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Spatial Distribution of *Plasmodium falciparum* and *Plasmodium vivax* in Northern Ethiopia by Microscopic, Rapid Diagnostic Test, Laboratory Antibody, and Antigen Data

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Abstract

Background.—Determining malaria transmission within regions of low, heterogenous prevalence is difficult. A variety of malaria tests exist and range from identification of diagnostic infection to testing for prior exposure. This study describes the concordance of multiple malaria tests using data from a 2015 household survey conducted in Ethiopia.

Methods.—Blood samples (n = 2279) from 3 regions in northern Ethiopia were assessed for *Plasmodium falciparum* and *Plasmodium vivax* by means of microscopy, rapid diagnostic test, multiplex antigen assay, and multiplex assay for immunoglobulin G (IgG) antibodies. Geospatial analysis was conducted with spatial scan statistics and kernel density estimation to identify malaria hot spots by different test results.

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Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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Results.—The prevalence of malaria infection was low (1.4% by rapid diagnostic test, 1.0% by microscopy, and 1.8% by laboratory antigen assay). For *P. falciparum*, overlapping spatial clusters for all tests and an additional 5 unique IgG clusters were identified. For *P. vivax*, clusters identified with bead antigen assay, microscopy, and IgG partially overlapped.

Conclusions.—Assessing the spatial distribution of malaria exposure using multiple metrics can improve the understanding of malaria transmission dynamics in a region. The relative abundance of antibody clusters indicates that in areas of low transmission, IgG antibodies are a more useful marker to assess malaria exposure.

Keywords

Plasmodium falciparum ; *Plasmodium vivax* ; infection test; antibodies; geospatial analysis; GIS; Ethiopia

Over the past decade, malaria cases and deaths have declined worldwide, though malaria still remains a major public health problem in many parts of the world [1]. In 2010, the World Health Organization recommended that all suspected cases of malaria be confirmed by microscopy or rapid diagnostic test (RDT) [2]. RDTs detect malaria antigens such as *Plasmodium falciparum* histidine-rich protein 2 (HRP2), and/or pan-*Plasmodium* lactate dehydrogenase (pLDH), and they allow for quick and practical detection of malaria infection [3]. The more traditional method to diagnose malaria is microscopic examination of blood films to identify *Plasmodium* parasites [4]. The sensitivity of microscopy for diagnosis varies (ranging from 50% to 90%) based on the transmission intensity in a region, expertise of the microscopist, and magnitude of parasitemia in the sample [5-8]. In addition to these diagnostic tests, antibody tests detect prior malaria exposure occurring months to years in the past.

Malaria elimination in a population requires identifying areas where transmission is occurring [9], and neither RDTs nor microscopy are able to detect very low parasite densities (<100/μL), which can be prevalent in low-transmission settings [10-13]. However, more sensitive laboratory-based tests are available to detect malaria infection [14, 15]. The multiplex bead antigen assay simultaneously detects multiple antigens from blood samples with concentrations approximately 200 pg/mL for PfLDH, 100 pg/mL for *Plasmodium* aldolase, and as low as 1 pg/mL for HRP2 (parasite density for all, <1/μL) [15, 16]. These detection limits provide sensitivity comparable to PCR. The presence of *Plasmodium* aldolase or pLDH antigens indicates active infection [17, 18], whereas the presence of HRP2 indicates active or recent infection, with HRP2 clearance in systemic circulation occurring 4–7 weeks after treatment [18, 19]. The presence of anti-*Plasmodium* antibodies can serve as a proxy for prior exposure to malaria parasites, and species-specific immunoglobulin G (IgG) antibodies are known to be produced against *P. falciparum* and *Plasmodium vivax* [20-22]. Assessing population-level antibody responses to *Plasmodium* species can provide an estimate of historical transmission intensity in a region [22-26].

Ethiopia has a very low malaria prevalence nationally (1.2% by RDT) [27] and aims to achieve malaria elimination by 2030 [28]. Even so, about 60% of the population remains at risk, and transmission is highly heterogenous throughout the country [27]. Compared with

the rest of the country, the northwest region has a relatively high burden of malaria [29]. Ethiopia is coendemic for *P. falciparum* and *P. vivax* malaria [1], with approximately 60% of cases due to *P. falciparum* and approximately 40% due to *P. vivax* [30]. To compare the results of multiple tests for malaria infection or exposure, RDT, microscopy, bead antigen assay, and antibody detection assay were conducted on each blood sample. The goal of this study was to assess concordance among the different test results and compare statistically significant hot spots (spatial clusters) of malaria based on the different tests to indicate areas of malaria transmission.

METHODS

Study Design

The study data were obtained from the 2015 Ethiopia Malaria Indicator Survey (MIS), which was conducted from 30 September to 10 December 2015 (coinciding with the high malaria transmission season). The MIS used a 2-stage cluster-randomized sampling technique to select 555 enumeration areas (kebeles/villages) proportional to population size and 25 households randomly selected per enumeration area. Every child <5 years (6–59 months) old in each selected household and all persons in every fourth household were eligible for malaria testing. Survey enumerators recorded the global positioning system (GPS) coordinates of each household [27]. Before enrollment in the MIS, informed consent form was read to each participant in the appropriate local language, and verbal informed consent was obtained. For children <5 years old, the parent's consent was obtained before any blood sample was collected.

The study protocol was approved by the Ethiopian Public Health Institute Scientific and Ethical Review Committee and the ethical review committees of Malaria Control and Elimination Partnership in Africa/PATH and the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia (no. 2015-244). Laboratory assays for antigen and IgG antibody detection were conducted at the CDC in Atlanta, and researchers did not have access to identifying information. Data are available on request to the corresponding author and approval from the Ethiopian Public Health Institute.

Study Area and Population

The study involved the 3 northern administrative regions of Ethiopia (Amhara, Tigray, Afar; Figure 1). The physical environment is heterogenous, including both mountains and desert and altitude ranges from below sea level to >2500 m above sea level [31]. The sample data set for analysis (n = 2279) consisted of persons who had results for all 4 tests (RDT, microscopy, multiplex bead antigen assay, and IgG antibody detection).

Microscopy and RDTs

Whole-blood samples were tested using the Carestart (AccessBio) multispecies RDT, testing for the presence of HRP2 and pLDH antigens. Participants with a positive RDT result were immediately treated for malaria according to the national malaria treatment guidelines [27]. Thick and thin blood smears for microscopy were made on one slide and stained with 3% Giemsa, and the slides were read by microscopists at the Ethiopian Public Health Institute.

A slide was considered negative if no *Plasmodium* asexual forms or gametocytes were identified after reading 100 fields. All positive slides and 5% of negative slides from each region were reread at the Adama Malaria Control Reference Center.

Multiplex Antigen and IgG Detection Assays

Reagent preparation and multiplex bead-based antigen detection [16] and IgG detection assay [29] were performed as described elsewhere. Briefly, a 6-mm punch equivalent to 10 μL of whole blood was used from each dried blood spot, with blood eluted overnight to a 1:20 concentration in blocking buffer (phosphate-buffered saline containing 0.5% bovine serum albumin, 0.05% Tween 20, 0.02% sodium azide, 0.5% polyvinyl alcohol, 0.8% polyvinylpyrrolidone, and 3-μg/mL *Escherichia coli* extract).

The 1:20 whole-blood dilution was used for the multiplex bead assay to detect HRP2, pLDH, and *Plasmodium* aldolase antigens. A 1:200 dilution of serum was used for the multiplex IgG detection assay to detect antibodies to a panel of 10 *Plasmodium* antigens. For *P. falciparum*, the following were tested: circumsporozoite protein (PfCSP), early transcribed membrane protein 5 antigen 1 (PfEtramp5Ag1), glutamate-rich protein (PfGLURP-R0), liver-stage antigen 1 (PfLSA1), schizont egress antigen-1 (PfSEA1), apical membrane antigen 1 N terminal region (PfAMA1), and merozoite surface protein 1 19-kD region (PfMSP1); for *P. vivax*: PvAMA1, PvMSP1, and a chimeric form of the PvMSP1 antigen with additional T- and B-cell epitopes (chPvMSP1). Threshold assay signal was dichot-omized into antigen or IgG positive or negative as appropriate, based on the log-normal mean plus 3 standard deviations of a panel of 92 known negative blood samples from US residents for all assays. For both antigen detection and IgG assays, each assay plate included a buffer blank and positive and negative controls to ensure appropriate assay data collection.

Statistical Analysis

Summary statistics were calculated using SAS software, version 9.4 (SAS Institute). Any RDT result positive for HRP2 was considered a *P. falciparum* infection, and any result positive for pLDH only was considered a *P. vivax* infection. Likewise, any multiplex bead antigen assay positive for HRP2 was considered a *P. falciparum* infection, and any test positive for pLDH or *Plasmodium* aldolase alone (and not HRP2) was considered a *P. vivax* infection. These assumptions were made owing to the low prevalence of *P. malariae* and *Plasmodium ovale* in Ethiopia [32, 33]. The 10 antigens assessed for IgG antibody detection were categorized into 3 groups: “short-term” *P. falciparum*, “long-term” *P. falciparum*, and *P. vivax* antibodies. Short-term antibodies were more likely to have been acquired in the past year and included antibodies to PfCSP, PfEtramp5Ag1, PfGLURP-R0, PfLSA1, and PfSEA1 [34, 35]. Long-term *P. falciparum* antibodies (acquired at any time in life) included antibodies to PfAMA1 and PfMSP1 [24, 35]. *P. vivax* antibodies to these antigens (PvAMA1, PvMSP1, and chPvMSP1) were all considered long-term. Although the long-term antibodies generally persist longer than the short-term antibodies, some of the short-term antibodies may last for years, especially in adults [34].

Geospatial Analysis and Mapping

Cluster analysis within each region was performed using the Kulldorff spatial scan statistic in SaTScan software (version 9.7). Statistically significant clusters were identified using circular and elliptical windows of varying sizes, from zero to a maximum of 40% of the total population at risk, discrete purely spatial Poisson modeling, and 999 Monte Carlo simulations. The window with the highest log-likelihood ratio was defined as the most likely cluster if the *P* value was <.05 [36]. A criterion of "no geographic overlap" was specified for identifying any secondary clusters [37]. The spatial clusters were mapped using QGIS 3.16.3 software [38].

In addition, kernel density estimation (KDE) was conducted to identify clusters of high malaria prevalence from smoothed prevalence surfaces. Two spatial intensity surfaces were compared: one surface for testing positive for the test of interest and another for all survey participants. The ratio of the 2 surfaces provided the kernel-smoothed prevalence surface map. The maximum bandwidth (approximately 35 km) was calculated using the maximal smoothing principle [39]. Only areas within 35 km of a kebele were considered for the kernel-smoothing procedure, to prevent oversmoothing or estimating prevalence very far from where data were collected. An adaptive bandwidth and the Diggle edge correction was used [40]. To identify areas of significantly higher prevalence, 1-sided asymptotic tolerance contours ($\alpha = .05$) were calculated [41]. The kernel-smoothed maps were created using R software, version 4.03.3 (R Foundation for Statistical Computing) [42].

RESULTS

Since enumeration areas were randomly selected, in proportion to population size, the number of participants varied between the 3 regions (Table 1). Tigray had the most participants included in this analysis, at 1133 (49.7%), followed by Amhara (742 [32.6%]) and Afar (404 [17.7%]). Nearly half of the samples were from children <5 years old (45.3%) and the second-largest age group was persons aged ≥25 years (24.6%). The prevalence of malaria infection (by RDT, microscopy, or multiplex bead antigen assay) was low (<2.0% overall), though evidence of prior infection via IgG detection was much higher. For *P. falciparum,* 30.8% of all participants were seropositive for short-term IgG antibodies, and 38.1% for long-term antibodies. For *P. vivax*, 39.9% of participants were seropositive for long-term antibodies. In this sample, for both *P. falciparum* and *P. vivax*, Amhara had a higher antibody prevalence than the other 2 regions.

Overall, concordance among the different malaria tests was varied (Figure 2A). For *P. falciparum*, only 5 participants (0.2%) tested positive with all tests: RDT, bead-antigen assay, microscopy, and short- and long-term antibodies. Among the 22 samples positive for *P. falciparum* with RDT, 8 (36.4%) also had a positive result with both bead antigen assay and microscopy. A high concordance was observed between short- and long-term *P. falciparum* antibodies; however, 394 people (17.3%) tested positive for long-term *P. falciparum* antibodies alone. Among those 394 persons, 51.0% were aged ≥15 years. About half of all participants (51.4%) tested negative for *P. falciparum* infection or exposure.

For *P. vivax*, only 1 sample (0.04%) tested positive with RDT, bead-antigen assay, microscopy, and *P. vivax* antibodies (Figure 2B). Among the 9 samples positive for *P. vivax* with RDT, only 1 (11.1%) also tested positive with bead antigen assay and microscopy. For IgG detection, 894 samples (39.2%) tested positive for anti–*P. vivax* antibodies alone. Most participants (59.9%) tested negative for any *P. vivax* infection or prior exposure.

When the 3 diagnostic tests for malaria infection (RDT, microscopy, and bead-based assay) were compared, the bead antigen assay provided the highest number of positive results. For all *Plasmodium* species, the bead antigen assay detected 40 positive samples (1.8%), compared with 31 (1.4%) for RDT and 23 (1.0%) for microscopy. For *P. falciparum* infection, the bead antigen assay identified 29 positive samples (1.3%), compared with 22 (0.97%) for RDT and 18 (0.79%) for microscopy, and for *P. vivax*, the bead antigen assay identified 11 positive samples (0.48%), compared with 9 (0.39%) and 4 (0.18%), respectively.

For seroprevalence of anti-*Plasmodium* IgG antibodies, higher prevalence estimates were found for the long-term antibodies than for the short-term antibodies (Supplementary Table 1). For all ages, the highest overall seroprevalence was found for the *P. vivax* chPvMSP1 antigen (35.8%) followed by *P. falciparum* MSP1 (34.4%). In addition, the levels of IgG antibodies against each antigen reliably increased with age among the 4 age groups (0–4, 5–14, 15–24, and ≥25 years) (Supplementary Figure 1).

Clusters of malaria cases were first assessed using the Kulldorff spatial scan statistic within each region independently to avoid large clusters between regions that would include large areas without data. Overlapping clusters of *P. falciparum* infection (RDT, microscopy, and antigen assay) were found in Amhara only (Figure 3). In Tigray and Afar, partially overlapping clusters of short- and long-term anti–*P. falciparum* antibodies were found. Also in Tigray, a cluster of anti–*P. vivax* antibodies was identified. Five of the 9 clusters identified from the spatial scan statistic were antibody clusters. Overall, there was clear spatial heterogeneity in malaria prevalence, particularly with the *P. falciparum* RDT, bead antigen assay, and microscopy (Supplementary Figure 2). Spatial heterogeneity was also found for *P. vivax* malaria infection and exposure (Supplementary Figure 3).

The kernel-smoothed spatial prevalence identified more clusters of smaller size than the SaTScan analysis (Figure 4). For *P. falciparum*, overlapping clusters were identified in northern Amhara for all tests (RDT, bead HRP2 antigen assay, microscopy, and short- and long-term antibodies) (Supplementary Figure 4 and Figure 4). Five unique clusters were identified for long-term *P. falciparum* antibodies, 2 of which overlap either completely or partially with the 2 short-term *P. falciparum* antibody clusters. The remaining long-term *P. falciparum* antibody clusters (in Tigray and Afar) coincide with the clusters identified by SaTScan within those regions. For the anti–*P. vivax* IgG, 3 clusters were identified by kernel smoothing (Figure 4). The antibody cluster in Tigray partially overlapped with the *P. vivax* bead antigen assay and microscopy clusters (Supplementary Figure 5, and Figure 4). Although no clusters of *P. vivax* infection were identified in any region by SaTScan, clusters for all 3 tests of active infection were identified in northwest Tigray by kernel smoothing.

DISCUSSION

Spatial clustering of *P. falciparum* and *P. vivax* was identified in northern Ethiopia by using data from independent assays for parasite exposure, providing evidence that malaria infection (or exposure) was not randomly distributed within the 3 regions. Overlapping clusters were identified for *P. falciparum* by all 4 tests, whereas for *P. vivax*, only 3 clusters (antibody, bead antigen assay, and microscopy) overlapped. Many antibody clusters were identified for both *P. falciparum* and *P. vivax*, indicating that in areas of low transmission, antibody data provide more information to assess malaria exposure in a population. Importantly, the combination of results from multiple test types provides more information —this is required in areas of high malaria heterogeneity.

Owing to the unique nature of the targets detected by each test, it was not surprising that some discordance was found among test results for malaria infection and exposure. The overall malaria infection prevalence with the bead antigen assay was 1.8%, which was 25% higher than with RDT and 57% higher than with microscopy. Evidence for malaria exposure in this part of Ethiopia appears to be low to moderate as measured by antibody prevalence (38.1% and 39.9% sero-positivity for long-lived IgG against *P. falciparum and P. vivax* antigens, respectively). As expected, overall seroprevalence was higher for long-lived than for short-lived IgGs [24, 35, 43], and by age 5 years, 4.2%–17.0% of children have IgG seroconverted to the *P. falciparum* and *P. vivax* targets on our panel.

Although overall seroprevalence was higher for the long-term IgG, in Amhara the prevalences of long-term and short-term IgG were very similar (47.0% and 50.1%, respectively). This is likely a result of more recent exposure in Amhara, as there was an increase in malaria cases presenting to health facilities in late 2014 and 2015 [44]. Seroprevalences to long-term *P. falciparum* and *P. vivax* antibodies were generally equivalent, in contrast with tests for infection, which found a predominance of *P. falciparum*. This pattern of slightly higher seroprevalence for *P. falciparum* compared with *P. vivax* has been found by previous studies in Ethiopia [29, 45, 46]. These contrasts may point to better diagnostic tests for *P. falciparum* than for *P. vivax*, as the HRP2 antigen target has a higher sensitivity than pLDH [47]; lower *P. vivax* infection densities that may still induce an antibody response; or the potential ability of *P. vivax* to induce IgG with longer half-lives.

Although overall concordance among the different tests was low, geospatial overlap of several different clusters of malaria was identified, providing evidence for a genuine finding of relatively higher malaria transmission in specific areas among these 3 Ethiopian regions. The geospatial overlap of *P. falciparum* clusters detected by RDT, bead antigen assay, microscopy, and antibodies in western Amhara indicate that relatively more *P. falciparum* transmission was occurring there. In Amhara, 4 kebeles accounted for all of the *P. falciparum* cases detected by RDT and 3 of those kebeles accounted for all of the *P. falciparum* cases detected by bead antigen assay. Such findings can assist programmatic decision making by highlighting the geographically heterogenous distribution of *P. falciparum* infection, which is common in low-endemic settings [29, 45].

For *P. vivax*, overlapping clusters for the bead antigen assay, microscopy, and antibody detection were found with KDE, but not with SaTScan. This is likely because clustering was assessed within each region with SaTScan, instead of comparing all 3 regions, as was done with KDE. In addition, SaTScan relies on circular or elliptical windows to identify clusters, which may miss more subtle clusters of varying shapes that can be identified by KDE. Within Tigray, the SaTScan analysis identified 1 large cluster of *P. vivax* antibody prevalence, coinciding in the same area identified by KDE. Almost no *P. vivax* infection was detected in Amhara, but there was a high prevalence of previous *P. vivax* exposure, as indicated by the presence of anti–*P. vivax* antibodies. This may indicate that *P. vivax* transmission has decreased in Amhara over time.

For both *P. falciparum* and *P. vivax*, antibody data provided more positive results and a more detailed representation of malaria transmission compared with other indicators. Five hot spots of long-term antibody prevalence for *P. falciparum* and 3 for *P. vivax* were identified with the kernel-smoothed maps (compared with 2 hot spots and 1 hot spot, respectively, with SaTScan analysis). In studies with similar age distributions, these findings accentuate the utility of using antibody data to estimate transmission intensity in settings of low endemicity [23-26]. As infections become more rare in a population, they are harder to identify because a higher proportion are asymptomatic [48]. In addition, antibody data are a more sensitive indicator of malaria transmission compared with indicators of active infection, because antibodies linger within a person, and there is therefore more time to measure exposure. Many studies have used spatial scan statistics to identify disease clusters [49, 50], but in areas with sparse data or disperse population density, KDE is useful for identifying more refined hot spots. However, more dense sampling of the population would inherently provide tighter clusters of positivity. Concordance between both spatial models would provide the highest confidence that malaria exposure in an area is relatively high.

Our analyses are subject to several limitations. The study samples were collected from a cross-sectional survey conducted in 2015. As malaria is dynamic by place and time [45, 50], the results presented here indicate malaria prevalence during the study period and likely do not indicate current malaria prevalence in northern Ethiopia. Future studies could estimate malaria prevalence using the tests described here at multiple time points during the year to assess how transmission varies. In Ethiopia, the MIS is conducted periodically (approximately every 4–5 years) during the peak malaria transmission season; therefore, these data could be compared with data from a future MIS, controlling for seasonality. In addition, the samples analyzed here were a subset of the original MIS; therefore, the sample may not be a statistically representative sample of the 3 administrative regions. Not all dried blood spots collected were shipped to the CDC, and not all could be assessed with the multiplex antigen detection or antibody detection assays. Samples are assumed to reflect malaria infection and exposure at their respective locations, and we did not account for population movement or individual travel history, although the reported rate of recent travel in this sample was very low (2.1%). A person was considered seropositive to short- or long-term IgG antibodies if their dried blood spot was IgG positive to any of the multiple targets designated as inducing a short- or long-term IgG response. For this reason, absolute seroprevalence estimates in this study would be more sensitive but potentially less specific.

Overlapping spatial clusters of different metrics provide more confidence that malaria transmission in a setting is truly higher than in surrounding areas. Antibody clusters, especially where short-term and long-term IgG clusters overlap, may be used to narrow down areas that warrant follow-up activities, including further surveys to investigate malaria transmission. For regions of low malaria endemicity and coendemic *Plasmodium* species, combining multiple measures of infection or exposure can provide a detailed assessment of transmission.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Disclaimer.

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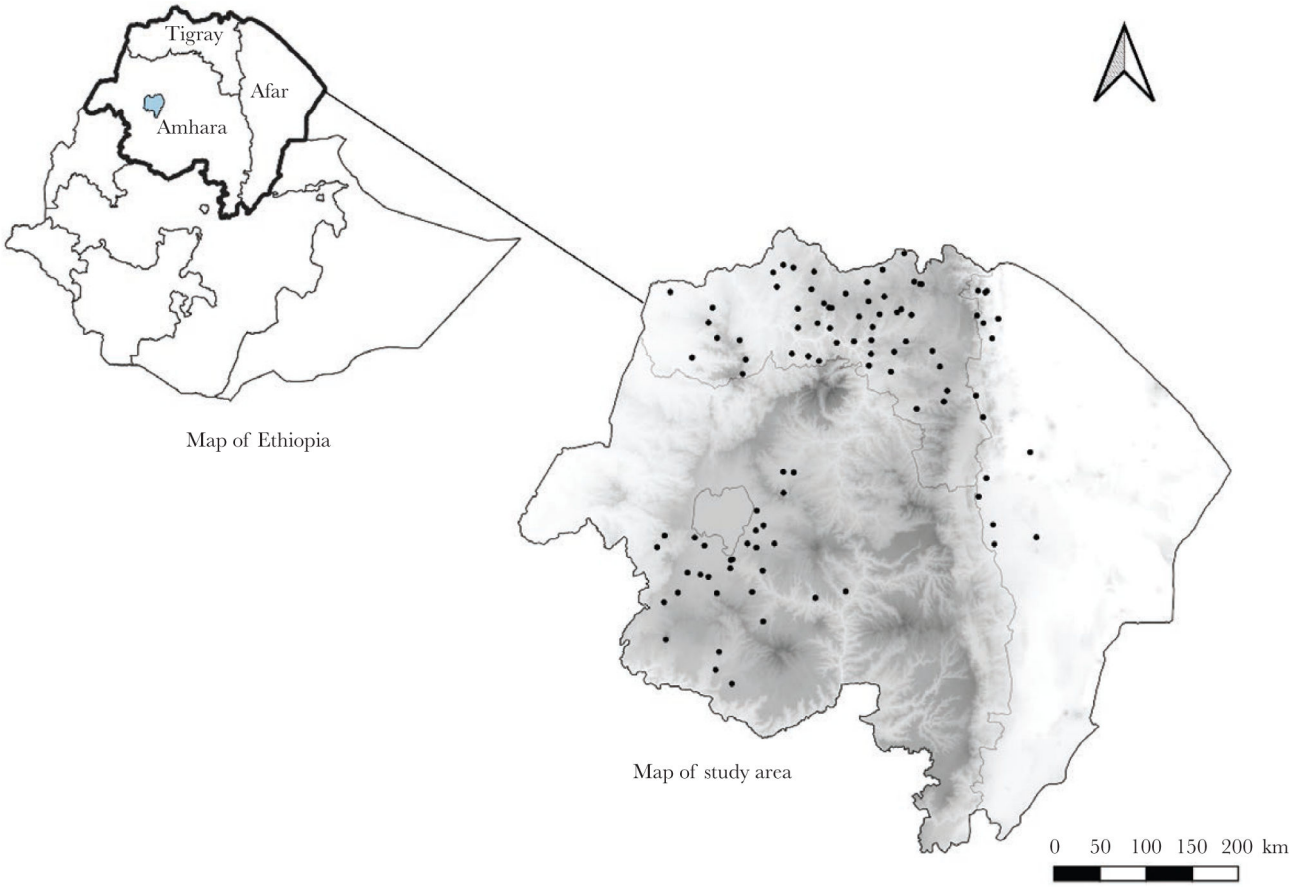

Figure 1.

Map of Ethiopia, highlighting the study regions (Amhara, Afar, and Tigray). Map on the right shows the distribution of locations where dried blood spot samples were collected in the 3 study regions. Each black dot represents 1 cluster of households in the enumeration area (kebele). Shading indicates elevation, with darker areas at a higher elevation (elevation range, from 131 m below to 4092 m above sea level).

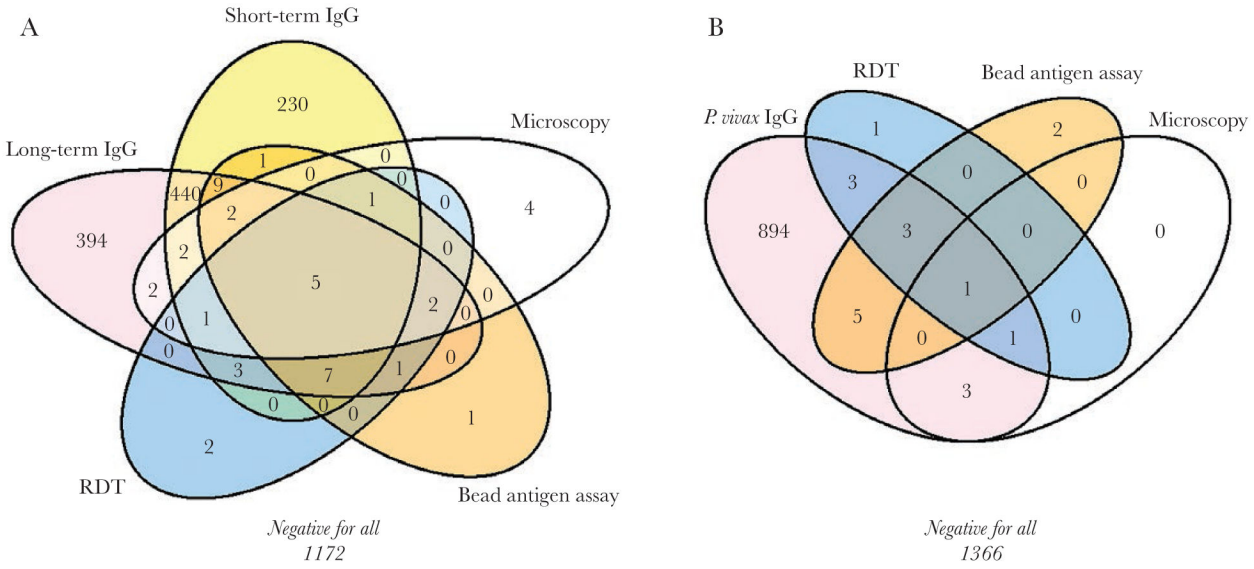

Figure 2.

Concordance of different tests for malaria infection or prior exposure to malaria, *Plasmodium falciparum* (*A*) or *Plasmodium vivax* (*B*). Results reflect field and laboratory samples from 2279 participants in the Malaria Indicator Survey, 2015. Abbreviations: IgG, immunoglobulin G; RDT, rapid diagnostic test.

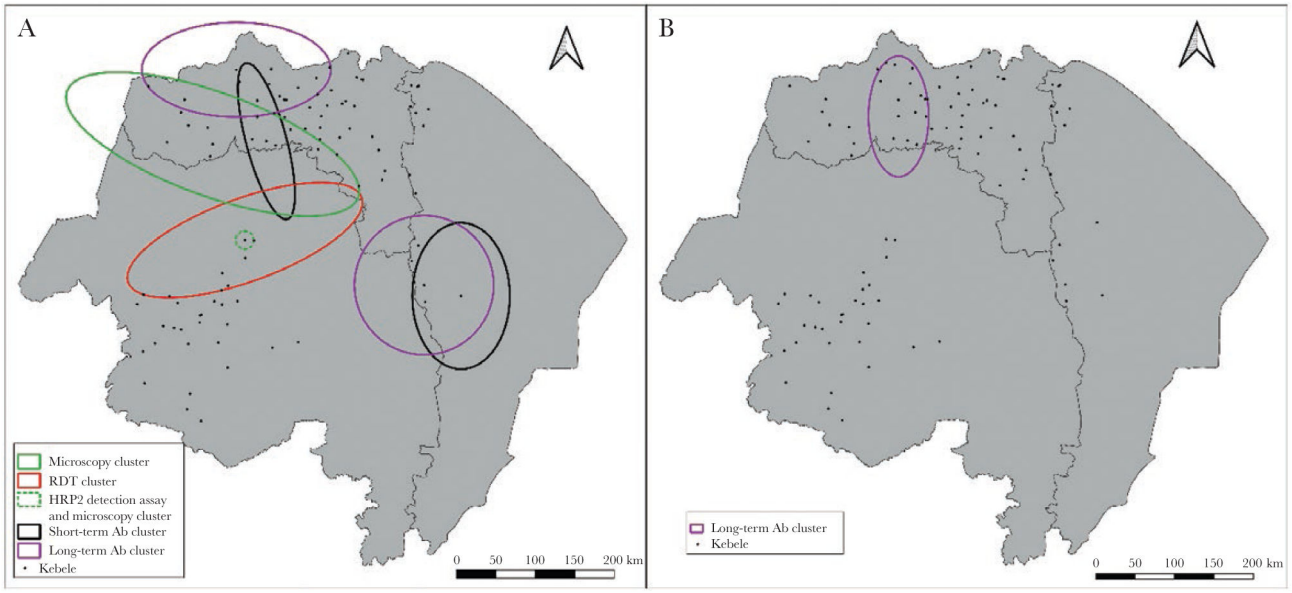

Figure 3.

All statistically significant spatial clusters for *Plasmodium falciparum* (*A*) and *Plasmodium vivax* (*B*) malaria. Clusters were assessed independently by region. Each cluster indicates a significant geospatial cluster of prevalence ($P < .05$). Abbreviations: Ab, antibody; HRP2, histidine-rich protein 2; RDT, rapid diagnostic test.

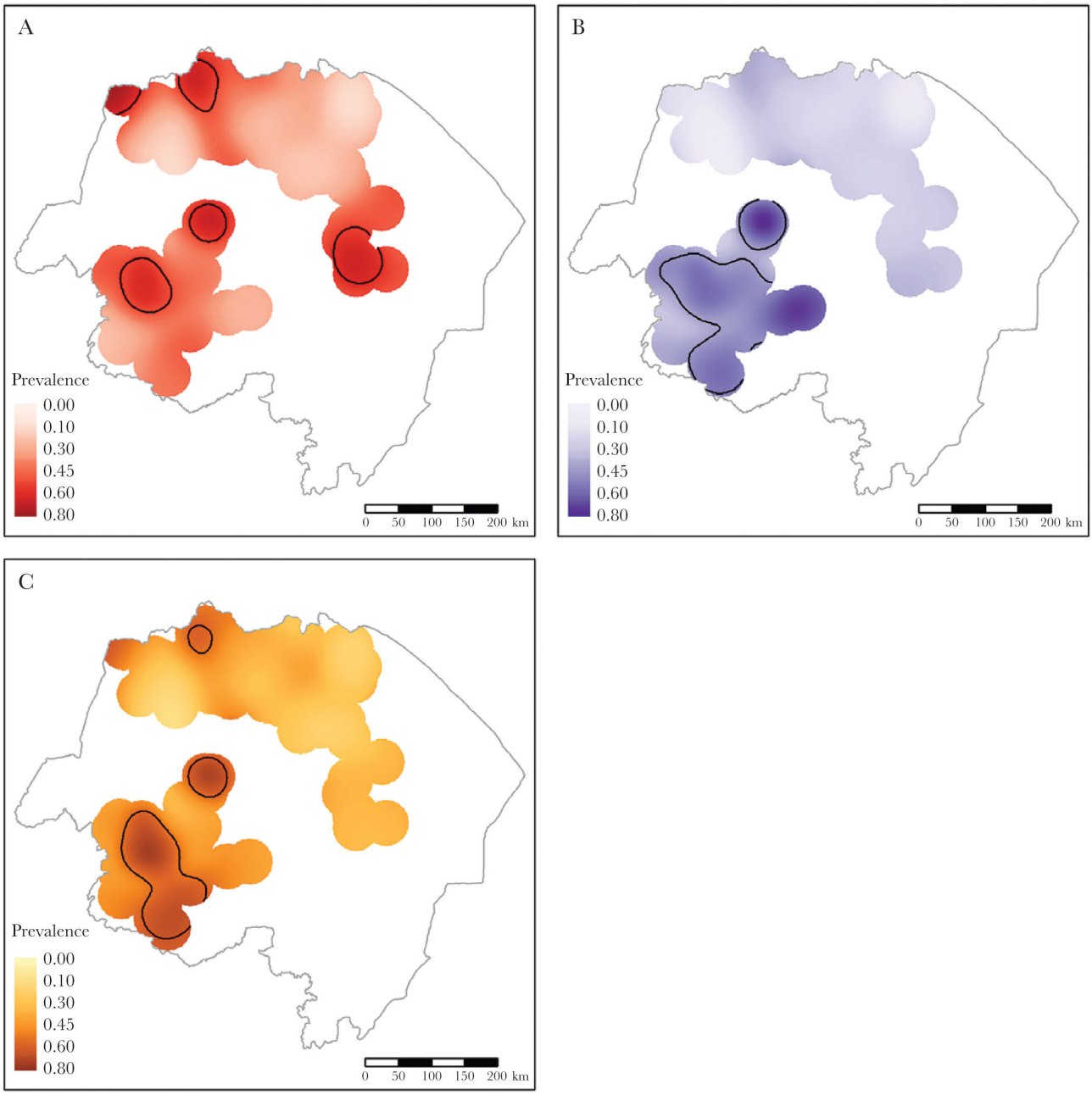

Figure 4.

Kernel-smoothed prevalence surfaces for long-term anti–*Plasmodium falciparum* antibodies (*A*), short-term anti-*P. falciparum* antibodies (*B*), and anti–*Plasmodium vivax* antibodies (*C*). Areas encircled by black lines represent areas of significantly higher prevalence (asymptotic $P < .05$).

Table 1.

Characteristics of the Study Population and Malaria Prevalence Estimates for Amhara, Afar, and Tigray Regions in 2015

Variable	All	Afar Region	Amhara Region	Tigray Region
Samples, no. (%)	2279 (100)	404 (17.7)	742 (32.6)	1133 (49.7)
Female sex, no. (%)	1188 (52.1)	201 (49.8)	367 (49.5)	620 (54.7)
Age group, no. (%)				
<5 y	1032 (45.3)	259 (64.1)	292 (39.4)	481 (42.5)
5–14 y	419 (18.4)	56 (13.9)	161 (21.7)	202 (17.8)
15–24 y	267 (11.7)	31 (7.7)	86 (11.6)	150 (13.2)
≥25 y	561 (24.6)	58 (14.4)	203 (27.4)	300 (26.5)
Result, no. (% [95% CI])				
RDT positive[a]	31 (1.4 [.9–1.8])	1 (0.3 [.0–.7])	10 (1.4 [.5–2.2])	20 (1.8 [1.–2.5])
Microscopy positive[a]	23 (1.0 [.6–1.4])	4 (1.0 [.0–2.0)	7 (.9 [.3–1.6])	12 (1.1 [.5–1.7])
Bead antigen assay positive[a]	40 (1.8 [1.2–2.3])	4 (1.0 [.0–2.0])	12 (1.6 [.7–2.5])	24 (2.1 [1.3– 3.0])
Short–term *Plasmodium falciparum* IgG positive	701 (30.8 [28.9–32.7])	82 (20.3 [16.4–24.2])	372 (50.1 [46.5–53.7])	247 (21.8 [19.4–24.2])
Long–term *P. falciparum* IgG positive	868 (38.1 [36.1–40.1])	137 (33.9 [29.3–38.5])	349 (47.0 [43.4–50.6])	382 (33.7 [31.0–36.6])
Plasmodium vivax IgG positive	910 (39.9 [37.9–41.9])	110 (27.2 [22.9–31.6])	403 (54.3 [50.7–57.9])	397 (35.0 [32.3–37.8])
Any IgG positive	1327 (58.2 [56.2–60.3])	195 (48.3 [43.4–53.1])	559 (75.3 [72.2–78.4])	573 (50.6 [47.7–53.5])
Negative for all tests	943 (41.4 [39.4–43.4])	205 (50.7 [45.9–55.6])	182 (24.5 [21.4–27.6])	556 (49.1 [46.2–52.0])

Abbreviations: CI, confidence interval; IgG, immunoglobulin G; RDT, rapid diagnostic test.

[a]Includes a positive result for any malaria antigen, including histidine-rich protein 2, pan-*Plasmodium* lactate dehydrogenase, and *Plasmodium* aldolase.