Rapid Epidemiological and Entomological Survey for Validation of Reported Indicators and Characterization of Local Malaria Transmission in Guinea, 2017

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Abstract. To confirm and investigate possible explanations for unusual trends in malaria indicators, a protocol for rapid, focal assessment of malaria transmission and control interventions was piloted in N'Zérékoré and Macenta Prefectures, which each reported surprisingly low incidence of malaria during the peak transmission months during 2017 in holoendemic Forested Guinea. In each prefecture, epidemiological and entomological cross-sectional surveys were conducted in two sub-prefectures reporting high incidence and one sub-prefecture reporting low incidence. Investigators visited six health facilities and 356 households, tested 476 children, performed 14 larval breeding site transects, and conducted 12 nights of human landing catches during the 2-week investigation. Rapid diagnostic test positivity in the community sample of children under five ranged from 23% to 68% by subprefecture. Only 38% of persons with fever reported seeking care in the public health sector; underutilization was confirmed by verification of health facility and community healthcare worker (CHW) registries. High numbers of *Anopheles* mosquitoes were collected in human landing collections in N'Zérékoré (38 per night in combined indoor and outdoor collections) and Macenta (87). Most of the detected breeding sites positive for *Anopheles* larvae (83%) were shallow roadside puddles. In the investigated prefectures, malaria rates remain high and the low reported incidence likely reflects low utilization of the public health-care sector. Strengthening the CHW program to rapidly identify and treat malaria cases and elimination of roadside puddles as part of routine cleanup campaigns should be considered. Systematic joint epidemiological/entomological investigations in areas reporting anomalous signals in routine data can allow control programs to respond with tailored local interventions.

INTRODUCTION

Malaria control in Guinea is based on several key interventions: long lasting insecticidal nets (LLINs), intermittent preventative treatment in pregnant women, seasonal malaria chemoprophylaxis, and rapid diagnostic tests (RDTs) and artemisinin-based combination therapy (ACT) for treatment. At the same time as these interventions have been approaching saturated coverage, contemporaneous investments in routine malaria information systems have allowed for improved characterization of malaria epidemiology, complementing surveys such as demographic and health surveys or malaria indicator surveys. Data reported through these routine systems have revealed a highly heterogeneous and focal nature of malaria transmission that contrasts with the uniformity of key interventions' implementation.

Guinea is entirely endemic for malaria. Since 2011, the country has performed two nationwide universal coverage LLIN campaigns, replaced clinical diagnosis with universal use of microscopy and RDTs, including at the community level, and replaced widespread chloroquine and sulfadoxine/pyrimethamine with ACTs for malaria treatment.^{1,2} These interventions are likely responsible for the significant drop in the proportion of children less than 5 years of age with slide-detectable parasitemia, which decreased from 44% in 2012³ to 15% in 2016,⁴ despite a disruptive Ebola epidemic in the intervening years.⁵ At the same time as the Guinea National

Malaria Control Program (NMCP) has scaled up malaria control interventions, it has also been building up its monitoring and evaluation arm, the basis of which is a system of monthly malaria reports completed by health facilities. The Guinea NMCP uses these reports to generate a monthly malaria bulletin, which summarizes health district-level indicators on malaria epidemiology, case management performance, and malaria commodity availability. These bulletins have indicated the highly heterogeneous nature of malaria transmission in the country. Just as the 2016 household survey showed that RDT positivity ranged from 2% to 58% by region, the monthly reported incidence data routinely show variation spanning several orders of magnitude.

The Guinea NMCP systematically analyzes the monthly data to look for anomalous signals that could indicate areas requiring extra attention. For example, in late 2016, Dabola and Dalaba prefectures (*syn.* Health Districts) in central Guinea began reporting unusually high malaria incidence rates. In response to this signal, the NMCP dispatched teams of epidemiologists to these prefectures to investigate possible reasons for the increase in incidence. The teams confirmed elevated incidence and identified potential vector control issues in Dabola, and identified reporting issues and problems with case management in Dalaba.

However, these investigations focused on the human and parasitic aspect of malaria transmission and lacked evaluations that would allow inferences about factors related to the *Anopheles* vectors and their contribution to the increase in cases. Consequently, the teams did not have all the desired data to make evidence-based recommendations regarding vector control interventions.

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To address this gap, the NMCP developed a joint epidemiological/entomological approach for investigation of unusual signals identified by the analysis of the routine data. The investigation protocol was separately piloted in N'Zérékoré and Macenta prefectures in Forested Guinea in September and October 2017. The prefectures were chosen because they were both consistently reporting unusually low crude incidence of malaria cases (unadjusted for health-care seeking), of 135/1,000 population in Macenta and 65/1,000 in N'Zérékoré in the months leading up to the investigations, compared with a national mean incidence of 197/1,000. The reported incidence was judged to be unusually low, given the fact that the two prefectures lie in the heart of holoendemic Forested Guinea, which had recorded the highest slide positivity of 30% in the 2016 nationwide household survey, double the national average of 15%. The objective of the investigation was, thus, to characterize local risk factors for malaria transmission, understand the heterogeneity in routine data, and propose tailored interventions.

METHODS

Survey design. The evaluation comprised a multilevel joint epidemiological/entomological survey. The evaluation included interviews with key stakeholders (i.e., health authorities, health facility and community healthcare workers [CHWs], and community leaders); review of health facility data; a household survey; and larval transects and adult mosquito collections. Investigation teams spent 1 week in each prefecture in September and October 2017.

Survey site and population. Within each prefecture, three subprefectures were purposefully selected with the prefectural health authorities: two subprefectures with “high” reported crude incidence and one subprefecture with “low” reported crude incidence. Routine malaria data submitted by the health center in each subprefecture in the chosen prefectures for the preceding 2 months were analyzed. In both prefectures, one of the chosen “high-incidence” subprefectures had high reported incidence of confirmed malaria cases, a high proportion of confirmed cases among all-cause outpatient consults, and a high proportion of patients tested for malaria among all-cause outpatient consults. The second chosen “high-incidence” subprefecture reported high incidence of confirmed malaria cases, a high proportion of confirmed cases among all-cause outpatient consults, but a lower proportion of patients tested for malaria among all-cause outpatient consults. In both prefectures, the chosen “low-incidence” subprefecture reported relatively low incidence of confirmed malaria cases, a low proportion of confirmed cases among all-cause outpatient consults, and a high proportion of patients tested for malaria among all-cause outpatient consults.

All of the selected subprefectures are supported by the NMCP with the standard portfolio of malaria control activities. Prevention activities include the distribution and use of LLINs and provision of intermittent preventative treatment in pregnant women attending antenatal clinic visits. According to NMCP guidelines, all fever cases seeking care are to be tested either by RDT or microscopy, and positive cases are to be treated with a first-line antimalarial. A network of CHWs attached to health facilities provide RDT testing and ACT treatment to children and adults in the community. Catchment

areas and populations for a CHW vary but generally represent roughly 1,000–2,000 individuals.

Within each subprefecture, the primary health center was selected for inclusion in the investigation. The head of the health center was invited to be interviewed. The head of the health center was also asked to indicate two representative villages from within the subprefecture, one deemed to be at high risk of malaria transmission, and one at low risk of malaria transmission based on the head of the health center's judgement.

Within each village, the CHW serving that village was selected for interview, as was one purposefully chosen community leader. Next, 30 households were randomly selected for inclusion in the household surveys. Assuming two children < 5 tested per household for a total of 180 children < 5 tested by prefecture, the survey was powered to have > 99% power to detect a difference of at least 20% in the prevalence of RDT positivity between the two subprefectures reporting high incidence and the subprefecture reporting low incidence in each prefecture.

Interviews with key stakeholders. Key stakeholders, including the prefectural health authorities, the chiefs of the selected health centers, and CHWs and community leaders in the selected villages were interviewed using standardized questionnaires. Interviewees were asked about their perceptions about recent changes in the number of malaria cases, common malaria control measures used in the community, recent emigrations or immigrations in the area, location of zones with high malaria risk and high numbers of mosquitoes, general health-care seeking behavior in their communities, and challenges in implementing malaria control. In addition, CHWs' registers were examined and RDT and ACT stock levels and the number of patients tested and treated by the CHW in the preceding month were recorded.

Health facility visits. Investigators previewed registry data from June, July, and August for 2016 and 2017 in the main health facility serving each sub-prefecture. The total numbers of outpatient consults, fever cases, malaria tests ordered and treatments prescribed, and malaria deaths were abstracted and recorded. Availability of malaria commodities, including RDTs, ACTs, LLINs, and sulfadoxine/pyrimethamine was verified from stock cards.

Household visits. In each selected village, the investigation team randomly selected 30 households according to the expanded program on immunization sampling methodology.⁶ During the household visits, investigators used standardized questionnaires to interview an adult member of the household about the household's access and use of malaria control measures. Investigators completed a sleeping space roster, recording the availability, location (hanging or stored), origin, brand, and self-reported use of any bed nets. Investigators also completed a roster of individuals routinely sleeping in the household, recording age, pregnancy status, LLIN use the previous night, occurrence of fever in the preceding 2 weeks, and health-care seeking behavior in response to the fever for each person. Up to four children < 5 years in each household were selected for RDT testing (Standard Diagnostics Inc., Bioline Pf, Yongin, Republic of Korea). If the household had more than four children < 5 present, four among them were randomly selected. If there were four or fewer children < 5 present, all were selected for testing, and additional persons (first pregnant women, then randomly selected adults and children age ≥ 5) were chosen with

the aim of testing four persons per house. All individuals testing positive received treatment according to national treatment guidelines.

Larval transects. A rapid larval survey was conducted by walking a minimum of two transects through each village to find and characterize bodies of standing water and to look for *Anopheles* larvae in these sites. Aerial imagery of the village to be visited was downloaded from Google Maps⁷ and transects were designated to cross as much of the village as possible, choosing the longest axes of the village for the transects. After transects were completed, local residents were questioned as to the location of any other bodies of standing water that might contain larvae and these were visited. The edges of any swamps or rice fields were sampled but these were not inspected fully. Sites containing *Anopheles* larvae were categorized as positive, sites without larvae were categorized as negative. No attempt was made to quantify larvae or identify larvae to species.

Mosquito collection. Adult mosquitoes were collected in each village in human landing collections and aspiration from households. In each village, two nights of human landing collections were made at one house, with a collector positioned outside the house, and a second collector inside the house. Collectors were recruited locally and were supervised by investigators. Collections were made between 1800 and 0700 hours. Mosquitoes were captured using glass tubes and were killed and identified in the field laboratory the following day using appropriate identification keys.^{8,9} Mosquitoes were stored for future laboratory analysis. The number of people in the courtyard at each hour (on the hour) was recorded by those conducting the human landing collections and was compared with the times when *Anopheles* mosquitoes were collected. By dividing the total number of person-hours recorded outdoors by the number of person-hours recorded outdoors when *Anopheles* mosquitoes were collected outdoors, the proportion of hours between 1800 and 0700 that residents were at risk of outdoor biting was calculated.

Data entry and analysis. Data were entered using Epi Info 7 (CDC, Atlanta, GA) on a daily basis, and analyzed using

Microsoft Excel[®] 2016 (Microsoft Corp., Redmond, WA) and R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria). Data from health facility registers were used to calculate the incidence of reported confirmed malaria and the proportion of confirmed malaria among all-cause consultations. The incidence of non-malaria consults was calculated subtracting confirmed malaria cases from all-cause consultations. Data abstracted from the registries were compared with data reported through the routine malaria information system, and the percent difference was calculated for key epidemiological and commodity availability indicators.

Data from household surveys were tabulated to calculate key epidemiological indicators, including RDT positivity, LLIN coverage and use, and health-care seeking behavior. Data from the entomological surveys were used to calculate indoor and outdoor mosquito densities and to construct biting time curves. Two comparisons were performed for epidemiological and entomological indicators: testing for a difference between the two subprefectures reporting high incidence combined and the subprefecture reporting low incidence, and testing for a difference between the three subprefectures individually. Differences were assessed using a χ^2 test for categorical variables and Student's *t* test for continuous variables.

Ethical considerations. Verbal consent was obtained before interviews with health authorities, health facility staff, and CHWs, and community leaders. Written informed consent was obtained from household members interviewed during the survey and any tested household members. Parents or guardians provided written permission on behalf of minors. The protocol was reviewed and classified as a non-research program evaluation by the CDC Center for Global Health Office of the Associate Director for Science (2017-347) and the Guinea Ministry of the Health.

RESULTS

Annualized incidence of reported confirmed malaria in the subprefectures selected as subprefectures reporting high incidence (Figure 1) ranged from 132 to 336 per 1,000

FIGURE 1. Location of subprefectures reporting high incidence (blue circles) and low incidence (yellow triangles) visited during joint epidemiological/entomological investigation in N'Zérékoré (A) and Macenta (B) Prefectures, Guinea. This figure appears in color at www.ajtmh.org.

TABLE 1

Key malaria indicators reported by selected subprefectures in months preceding joint entomological/epidemiological investigation, Guinea, 2017

Subprefecture	Annualized incidence of confirmed malaria (per 1,000)	Proportion of malaria among all-cause consultations (%)	Testing proportion (%)
Macenta prefecture			
Palé (reporting high incidence)	264	61	72
Koropara (reporting high incidence)	132	47	16
Samóé (reporting low incidence)	66	31	43
N'Zérékoré prefecture			
Daro (reporting high incidence)	201	63	43
Bofossou (reporting high incidence)	336	62	14
Séredou (reporting low incidence)	171	32	33

inhabitants, respectively, in the months preceding the investigation (Table 1). The proportion of malaria among all-cause outpatients was similarly high, ranging from 47% to 61%. The proportion of outpatients tested for malaria reflected the a priori sampling strategy of choosing one subprefecture with a high testing rate and one subprefecture with a lower testing rate in each prefecture. The incidence and proportion of malaria among all-cause outpatients was lower in the subprefectures reporting low incidence, with intermediate testing rates.

Teams visited 176 households in N'Zérékoré and 180 in Macenta, comprising 485 and 613 sleeping spaces, respectively (Table 2). During the household surveys, teams tested 466 individuals in N'Zérékoré and 577 individuals in Macenta, and collected data on reported fever and health-care seeking behavior on 634 and 1148 individuals. Investigators also interviewed six health center staff, 12 CHWs, and 12 community representatives.

Household surveys. The proportion of children < 5 testing positive by RDT was high in all subprefectures in N'Zérékoré, ranging from 63% to 68%, and did not differ significantly by subprefecture (Table 3). By contrast, in Macenta, although there was a high prevalence of RDT positivity in children < 5 in the subprefectures reporting high incidence (51% in both), RDT positivity in children < 5 was significantly lower at 23% in the subprefecture reporting low incidence (P value < 0.01). The prevalence of RDT positivity in older children and adults in all six sub-prefectures in both prefectures ranged from 36% to 49%, with no significant difference between subprefectures.

Ownership (93–95% versus 70%) of, access to (80–88% versus 48%), and use (78–82% versus 48%) of LLINs was higher in the subprefectures reporting high incidence in N'Zérékoré compared with the subprefecture reporting low incidence (all P values < 0.01). The same trend was reflected in the reported use of LLINs the previous night in children < 5 (72–87% versus 69%) and older children and adults (72–88% versus 55%). In Macenta, LLIN ownership was slightly lower in the subprefecture reporting low incidence (87%) compared

with 95–97% in the subprefectures reporting high incidence (P value 0.03), but there was no statistically significant difference between subprefectures reporting high and low incidence in terms of LLIN access or reported use in children < 5 (P values 0.08–0.6). A larger proportion of older children and adults reported sleeping under an LLIN in the subprefectures reporting high incidence (78–84%) compared with the subprefectures reporting low incidence (68%) (P value < 0.01).

The rate of reported fever in the previous 2 weeks was consistent across all subprefectures in both prefectures, ranging from 15% to 21%. However, health-care seeking behavior was much more variable (Table 3; Figure 2). In N'Zérékoré, in both subprefectures reporting high incidence, most people (64–69%) with fever reported seeking care at the nearest health facility, compared with only 12% in the subprefecture reporting low incidence (P value < 0.01), where people were more likely to either seek no care (40%) or seek care at a private pharmacy (36%). In Macenta, healthcare seeking at the public sector was uniformly low in three subprefectures, ranging from 21% to 31% (P value 0.3). There was a high rate of seeking care at private health facilities in the subprefecture reporting low incidence (18%) compared with the two subprefectures reporting high incidence combined (7%, P value 0.02).

Community health-care workers were rarely accessed across all six subprefectures in both prefectures, with only 0–12% of febrile cases seeking care at the CHW level (Table 3). This finding was confirmed by data obtained from review of CHW registers. With the exception of the Palé subprefecture reporting high incidence in N'Zérékoré, the number of people tested in the previous month ranged from 5 to 10, and the number of people treated ranged from two to eight (Table 4). Community health workers on average had less than one box of RDTs (< 25 tests) and the median number of ACT treatments available was 10. In N'Zérékoré, one of the subprefectures reporting high incidence reported higher rates of testing (36 per month) and treating (34 per month) by CHWs, as well as higher availability of RDTs (25) and ACTs (63).

TABLE 2

Sample size for interviews and household surveys conducted during joint epidemiological-entomological investigation, Guinea, 2017

	N'Zérékoré			Macenta		
	Palé (reporting high incidence)	Koropara (reporting high incidence)	Samóé (reporting low incidence)	Bofossou (reporting high incidence)	Daro (reporting high incidence)	Séredou (reporting low incidence)
Household visited	56	60	60	60	60	60
Sleeping spaces recorded	142	179	164	191	197	225
Persons tested for malaria	148	149	169	168	203	206
Community health-care workers interviewed	2	2	2	2	2	2

TABLE 3
Key malaria epidemiological indicators measured during household surveys as part of joint epidemiological-entomological investigation, Guinea, 2017

	N'Zérékoré				Macenta					
	Palé (reporting high incidence)	Koropara (reporting high incidence)	Samodé (reporting low incidence)	P value*	P value†	Bofossou (reporting high incidence)	Daro (reporting high incidence)	Sérédou (reporting low incidence)	P value*	P value†
RDT positivity in children < 5	31/49 (63%)	53/81 (65%)	61/90 (68%)	0.7	0.9	39/76 (51%)	38/75 (51%)	24/105 (23%)	< 0.01	< 0.01
RDT positivity in children ≥ 5 and adults	40/99 (40%)	22/61 (36%)	39/79 (49%)	0.1	0.3	44/92 (48%)	63/128 (49%)	38/101 (38%)	0.07	0.2
LLIN household ownership (≥ 1 LLIN per household)	53/56 (95%)	56/60 (93%)	42/60 (70%)	< 0.01	< 0.01	56/59 (95%)	58/60 (97%)	52/60 (87%)	0.03	0.1
LLIN access (proportion of sleeping spaces covered by LLIN)	125/142 (88%)	144/179 (80%)	79/164 (48%)	< 0.01	< 0.01	154/191 (81%)	135/197 (69%)	163/225 (72%)	0.6	0.02
LLIN hanging	111/135 (82%)	134/171 (78%)	76/160 (48%)	< 0.01	< 0.01	144/184 (78%)	130/194 (67%)	147/221 (67%)	0.1	0.02
LLIN use in children < 5 previous night	46/53 (87%)	64/89 (72%)	66/96 (69%)	0.1	0.04	82/91 (90%)	69/95 (73%)	94/130 (72%)	0.08	< 0.01
LLIN use in children ≥ 5 and adults	94/107 (88%)	87/120 (72%)	96/176 (55%)	< 0.01	< 0.01	205/244 (84%)	222/284 (78%)	213/315 (68%)	< 0.01	< 0.01
Households visited by CHW in preceding month	34/56 (61%)	12/60 (20%)	26/60 (43%)	0.7	< 0.01	24/60 (40%)	41/60 (68%)	40/60 (67%)	0.1	< 0.01
Proportion of household members with fever in preceding 2 weeks	23/158 (15%)	34/214 (16%)	51/262 (19%)	0.2	0.4	59/327 (18%)	69/378 (18%)	91/443 (21%)	0.3	0.6
Sought care in public health facility	9/14 (64%)	22/32 (69%)	6/50 (12%)	< 0.01	< 0.01	15/59 (25%)	14/68 (21%)	28/90 (31%)	0.2	0.3
Sought care in private facility	1/14 (7%)	0/32 (0%)	5/50 (10%)	0.2	0.2	4/59 (7%)	5/68 (7%)	16/90 (18%)	0.02	0.07
Sought care at traditional healer	4/14 (29%)	1/32 (3%)	2/50 (4%)	0.3	0.01	0/59 (0%)	0/68 (0%)	2/90 (2%)	0.2	0.3
Sought care at pharmacy	1/14 (7%)	3/32 (9%)	18/50 (36%)	< 0.01	< 0.01	7/59 (12%)	23/68 (34%)	27/90 (30%)	0.3	< 0.01
Sought care with CHW	1/14 (7%)	0/32 (0%)	1/50 (2%)	1	0.4	7/59 (12%)	8/68 (12%)	8/90 (9%)	0.7	0.8
Did not seek care	3/14 (21%)	7/32 (22%)	20/50 (40%)	0.08	0.2	26/59 (44%)	20/68 (29%)	10/90 (11%)	< 0.01	< 0.01

CHW = community healthcare worker; LLIN = long-lasting insecticidal net; RDT = rapid diagnostic test. P values < 0.05 in bold.

*Significant difference between zones reporting high and low incidence.

†Significant difference between all three zones.

FIGURE 2. Distribution of reported health-care seeking behavior in community members reporting fever in the preceding 2 weeks, assessed during joint entomological/epidemiological investigation in N'Zérékoré (A) and Macenta (B) Prefectures, Guinea. This figure appears in color at www.ajtmh.org.

Health center data. The number of total outpatients seen at the public health-care sector per 1,000 population differed by subprefecture in N'Zérékoré, and this difference continued even after the exclusion of confirmed malaria cases (Figure 3). By contrast, although subprefectures reporting high incidence in Macenta showed a higher rate of all-cause outpatient visits than the subprefecture reporting low incidence, this difference disappeared after exclusion of confirmed malaria cases. Using data verified from registries, in Macenta the subprefecture reporting low incidence had a lower incidence of confirmed malaria than the two subprefectures reporting high incidence (Table 5). In N'Zérékoré, the Palé subprefecture reporting high incidence had a higher rate of confirmed malaria incidence than the remaining two subprefectures.

Comparison of registry data and reported data revealed significant discrepancies in all health facilities visited in N'Zérékoré, with the percent difference in key indicators ranging from -51% to +36% (Table 6). Notably, the subprefecture reporting low incidence underreported the incidence of confirmed malaria cases (-30%) and the proportion of malaria among all-cause consults at the health center and health post level (-36%) as well as the community level (-51%). There was also significant discordance between the stock cards and the reported commodity availability data in all three health facilities, ranging from -27% to +62%.

Data quality in Macenta was very good in the subprefecture reporting low incidence, with the percent difference between the verified and reported data only ranging from 0% to 5% in the preceding 3 months. Data quality in the Daro subprefecture reporting high incidence was intermediate, whereas

the Bofossou subprefecture reporting high incidence substantially overreported the incidence of all-cause outpatient consults (+51%), the incidence of confirmed malaria cases (+57%), and the proportion of malaria among all-cause consults at the health center and health post level (+17%) as well as the community level (+7%).

Entomological monitoring. The larval transects detected *Anopheles* larval habitats in all villages in both prefectures with the exception of the subprefecture reporting high incidence in N'Zérékoré. As a result, in N'Zérékoré there was a significant difference in the proportion of potential larval sites found to be positive for *Anopheles* larvae between the two subprefectures reporting high incidence and the subprefecture reporting low incidence (P value 0.02) (Table 7). This difference was not significant in Macenta, where the proportion of sites positive for *Anopheles* larvae sites were similar in all three villages (P value 0.69). Across the ensemble of positive sites in the six visited villages, 18 of 87 (21%) potential larval breeding sites were positive for *Anopheles* larva. Fifteen of these (83%) were shallow puddles associated with roads: roadside ditches, tire tracks in the mud roads, or puddles in the roads. The three remaining sites (17%) that were not associated with roads were a puddle in front of a house (1), a puddle near a brick pile (1), and water that had collected in the foundation of a house under construction (1).

The mosquitoes collected in human landing collections were primarily composed of *Anopheles gambiae* s.l. and *Anopheles funestus* s.l. (Table 8). In N'Zérékoré, an average of 30 *A. gambiae* s.l. and eight *A. funestus* s.l. were collected per night. In Macenta, an average of 87 *A. gambiae* s.l. and 1

TABLE 4
Indicators for community healthcare worker testing and treatment activities and availability of malaria commodities assessed during interviews as part of joint epidemiological-entomological investigation, Guinea, 2017

Indicator	N'Zérékoré			Macenta		
	Palé (reporting high incidence)	Koropara (reporting high incidence)	Samoué (reporting low incidence)	Bofossou (reporting high incidence)	Daro (reporting high incidence)	Séradou (reporting low incidence)
Persons tested by CHW previous month	36	10	7	10	7	5
Persons treated by CHW previous month	34	8	5	7	6	2
Number of rapid diagnostic tests available to CHW	25	16	14	23	24	9
Number of artemisinin-based combination therapies available to CHW	63	13	40	6	10	6

CHW = community health worker.

FIGURE 3. Counts of monthly all-cause patient consultations recorded by community health-care workers (CHWs), health facilities, and health posts, assessed during joint entomological/epidemiological investigation in N'Zérékoré (A) and Macenta (C) Prefectures, Guinea; and counts of monthly all-cause patient consultations after exclusion of confirmed malaria cases in N'Zérékoré (B) and Macenta (D) Prefectures, Guinea. This figure appears in color at www.ajtmh.org.

A. funestus s.l. collected per night. The numbers collected in the villages in the subprefectures reporting high and low incidence were not significantly different in either prefecture (P value 0.61 in both prefectures).

The biting time of mosquitoes was compared with the human behavior (presence outdoor as observed by the collectors) (Figure 4, Table 9). In N'Zérékoré, the greatest percentage of person-hours observed during hours when *Anopheles* were

active was found in the Palé subprefecture reporting high incidence (44%), compared with the Koropara subprefecture reporting high incidence (8%) ($P < 0.01$), or the Samoé subprefecture reporting low incidence (17%) ($P < 0.01$). In Macenta, the percentage of person-hours outdoors when *Anopheles* were active were greater in the Bofossou subprefecture reporting high incidence (63%) than in the Séredou subprefecture reporting low incidence (44%) ($P < 0.01$). The

TABLE 5

Key malaria epidemiological indicators estimated from registry data in health facilities visited during epidemiological/entomological investigation, Guinea, 2017

	N'Zérékoré					Macenta				
	Palé (reporting high incidence)	Koropara (reporting high incidence)	Samoé (reporting low incidence)	P value*	P value†	Bofossou (reporting high incidence)	Daro (reporting high incidence)	Séredou (reporting low incidence)	P value*	P value†
Annualized incidence of confirmed malaria (per 1,000)	267	105	103	< 0.01	< 0.01	255	199	96	< 0.01	< 0.01
Proportion of malaria among all-cause consultations at the health center level	35%	49%	64%	< 0.01	< 0.01	41%	80%	44%	< 0.01	< 0.01
Proportion of malaria among all-cause consultations at the health post level	—	43%	44%	0.97	—	74%	37%	34%	0.02	< 0.01
Proportion of malaria among all-cause consultations* at the community level	—	—	—	—	—	—	70%	79%	0.53	—

P values < 0.05 in bold.

* Significant difference between zones reporting high and low incidence.

† Significant difference between all three zones.

TABLE 6
Comparison of health facility data from registers and data reported through routine monthly reports during the period June–August 2017

Indicator	Difference between registry/stock card data and reported data											
	N'Zérékoré						Macenta					
	Palé (reporting high incidence)		Koropara (reporting high incidence)		Samoé (reporting low incidence)		Bofossou (reporting high incidence)		Daro (reporting high incidence)		Sérédou (reporting low incidence)	
	Difference	%	Difference	%	Difference	%	Difference	%	Difference	%	Difference	%
Annualized incidence of all-cause outpatient consults (per 1,000)	120	33	64	36	26	13	188	51	–42	–12	4.6	2
Annualized incidence of confirmed malaria (per 1,000)	34	13	23	29	–35	–30	153	57	–1.3	5	4.3	4
Proportion of malaria among all-cause consultations* at the health facility level	–1	–2	–5.6	–12	–19	–36	7.4pp	17	10pp	25	1.1pp	3
Proportion of malaria among all-cause consultations* at the community level	–	–	14	18	–45	–51	5.3pp	7	–0.72pp	0	1.3pp	2
Health facility rapid diagnostic test stock at beginning of month	–140	–27	206	28	–119	–	–543	–	–	–	0	0
Health facility artemisinin-based combination therapy stock at beginning of month	133	62	–106	–	57	51	–68	–	–20	–11	0.83	5

pp = percentage points.

* Confirmed malaria/all-cause outpatient consults.

difference between the Daro subprefecture reporting high incidence (54%) and the Sérédou subprefecture reporting low incidence site was not significant ($P = 0.16$). In all sites in N'Zérékoré and Macenta, the percentage of outdoor human hours “at risk” was higher the first night than during the second night. This was discussed with those conducting the human landing collections (K. K., D. C., and Y. B.), who confirmed that people seemed to be observing the collections or chatting with the collectors the first night, suggesting that the second night of observations likely represented a more realistic pattern of human behavior.

DISCUSSION

The investigation sought to understand two things—the quality of routinely reported data and the situation with respect to malaria transmission and malaria control efforts in the chosen subprefectures. The results from all sites are generally consistent with a cycle of holoendemic malaria transmission in the region. In N'Zérékoré, all sites conformed to this pattern of transmission, and there was no difference in RDT positivity between subprefectures reporting high and low incidence.

The investigation found poor data quality at health centers in N'Zérékoré, particularly the subprefecture which had ostensibly lower incidence. At the community level in N'Zérékoré, the investigation found low utilization of public sector facilities and services in all three areas, in particular in the subprefecture reporting low incidence. Taken together, the results of the investigation support the hypothesis that the low incidence routinely reported by N'Zérékoré is attributable to underutilization of the public health sector and under-reporting of malaria cases due to low data quality. Moreover, the poor data quality and differential utilization of the public health sector by subprefecture belie relatively homogeneous malaria transmission, and could explain the heterogeneity in incidence noted during analysis of the routine data. These findings reinforce the importance of interpreting crude incidence rates in the context of potential large biases introduced because of heterogeneities in how cases seek care, are tested, and are reported.

For Macenta, in contrast to N'Zérékoré, the investigation results confirmed lower transmission in the subprefecture reporting low incidence than the subprefectures reporting high incidence. There was some evidence of lower exposure

TABLE 7
Results from larval transects conducted in villages assessed during epidemiological-entomological investigation, Guinea, 2017

	N'Zérékoré						Macenta					
	Palé (reporting high incidence)	Koropara (reporting high incidence)	Samoé (reporting low incidence)	<i>P</i> value*	<i>P</i> value†		Bofossou (reporting high incidence)	Daro (reporting high incidence)	Sérédou (reporting low incidence)	<i>P</i> value*	<i>P</i> value†	
Number of transects conducted	2	2	2	–	–		4	2	2	–	–	
Number of potential <i>Anopheles</i> larval sites inspected	12	12	13	–	–		22	13	15	–	–	
Number of sites positive for presence of <i>Anopheles</i> larvae	1	4	0	–	–		4	4	5	–	–	
Percentage of potential larval sites positive	8%	33%	0%	0.14	0.02		18%	31%	33%	0.86	0.69	

P values < 0.05 in bold.

* Significant difference between zones reporting high and low incidence.

† Significant difference between all three zones.

TABLE 8

Mean number of *Anopheles* mosquitoes collected in human landing collections (two nights per village) during epidemiological-entomological investigation, Guinea, 2017

	N'Zérékoré					Macenta				
	Palé (reporting high incidence)	Koropara (reporting high incidence)	Samoé (reporting low incidence)	P value*	P value†	Bofossou (reporting high incidence)	Daro (reporting high incidence)	Séradou (reporting low incidence)	P value*	P value†
<i>Anopheles gambiae</i> s.l.	47.5	18.0	25.0	0.75	0.54	99.5	67.0	93.5	0.72	0.58
<i>Anopheles funestus</i> s.l.	12.0	6.5	6.0	0.62	0.70	1.0	0.5	0	-0.36	-0.60

* Significant difference between zones reporting high and low incidence.

† Significant difference between all three zones.

to outdoor biting in the areas reporting low incidence, as well as a higher utilization of the private health sector in the zones. Nevertheless, like in N'Zérékoré, the investigation found substantial underutilization of the public health sector in all three zones. Ultimately, this seems to be the most likely explanation for the low incidence reported by Macenta. The heterogeneity in reported incidence by subprefecture in Macenta could potentially stem from differences in use of the private health sector or differences in outdoor exposure to mosquitoes.

Despite numerous and nuanced differences between all 12 communities visited during the investigation, three important observations were common in all areas. Firstly, high levels of parasite carriage were observed on a background of very high LLIN use, with LLIN access and use in several instances higher

in the subprefectures reporting high incidence compared with the subprefectures reporting low incidence. In these areas the numbers of mosquitoes collected while people were outdoors were sufficiently high that even consistent use of LLINs is likely not enough to completely cut short the cycle of malaria transmission and prevent new infections.

In this context, it is imperative to find and treat any infections that may arise. However, the second important finding common to all zones is that in four of the six visited communities, most individuals reporting fever in the last 2 weeks did not seek care at a public health facility or with a CHW. A consequence of this is that most incident malaria infections are not seen by the public health system and, thus, are not captured and counted in estimates of incidence. Moreover, cases that are undiagnosed and/or inadequately treated contribute to

FIGURE 4. Mean number of *Anopheles* collected in human landing collections and human behavioral observations conducted in N'Zérékoré and Macenta. Two human landing collections (comprised indoor and outdoor collections) were made per night in the subprefectures of: (A) Palé, (B) Koropara, (C) Samoé, (D) Bofossou, (E) Daro, and (F) Séradou. The second night of human behavioral observations (number of people observed in the courtyard at each hour) is presented here.

TABLE 9

Person-hours spent in courtyard between 1800 and 0700 when *Anopheles* adults were collected in human landing collections during epidemiological-entomological investigation, Guinea, 2017

	N'Zérékoré						Macenta					
	Palé (reporting high incidence)		Koropara (reporting high incidence)		Samoué (reporting low incidence)		Bofossou (reporting high incidence)		Daro (reporting high incidence)		Sérédou (reporting low incidence)	
	First night	Second night	First night	Second night	First night	Second night	First night	Second night	First night	Second night	First night	Second night
Person-hours recorded in courtyard	50	35	50	30	69	6	88	24	110	11	60	18
Number of person-hours in which at least one <i>Anopheles</i> mosquito was collected	29	8	6	0	12	1	58	13	61	4	30	4
Percentage of recorded person-hours potentially exposed to <i>Anopheles</i> adults	58%	23%	12%	0%	17%	17%	66%	54%	55%	36%	50%	22%
Percentage (both nights combined)	44%		8%		17%		63%		54%		44%	

malaria mortality and increased malaria transmission. Substantial health-care seeking in the private sector also highlights the importance of incorporating the private sector in the routine data reporting systems.

In a finding remarkably consistent across the 12 communities, between 15% and 20% of the population reported having a fever in the last 2 weeks. Extrapolating from this, in a village or group of villages of 1,000 inhabitants, a conservative estimate of the catchment population of a CHW, there should be roughly between 300 and 400 cases of fever per month. Assuming that a CHW could test half of these cases, a CHW in such a community could conduct up to 150–200 RDTs per month. Community health-care workers supplied with a box of RDTs a month at most can test 20% of the number they might test if optimally supplied and motivated.

Finally, most larval breeding sites identified in the six villages during the entomological investigation were in the form of ditches beside or ponds on the unpaved roads. There could be other breeding sites that were not identified during the surveys, given the punctual nature of the survey or sites such as swamps or rice fields, which we only sampled at the edges. However, the preponderance of positive sites that were on or near roads suggests that road sites may contribute to malaria transmission in these areas.

Taken together, these findings support the implementation of several tailored interventions for these prefectures that might optimize malaria control efforts in these areas. First, health authorities might consider changing the way in which CHWs are supplied. Instead of supplying CHWs on a month-to-month basis, authorities in Guinea could consider providing a larger, two- or three-month supply of RDTs and ACTs until the supply chain in Guinea is strong enough to consistently guarantee adequate RDT and ACT stocks to CHWs on a monthly basis. Moreover, targeted messaging aimed at the community could be implemented to encourage healthcare seeking among the population. The NMCP could also consider tracking CHW activity by prefecture as part of the monthly bulletin to assess different prefectures' performance in implementing community case management. Finally, local authorities could consider integrating filling in puddles in and next to roads as part of existing routine "cleanup" (environmental management) campaigns, which currently focus on removal of garbage and cutting of grass around houses.

These recommendations should be interpreted in the context of the limitations of the investigation. The investigation was executed over the course of a week in each prefecture, in a limited number of villages. By definition, the investigation was not designed to be either exhaustive or representative but rather to provide a rapid snapshot of the malaria epidemiology in the area. The limited geographical and temporal scope of the investigation limits extrapolation of the results to a larger geographic area or to the entire year. Data on health-care seeking rates could have been biased by the delay in seeking care, with a proportion of people reporting not seeking care potentially seeking care after the survey. The unavailability or poor condition of certain outpatient registers and stock cards hindered comparison of reported and recorded data. Similarly, the absence of insecticide resistance data limited investigators' ability to make inference regarding differential mosquito resistance as a factor for the observed heterogeneity in malaria incidence by area. Finally, the limited scope of the investigation meant that it was not able to explore in-depth the factors influencing poor health-care seeking in the investigated areas or to assess the factors behind the poor quality of routine data in several of the visited health facilities, and future investigations would benefit from questions designed to gather more data on these underlying factors.

Despite the lack of generalizability of these results to other contexts, the methodology itself shows promise for use in other settings. A systematic, comprehensive set of procedures in place for investigation of trends might give other national control programs the opportunity to explore and characterize the inherent heterogeneity of local malaria epidemiology. The increasing availability and quality of routine data offer the opportunity for malaria programs to interrogate the routine data, identify signals, and respond with field visits to tease out more nuanced risk factors. Targeted investigations aimed at characterizing local conditions for malaria transmission have played an important role in malaria control.^{10,11} The joint epidemiological/entomological approach described here allows for systematic characterization of the myriad factors that influence malaria epidemiology in an area, from the *Anopheles* larvae, the interaction of the adults with the human host, the level of carriage in the human population, the population's access and use of mosquito prevention measures, to the management and recording and

reporting of malaria cases in the health-care setting. Moreover, the experience of assessing the spectrum of factors influencing malaria transmission is a unique opportunity for capacity building for local-, regional-, and national-level malaria control staff.

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REFERENCES

1. Guinea National Malaria Control Program, 2017. *National Malaria Strategic Plan 2018–2022*. Conakry, Guinea: Ministry of Health.
2. President's Malaria Initiative, 2017. *Guinea Malaria Operational Plan FY 2018*. Washington, DC: U.S. President's Malaria Initiative.
3. Guinea National Statistics Institute, 2012. *Demographic Health Survey*. Conakry, Guinea: Guinea National Statistics Institute.
4. Guinea National Statistics Institute, 2016. *Multiple Indicator Cluster Survey*. Conakry, Guinea: Guinea National Statistics Institute.
5. Plucinski MM et al., 2015. Effect of the Ebola-virus-disease epidemic on malaria case management in Guinea, 2014: a cross-sectional survey of health facilities. *Lancet Infect Dis* 15: 1017–1023.
6. Expanded Programme on Immunization, 2008. *Training for Mid-Level Managers*. Geneva, Switzerland: World Health Organization.
7. Google Maps, 2017. Available at: <https://www.google.com/maps/@33.796096,-84.3259904,15z>. Accessed September 19, 2017.
8. Gillies MT, De Meillon B, 1968. *The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region)*. Johannesburg, South Africa: South African Institute for Medical Research.
9. Gillies M, Coetzee M, 1987. *A Supplement to the Anophelinae of Africa South of the Sahara (Afrotropical Region)*. Johannesburg, South Africa: South African Institute for Medical Research.
10. McArthur J, 1947. The transmission of malaria in Borneo. *Trans R Soc Trop Med Hyg* 40: 537–558.
11. Bannister-Tyrrell M et al., 2018. Micro-epidemiology of malaria in an elimination setting in central Vietnam. *Malar J* 17: 119.