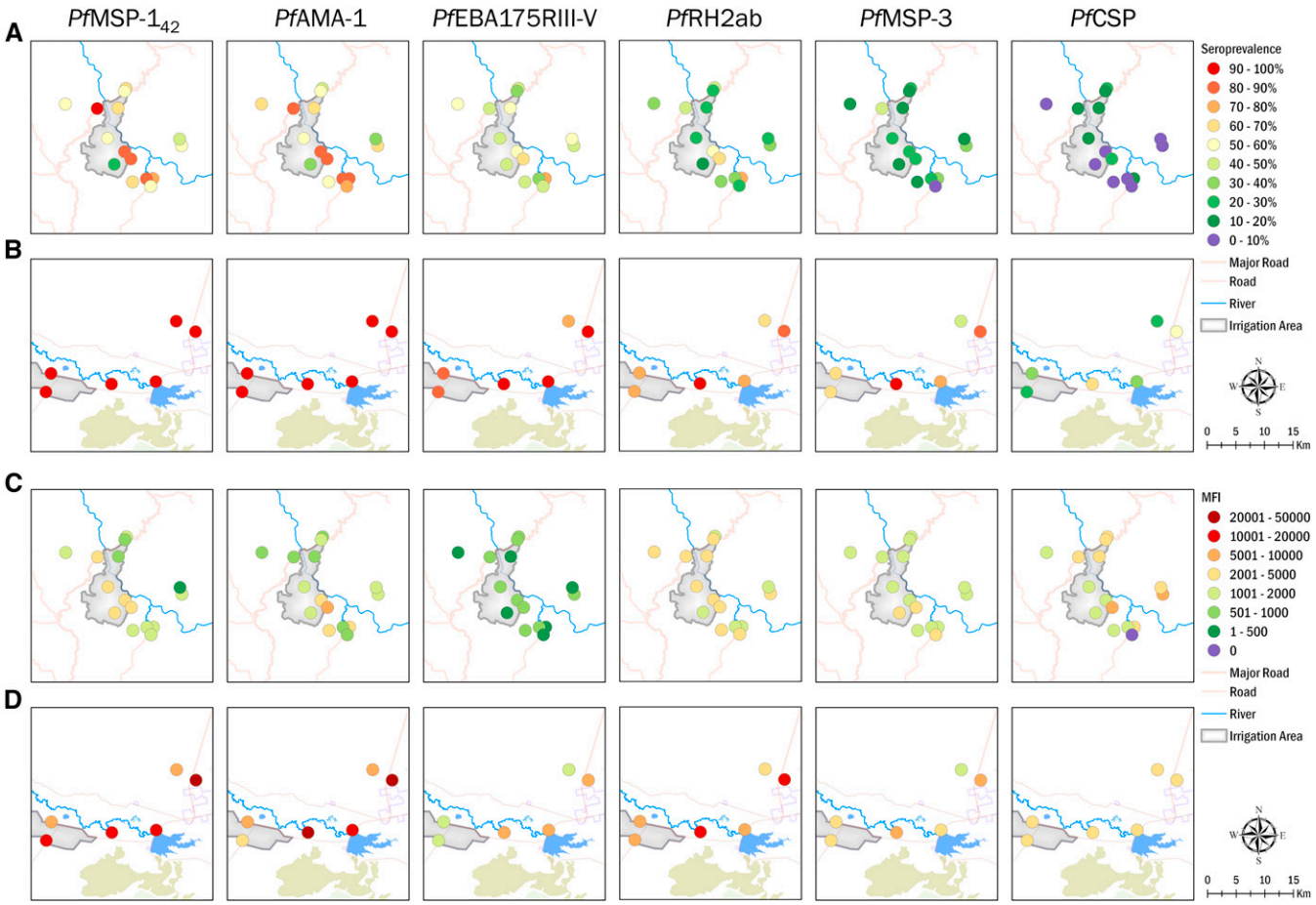

FIGURE 3. Cluster-level seroprevalence of antibodies against *Plasmodium falciparum* antigens in (**A**) Arjo (low-transmission area) and (**B**) Gambella (high-transmission area). Cluster-level median *P. falciparum* antibody mean fluorescence intensity (MFI) values in (**C**) Arjo and (**D**) Gambella in October 2018.

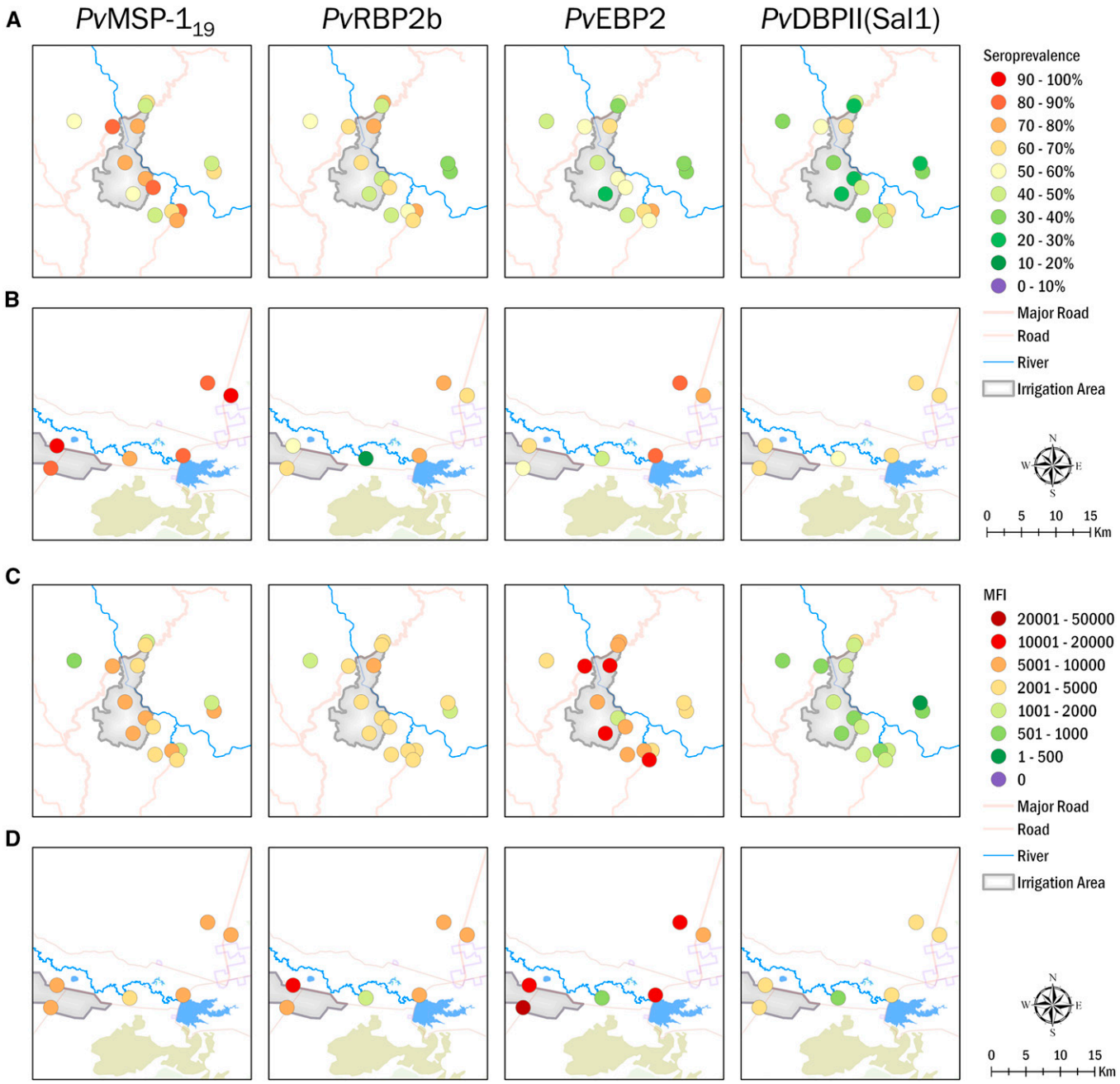

FIGURE 4. Cluster-level seroprevalence of antibodies against *Plasmodium vivax* antigens in (**A**) Arjo (low-transmission area) and (**B**) Gambella (high-transmission area). Cluster-level median *P. vivax* antibody mean fluorescence intensity (MFI) values in (**C**) Arjo and (**D**) Gambella in October 2018.

$P < 0.001$) except *Pf*CSP and *Pv*EBP2 (Figure 5). Spatial heterogeneity was observed in antibody responses against *P. falciparum* (Figure 3C and D) and *P. vivax* (Figure 4C and D) antigens across study clusters in Arjo and Gambella.

Predictors of *P. falciparum* antibody seropositivity. In Arjo, the odds of seropositivity for *Pf*AMA-1, *Pf*MSP-1$_{42}$, *Pf*MSP-3, and *Pf*RH2ab antibodies were higher among individuals aged 30 years or older compared with people 15–29 years old (Table 3). Male sex was also predictive of odds of seropositivity for these four antibodies. Agricultural occupation was associated with antibody seropositivity, but the association was only statistically significant for *Pf*AMA-1 and *Pf*RH2ab. No statistically significant associations were observed between antibody seropositivity and household-level covariates (i.e., distance to irrigation scheme and IRS within the past year) or personal protective measures (i.e., bednet usage). In Gambella, age (≥ 30 years old) was associated with increased odds of seropositivity, but the association was only statistically significant for *Pf*AMA-1 and *Pf*EBA175RIII-V antibodies (Table 4). The odds of *Pf*MSP-3 seropositivity were lower among participants who used bednets compared with those who did not (AOR: 0.41; 95% CI: 0.23–0.73). No other statistically significant associations were observed between the covariates of interest and

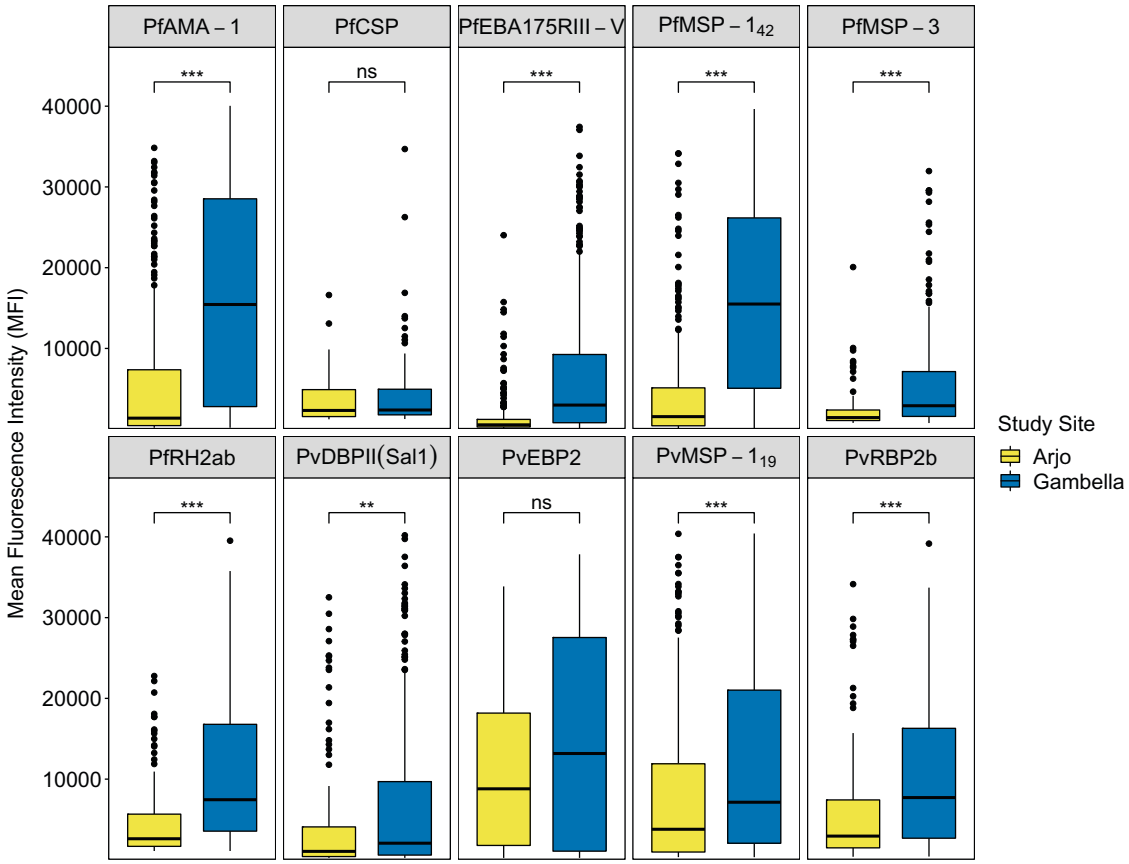

FIGURE 5. Antibody levels, expressed as mean fluorescence intensities (MFIs), among seropositive individuals in Arjo (yellow) and Gambella (blue) in October 2018. Asterisks indicate *P* values adjusted for multiple comparisons (ns = not significant; $^{**}P < 0.01$, $^{***}P < 0.001$).

seropositivity for any of the six *P. falciparum* antibodies in our serological panel.

Predictors of *P. vivax* antibody seropositivity. In Arjo, the odds of *P. vivax* seropositivity were greater among individuals 30 years or older, but the association between age and seropositivity was only statistically significant for *Pv*DBPII(Sal1) (AOR: 1.76; 95% CI: 1.14–2.73). Men were more likely to be seropositive for *P. vivax* antibodies, particularly for *Pv*EBP2 (AOR: 2.10; 95% CI: 1.39–3.19) and *Pv*RBP2b (AOR: 1.51; 95% CI: 1.01–2.26; Table 3). Similarly, in Gambella, the odds of *P. vivax* seropositivity trended higher among individuals aged 30 years or older compared with people under 30, among men compared with women, and among residents living within 500 m of an irrigation scheme compared with residents who lived ≥ 500 m from an irrigation scheme; however, none of the associations were statistically significant. Participants residing in a household that underwent IRS within the year leading up to the serological survey were two to three times more likely to be seropositive for *Pv*DBPII(Sal1), *Pv*EBP2, and *Pv*RBP2b (Table 4).

DISCUSSION

The study adds to the currently limited literature on serological studies concurrently evaluating *P. falciparum* and *P. vivax* in Ethiopia.[27–31] Here, we demonstrate the complementary value that our serological panel of six *P. falciparum* and four *P. vivax* antigens adds to characterizing malaria in declining transmission settings.

We identified only a few blood-stage *P. falciparum* and *P. vivax* infections in Arjo and Gambella, despite using highly sensitive and species-specific qPCR. Because antibodies persist beyond the duration of an active infection, antibody data collected from the same participants can potentially complement surveillance data and serve as sensitive markers of malaria exposure. Despite the low infection prevalence in both study sites, antibody seropositivity suggested differences in recent and historic malaria exposure. Seroprevalence was higher in Gambella than in Arjo for all 10 antibodies, as expected considering the higher prevalence of infection observed in Gambella. Fold differences in antibody seroprevalence (Gambella versus Arjo) were greatest for *Pf*CSP (3.49-fold) and *Pf*MSP-3 (2.96-fold), suggesting that *Pf*CSP and *Pf*MSP-3 may be associated with recent exposure and higher transmission intensity. However, longitudinal studies characterizing the duration of antibody responses and prospective studies capturing newly acquired infections are necessary to investigate this potential association. Our observations align with a 2015 study in three administrative regions of northern Ethiopia that found relatively high antibody seropositivity despite low infection prevalence.[28]

Individual-level risk factors of seropositivity varied by antigen target as well as study site. Notably, IRS in the previous year was associated with increased odds of *P. vivax* seropositivity only in Gambella. Increased odds of malaria exposure in households that underwent IRS may reflect the vector species composition, vectorial capacity, and outdoor biting behavior of malaria vectors in the study area. Although effective IRS

TABLE 3

AORs and 95% CIs from multivariable mixed effects logistic regressions of antibody seropositivity in Arjo, Ethiopia, October 2018

	Plasmodium falciparum antibody seropositivity, AOR (95% CI)						*Plasmodium vivax* antibody seropositivity, AOR (95% CI)			
Characteristic	*Pf*AMA-1	*Pf*CSP	*Pf*EBA175RIII-V	*Pf*MSP-1_{42}	*Pf*MSP-3	*Pf*RH2ab	*Pv*DBPII(Sal1)	*Pv*EBP2	*Pv*MSP-1_{19}	*Pv*RBP2b
Age										
Ref: < 30 years old	**2.62** (1.59, 4.30)	1.47 (0.75, 2.89)	1.34 (0.89, 2.02)	**2.10** (1.28, 3.45)	**1.78** (1.09, 2.91)	**1.64** (1.05, 2.58)	**1.76** (1.14, 2.73)	1.45 (0.94, 2.22)	1.50 (0.97, 2.33)	1.30 (0.85, 1.99)
Sex										
Ref: female	**1.88** (1.18, 2.98)	1.23 (0.65, 2.33)	1.46 (0.99, 2.16)	**3.04** (1.87, 4.95)	**1.86** (1.17, 2.97)	**2.54** (1.66, 3.91)	1.39 (0.91, 2.11)	**2.10** (1.39, 3.19)	1.57 (1.04, 2.39)	**1.51** (1.01, 2.26)
Occupation										
Ref: nonagricultural worker	**1.85** (1.12, 3.05)	1.88 (0.90, 3.93)	1.51 (0.98, 2.33)	1.60 (0.95, 2.70)	1.42 (0.83, 2.43)	**2.07** (1.27, 3.38)	1.37 (0.85, 2.21)	0.94 (0.60, 1.50)	1.47 (0.92, 2.34)	0.92 (0.59, 1.46)
Bednet usage										
Ref: no usage	1.00 (0.57, 1.74)	1.39 (0.64, 2.99)	0.85 (0.57, 1.27)	1.21 (0.68, 2.17)	0.85 (0.51, 1.43)	0.81 (0.49, 1.37)	1.24 (0.76, 2.01)	1.37 (0.85, 2.19)	1.44 (0.88, 2.33)	1.34 (0.85, 2.12)
Distance to irrigation scheme										
Ref: ≥ 500 m	1.55 (0.71, 3.41)	2.10 (0.86, 5.13)	1.08 (0.68, 1.72)	1.44 (0.59, 3.51)	1.13 (0.60, 2.14)	1.45 (0.76, 2.78)	1.12 (0.59, 2.13)	1.23 (0.70, 2.17)	1.56 (0.81, 3.01)	1.18 (0.66, 2.11)
IRS in the past year										
Ref: no IRS	0.83 (0.46, 1.51)	1.29 (0.53, 3.12)	1.24 (0.78, 1.97)	0.58 (0.31, 1.09)	0.99 (0.54, 1.78)	0.74 (0.41, 1.31)	0.99 (0.57, 1.71)	0.69 (0.40, 1.17)	0.89 (0.52, 1.53)	0.88 (0.52, 1.49)

AOR = adjusted odds ratio; 95% CIs = 95% confidence intervals; IRS = indoor residual spraying. Bolded AORs indicate that the corresponding 95% CIs do not include the null value of 1.0.

TABLE 4

AORs and 95% CIs from multivariable mixed effects logistic regressions of antibody seropositivity in Gambella, Ethiopia, October 2018

	Plasmodium falciparum antibody seropositivity, AOR (95% CI)						*Plasmodium vivax* antibody seropositivity, AOR (95% CI)			
Characteristic	*Pf*AMA-1	*Pf*CSP	*Pf*EBA175RIII-V	*Pf*MSP-1_{42}	*Pf*MSP-3	*Pf*RH2ab	*Pv*DBPII(Sal1)	*Pv*EBP2	*Pv*MSP-1_{19}	*Pv*RBP2b
Age										
Ref: < 30 years old	**5.34** (1.05, 27.17)	1.61 (0.97, 2.69)	**2.18** (1.01, 4.72)	6.18 (0.65, 58.60)	1.29 (0.74, 2.24)	1.77 (0.96, 3.26)	1.15 (0.69, 1.89)	1.01 (0.59, 1.72)	1.80 (0.78, 4.12)	1.41 (0.81, 2.43)
Sex										
Ref: female	0.85 (0.23, 3.09)	0.88 (0.52, 1.51)	1.59 (0.74, 3.45)	2.08 (0.44, 9.72)	1.14 (0.63, 2.06)	1.35 (0.71, 2.55)	1.20 (0.71, 2.04)	1.16 (0.66, 2.05)	1.80 (0.78, 4.16)	1.03 (0.58, 1.83)
Occupation										
Ref: nonagricultural worker	0.57 (0.15, 2.10)	0.94 (0.50, 1.77)	0.98 (0.41, 2.53)	0.32 (0.06, 1.59)	1.82 (0.83, 3.99)	1.61 (0.70, 3.69)	1.26 (0.70, 2.27)	1.41 (0.72, 2.75)	2.63 (0.99, 6.94)	0.92 (0.45, 1.87)
Bednet usage										
Ref: no usage	0.25 (0.05, 1.24)	1.11 (0.66, 1.88)	0.93 (0.45, 1.92)	0.19 (0.020, 1.74)	**0.41** (0.23, 0.73)	0.55 (0.29, 1.03)	0.65 (0.39, 1.09)	0.85 (0.49, 1.44)	0.80 (0.35, 1.85)	1.03 (0.60, 1.78)
Distance to irrigation scheme										
Ref: ≥ 500 m	1.69 (0.33, 8.63)	0.41 (0.13, 1.31)	0.41 (0.11, 1.47)	0.03 (0.0005, 2.45)	0.44 (0.10, 1.83)	0.90 (0.15, 5.35)	1.84 (0.83, 4.07)	1.33 (0.45, 3.93)	2.10 (0.62, 7.10)	3.47 (0.64, 18.89)
IRS in the past year										
Ref: no IRS	3.09 (0.63, 15.08)	0.59 (0.28, 1.26)	0.72 (0.21, 2.46)	0.16 (0.0024, 11.03)	0.69 (0.28, 1.72)	2.07 (0.78, 5.47)	**2.39** (1.17, 4.90)	**2.55** (1.17, 5.55)	1.35 (0.46, 3.99)	**3.79** (1.62, 8.90)

AOR = adjusted odds ratio; 95% CIs = 95% confidence intervals; IRS = indoor residual spraying. Bolded AORs indicate that the corresponding 95% CIs do not include the null value of 1.0.

can reduce indoor biting rates, outdoor biting by anopheline mosquitoes can continue to drive malaria transmission. In vector surveys conducted within the same year as the present study, we found that *Anopheles gambiae* s.l. was the dominant vector in both Arjo and Gambella, followed by the *Anopheles coustani* group in Arjo and *Anopheles pharoensis* in Gambella.[18,32,33] Mosquito densities, particularly of *An. pharoensis* and *An. coustani*, were greater outdoors than indoors, suggesting that these mosquitoes were less likely to be targeted effectively through IRS.[18,33] The entomological inoculation rates of the major anopheline species were not measured in Arjo.[32,33] In Gambella, the highest monthly *P. falciparum* EIR was 3.8 infective bites/person/month for both *An. gambiae* s.l. as well as the *Anopheles funestus* group. The highest monthly *P. vivax* EIR was 16.0 infective bites/person/month for *An. pharoensis*.[18] Collectively, these findings could potentially explain the differences in *P. vivax* exposure that we observed at the site level and by household IRS status within Gambella.

We did not find any statistically significant increases in the odds of *P. falciparum* seropositivity at either site among residents in IRS households. However, a study conducted in Oromia Regional State in Ethiopia found that IRS was associated with increased risk of both *P. falciparum* and *P. vivax* infection in school-based surveys, likely because of the fact that residing in an area in which the National Malaria Control Program conducts IRS is predictive of exposure to *Plasmodium* infection.[34] In Ethiopia, IRS is typically performed in targeted districts by the government or nongovernmental agencies (D. Y., unpublished data). Interestingly, more than half of the study participants in both sites reported that their houses were not sprayed in the previous year despite residing in IRS-targeted districts. Because residents were required to vacate their homes and remove household items for IRS, recall bias seems an unlikely cause of the discrepancy observed. The high proportion of "no IRS" responses we noted might instead reflect knowledge gaps regarding this form of vector control. Additionally, participants who responded "no" to their homes being sprayed may differ from participants who responded "yes" on factors such as working in agriculture or travel away from home, which would similarly affect their risk of malaria exposure.[35] More detailed survey questions regarding participants' experiences with IRS paired with qualitative approaches (e.g., focus group discussions or in-depth interviews) could reduce recall bias while providing insight into the potential receptiveness of these communities to future rounds of IRS or other vector control measures. Interrogating travel history and human behavior could additionally shed light on risk factors of exposure that were missed in our study.

We did not observe significant associations between living within 500 m of an irrigation scheme and odds of seropositivity for any *P. falciparum* or *P. vivax* antibodies in Arjo or Gambella. One potential explanation for the lack of association between household proximity to an irrigation scheme and antibody seropositivity could be that exposure to *Plasmodium* occurs away from the home. Although there were too few malaria infections to be included in our statistical analyses, we observed that the few infections in Arjo were not geographically clustered. In most clusters, there were no *P. falciparum* or *P. vivax* infections found, yet cluster-level antibody seroprevalence varied widely (Supplemental Tables 4A and 5A). Interestingly, malaria infections in Gambella appeared to cluster near the irrigation scheme (clusters BRA and GRC) and Alwero Dam (cluster TER). Whereas seroprevalence for all six *P. falciparum* targets was similar among clusters BRA, GRC, and TER, higher seroprevalences were observed in clusters TER and V13, where there was only one infection and no infections detected, respectively (Supplemental Table 4B). Similarly, most *P. vivax* infections were found in clusters BRA and GRC, yet the seroprevalences for all four *P. vivax* targets were highest in clusters where no infections were found (clusters V12 and V13; Supplemental Table 5B). The lack of significant association between village-level seroprevalence and parasite prevalence for *P. falciparum* or *P. vivax* was also observed in a 2017 study conducted in Babile district, Oromia.[29] Our findings underscore the need for using multiple metrics of infection and exposure to improve our understanding of the spatial heterogeneities in malaria transmission.

Currently, there are no international standards for defining seropositivity. The use of multiple methods (e.g., receiver operating curves, finite mixture models, and quantiles) and technical variability across laboratories can lead to inconsistencies in deriving and interpreting results.[36] In this study, we used a presumed unexposed (i.e., malaria-naïve) control group from North America to determine seropositivity cutoff values, which may have resulted in misclassification of serostatus. For example, the control group may have differed from the study population in terms of genetic factors and nutritional status that could have affected immune responses.[36] The control group may also have unknowingly been exposed to *Plasmodium* spp. or other pathogens that elicited cross-reactive antibodies. Given that most of Ethiopia's population is at risk of malaria, an endemic seronegative control group more closely matched study participants on the aforementioned factors would have been difficult to identify. Thus, the use of sera from individuals who, to the best of our knowledge, had no prior exposure to *Plasmodium* spp. was a practical, logistically feasible approach.

Population-level variations in kinetics and seroprevalence can be used to measure the impact of interventions, determine risk stratification, and identify foci to target with immediate action (e.g., IRS, mass drug administration, or point-of-contact testing).[11] One key limitation of our study was the absence of serological data that span all ages, which limits generalizability of our findings to the broader community even within our two study sites. Age-specific seropositivity rates can be used to calculate seroconversion rates.[10] Because children are less likely to have acquired infections outside of the study area than adults, antibody responses in children could provide insight into transmission exposure within the study area as opposed to exposure encountered beyond the study area, away from home. Our study originally intended to capture antibody responses across the age spectrum. However, parents and guardians in both sites did not provide consent for individuals under 15 to participate in blood surveys. Although there was a clear bias toward individuals aged 15 and older in our study populations, one strength of our study was that no participants exhibited any symptoms of malaria during the survey. Thus, we do not believe there was any systematic bias in our study recruitment on the basis of symptomology or parasite density.

Another limitation of this study was the lack of follow-up data. Although antibody seropositivity is an easily interpretable

metric that can complement standard disease surveillance, it is subject to the biases described above. Antibody responses can further augment our understanding of malaria transmission, but measurements at a single time point are insufficient for determining antibody half-lives and inferring changes in transmission intensity. With longitudinal sampling, fluctuations in antibody response levels can be evaluated in the context of reinfection events and used to identify immune factors that mediate protection against *Plasmodium* infection.[37] For highly immunogenic antigens (e.g., *Pf*AMA-1 and *Pf*MSP-1_{42} in our study), antibody levels are likely to be more sensitive to changes over time compared with antibody seropositivity; antibody seropositivity is more informative than antibody response levels when less immunogenic antigens (e.g., *Pf*CSP) are concerned.[9,38] In instances where longitudinal sampling is not feasible, meaningful inferences can still be drawn from cross-sectional surveys. Arnold et al. demonstrated consistency in estimates of *Pf*MSP-1_{42} seroconversion rates derived from a longitudinal, prospective cohort study and a concurrent cross-sectional survey in Haiti, suggesting that cross-sectional data, as were collected in our study, can be leveraged in monitoring malaria transmission following deployment of interventions.[39]

CONCLUSION

To date, few serological studies have concurrently evaluated responses against *P. falciparum* and *P. vivax* antigen targets in Ethiopia.[27–31] Our findings provide baseline data on antibody responses at two National Institutes of Health-supported sub-Saharan Africa International Center of Excellence for Malaria Research study sites where anthropogenic environmental modifications, shifting agricultural practices, and scaling-up of malaria control efforts continue to alter malaria transmission and epidemiology. The observed variations in antibody prevalence and levels between and within sites of contrasting transmission intensity suggest the potential for adapting this serological panel for monitoring spatiotemporal trends of malaria transmission intensities after the implementation of control interventions. Our study illustrates the specificity of serological responses to particular geographic areas and populations. This study also revealed unanticipated challenges in sampling that must be addressed via enhanced community engagement. Future studies should aim to assess additional sociodemographic and behavioral factors associated with exposure to *Plasmodium* parasites, measure the duration of antibody responses, and identify antibody correlates of protection against clinical disease.

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REFERENCES

1. Ketema T, Bacha K, Getahun K, del Portillo HA, Bassat Q, 2021. *Plasmodium vivax* epidemiology in Ethiopia 2000–2020: a systematic review and meta-analysis. *PLoS Negl Trop Dis 15:* e0009781.
2. Price RN, Commons RJ, Battle KE, Thriemer K, Mendis K, 2020. *Plasmodium vivax* in the era of the shrinking *P. falciparum* map. *Trends Parasitol 36:* 560–570.
3. World Health Organization, 2018. *World Malaria Report 2018.* Geneva, Switzerland: WHO.
4. Lo E et al., 2017. Transmission dynamics of co-endemic *Plasmodium vivax* and *P. falciparum* in Ethiopia and prevalence of antimalarial resistant genotypes. *PLoS Negl Trop Dis 11:* e0005806.
5. Hailemeskel E et al., 2019. Prevalence of *Plasmodium falciparum* Pfcrt and Pfmdr1 alleles in settings with different levels of *Plasmodium vivax* co-endemicity in Ethiopia. *Int J Parasitol Drugs Drug Resist 11:* 8–12.
6. Ethiopian Public Health Institute, 2016. *Ethiopia National Malaria Indicator Survey 2015.* Addis Ababa, Ethiopia: Ethiopian Public Health Institute, Ministry of Health.
7. Tusting LS, Bousema T, Smith DL, Drakeley C, 2014. Measuring changes in *Plasmodium falciparum* transmission: precision, accuracy and costs of metrics. *Adv Parasitol 84:* 151–208.
8. Auburn S, Cheng Q, Marfurt J, Price RN, 2021. The changing epidemiology of *Plasmodium vivax*: insights from conventional and novel surveillance tools. *PLoS Med 18:* e1003560.
9. Cook J, Kleinschmidt I, Schwabe C, Nseng G, Bousema T, Corran PH, Riley EM, Drakeley CJ, 2011. Serological markers suggest heterogeneity of effectiveness of malaria control interventions on Bioko Island, Equatorial Guinea. *PLoS One 6:* e25137.
10. Pothin E, Ferguson NM, Drakeley CJ, Ghani AC, 2016. Estimating malaria transmission intensity from *Plasmodium falciparum* serological data using antibody density models. *Malar J 15:* 79.
11. Greenhouse B et al., 2019. Priority use cases for antibody-detecting assays of recent malaria exposure as tools to achieve and sustain malaria elimination. *Gates Open Res 3:* 131.
12. Corran P, Coleman P, Riley E, Drakeley C, 2007. Serology: a robust indicator of malaria transmission intensity? *Trends Parasitol 23:* 575–582.
13. King CL, Davies DH, Felgner P, Baum E, Jain A, Randall A, Tetteh K, Drakeley CJ, Greenhouse B, 2015. Biosignatures of exposure/transmission and immunity. *Am J Trop Med Hyg 93:* 16–27.

14. Aka KG et al., 2021. Influence of host-related factors and exposure to mosquito bites on the dynamics of antibody response to *Plasmodium falciparum* antigens. *Trop Med Infect Dis 6:* 185.
15. Weber GE et al., 2017. Sero-catalytic and antibody acquisition models to estimate differing malaria transmission intensities in western Kenya. *Sci Rep 7:* 16821.
16. Duffy PE, Patrick Gorres J, 2020. Malaria vaccines since 2000: progress, priorities, products. *NPJ Vaccines 5:* 48.
17. Hawaria D, Getachew H, Zhong G, Demissew A, Habitamu K, Raya B, Lee M-C, Yewhalaw D, Yan G, 2019. Ten years malaria trend at Arjo-Didessa sugar development site and its vicinity, southwest Ethiopia: a retrospective study. *Malar J 18:* 145.
18. Haileselassie W et al., 2021. The effect of irrigation on malaria vector bionomics and transmission intensity in western Ethiopia. *Parasit Vectors 14:* 516.
19. Degife AW, Zabel F, Mauser W, 2021. Climate change impacts on potential maize yields in Gambella Region, Ethiopia. *Reg Environ Change 21:* 60.
20. Bereczky S, Mårtensson A, Gil JP, Färnert A, 2005. Short report: rapid DNA extraction from archive blood spots on filter paper for genotyping of *Plasmodium falciparum*. *Am J Trop Med Hyg 72:* 249–251.
21. Zhong D, Hemming-Schroeder E, Wang X, Kibret S, Zhou G, Atieli H, Lee M-C, Afrane YA, Githeko AK, Yan G, 2020. Extensive new *Anopheles* cryptic species involved in human malaria transmission in western Kenya. *Sci Rep 10:* 16139.
22. Longley RJ et al., 2020. Development and validation of serological markers for detecting recent *Plasmodium vivax* infection. *Nat Med 26:* 741–749.
23. Mazhari R et al., 2020. A comparison of non-magnetic and magnetic beads for measuring IgG antibodies against *Plasmodium vivax* antigens in a multiplexed bead-based assay using Luminex technology (Bio-Plex 200 or MAGPIX). *PLoS One 15:* e0238010.
24. Ondigo BN, Park GS, Gose SO, Ho BM, Ochola LA, Ayodo GO, Ofulla AV, John CC, 2012. Standardization and validation of a cytometric bead assay to assess antibodies to multiple *Plasmodium falciparum* recombinant antigens. *Malar J 11:* 427.
25. Perraut R, Varela M-L, Mbengue B, Guillotte M, Mercereau-Puijalon O, Vigan-womas I, 2015. Standardization of a multiplex magnetic bead-based for simultaneous detection of IgG to *Plasmodium* antigens. *J Immunol Tech Infect Dis 4:* 1–8.
26. Gebremariam EB, 2017. The politics of youth employment and policy processes in Ethiopia. *IDS Bull 48:* 3.
27. Feleke SM, Brhane BG, Mamo H, Assefa A, Woyessa A, Ogawa GM, Cama V, 2019. Sero-identification of the aetiologies of human malaria exposure (*Plasmodium* spp.) in the Limu Kossa district of Jimma zone, south western Ethiopia. *Malar J 18:* 292.
28. Leonard CM, Assefa A, Sime H, Mohammed H, Kebede A, Solomon H, Drakeley C, Murphy M, Hwang J, Rogier E, 2022. Spatial distribution of *Plasmodium falciparum* and *Plasmodium vivax* in northern Ethiopia by microscopic, rapid diagnostic test, laboratory antibody, and antigen data. *J Infect Dis 225:* 881–890.
29. Keffale M et al., 2019. Serological evidence for a decline in malaria transmission following major scale-up of control efforts in a setting selected for *Plasmodium vivax* and *Plasmodium falciparum* malaria elimination in Babile district, Oromia, Ethiopia. *Trans R Soc Trop Med Hyg 113:* 305–311.
30. Assefa A et al., 2019. Multiplex serology demonstrate cumulative prevalence and spatial distribution of malaria in Ethiopia. *Malar J 18:* 246.
31. Ashton RA et al., 2015. Geostatistical modeling of malaria endemicity using serological indicators of exposure collected through school surveys. *Am J Trop Med Hyg 93:* 168–177.
32. Hawaria D, Demissew A, Kibret S, Lee M-C, Yewhalaw D, Yan G, 2020. Effects of environmental modification on the diversity and positivity of anopheline mosquito aquatic habitats at Arjo-Dedessa irrigation development site, southwest Ethiopia. *Infect Dis Poverty 9:* 9.
33. Demissew A, Hawaria D, Kibret S, Animut A, Tsegaye A, Lee M-C, Yan G, Yewhalaw D, 2020. Impact of sugarcane irrigation on malaria vector *Anopheles* mosquito fauna, abundance and seasonality in Arjo-Didessa, Ethiopia. *Malar J 19:* 344.
34. Ashton RA, Kefyalew T, Tesfaye G, Pullan RL, Yadeta D, Reithinger R, Kolaczinski JH, Brooker S, 2011. School-based surveys of malaria in Oromia Regional State, Ethiopia: a rapid survey method for malaria in low transmission settings. *Malar J 10:* 25.
35. Alemu K, Worku A, Berhane Y, Kumie A, 2014. Men traveling away from home are more likely to bring malaria into high altitude villages, northwest Ethiopia. *PLoS One 9:* e95341.
36. Chan Y, Fornace K, Wu L, Arnold BF, Priest JW, Martin DL, Chang MA, Cook J, Stresman G, Drakeley C, 2021. Determining seropositivity – a review of approaches to define population seroprevalence when using multiplex bead assays to assess burden of tropical diseases. *PLoS Negl Trop Dis 15:* e0009457.
37. Markwalter CF, Nyunt MH, Han ZY, Henao R, Jain A, Taghavian O, Felgner PL, Han KT, Nyunt MM, Plowe CV, 2021. Antibody signatures of asymptomatic *Plasmodium falciparum* malaria infections measured from dried blood spots. *Malar J 20:* 378.
38. Ondigo BN, Hamre KES, Frosch AEP, Ayodo G, White MT, John CC, 2020. Antibody profiles to *P. falciparum* antigens over time characterize acute and long-term malaria exposure in an area of low and unstable transmission. *Am J Trop Med Hyg 103:* 2189–2197.
39. Arnold BF, Priest JW, Hamlin KL, Moss DM, Colford JM Jr, Lammie PJ, 2014. Serological measures of malaria transmission in Haiti: comparison of longitudinal and cross-sectional methods. *PLoS One 9:* e93684.