*Am. J. Trop. Med. Hyg.*, 92(3), 2015, pp. 497–500
doi:10.4269/ajtmh.14-0678


# Evidence of Recent Dengue Exposure among Malaria Parasite-Positive Children in Three Urban Centers in Ghana

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*Abstract.* Blood samples of 218 children ages 2–14 years old with confirmed malaria in hospitals across Ghana were tested for dengue virus exposure. We detected dengue-specific immunoglobulin M (IgM) antibodies in 3.2% of the children, indicating possible coinfection, and IgG antibodies in 21.6% of them, which suggests previous exposure. Correlates of exposure are discussed.

## INTRODUCTION

Misdiagnosis of febrile illnesses as malaria remains a challenge in Africa,[1] despite evidence that the proportion of fevers attributable to malaria has decreased over the last two decades.[2] Although urbanization has suppressed the global malaria burden,[3] it has also driven the emergence of many arboviral diseases, including dengue fever,[4,5] and newly revised estimates of the global dengue burden suggest that it is probably significantly larger than previously estimated.[6,7] Recent seroprevalence surveys have uncovered dengue exposure throughout sub-Saharan Africa,[7] and west Africa has been identified as a potential hotspot for transmission because of the existence of the *Aedes* mosquito vector, historical arbovirus transmission, rapid urbanization with inadequate sanitation, and low clinical knowledge of flavivirus infections.[8]

Malaria transmission is endemic in Ghana, and healthcare practitioners at many urban healthcare facilities presumptively diagnose malaria using clinical algorithms. Between 2001 and 2006, 47% of healthcare facility visits by children and 37% of healthcare facility visits by adults resulted in a clinical diagnosis of malaria.[9] Because of resource constraints, fewer than 10% of malaria diagnoses were confirmed by blood examination between 2007 and 2011.[10] Data from 2013 suggest that less than one-third of all national malaria diagnoses are currently confirmed by blood analysis, and clinical staff diagnose approximately 40–50% of all sick children—and about 40% of all outpatients—with malaria.[11] In a recent study of 605 feverish children who sought care at a hospital in Accra, it was revealed that only 11% tested positive for malaria by microscopy after 80% had been diagnosed with malaria and treated with antimalarials.[12] These findings show that the etiology of febrile illness includes much more than *Plasmodium* infections and underscore the urgent need to begin examining the possible role of other pathogens.

This study was implemented as a pilot for a broader population study of the etiology of febrile illness in Ghana and began by testing for evidence of dengue exposure in 218 children with laboratory-confirmed malaria.

## METHODS

This study investigated the possible role of dengue virus (DENV) in febrile illness by testing archived plasma samples that were collected from children ages 2–14 years old who tested positive for malaria at local health facilities in three

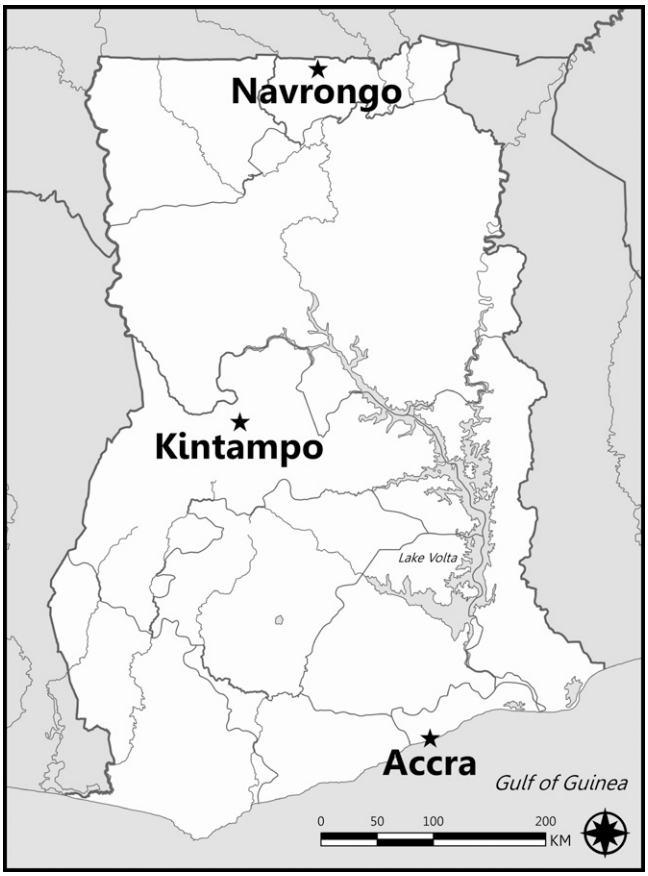


FIGURE 1. The three urban centers comprising the study area in Ghana. 2010 population census estimates for Accra, Kintampo, and Navrongo were 2.07 million, 42,957, and approximately 27,000, respectively.[18]

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ecological zones of Ghana: Ledzokuku-Krowor Municipal Assembly Hospital, Kpeshie, in the coastal savannah Greater Accra Region containing the national capital Accra; Kintampo Municipal Hospital, Kintampo, in the tropical-forested transition zone of Brong-Ahafo Region; and War Memorial Hospital, Navrongo, in the Upper East Region's dry southern savannah climate belt (Figure 1). This study was approved by the respective Institutional Review Boards of the Ghana Health Service, Navrongo Health Research Centre, Kintampo Health Research Centre, Noguchi Memorial Institute for Medical Research (University of Ghana), and the University of Miami. A parent or guardian gave written informed consent for each child who participated in the study. The samples were collected from 2011 to 2014 as part of a study at the University of Ghana investigating erythrocyte invasion mechanisms of *Plasmodium falciparum*, and therefore, only malaria-positive samples were available.

The inclusion criteria for enrollment were (1) resident in the respective community for at least the preceding 6 months, (2) age between 2 and 14 years old, (3) positive for malaria by rapid diagnostic test (RDT), and (4) having a parent or guardian who willingly signed the study consent form for the child's participation and answered a short questionnaire. Venous blood was collected into heparinized tubes and centrifuged, and plasma was harvested and stored at −80°C until use.

Dengue-specific immunoglobulin M (IgM) and IgG were detected in plasma samples using the Capture DxSelect enzyme-linked immunosorbent assay (ELISA; Focus Diagnostics, Inc., Cypress, CA). The sensitivity of the IgM DxSelect Kit assessed by assaying well-characterized sera was reported as 96% (73 of 76), with specificity pooled from multiple sites reported as 97% (296 of 306) and a 27% (9 of 33) cross-reactivity rate with other flavivirus infections.[13] The sensitivity of the IgG DxSelect Kit for well-characterized sera was 96% (76 of 79), with pooled specificity reported as 93% (253 of 271) and a 61% (20 of 33) cross-reactivity with other flaviviruses.[14] In addition, viral RNA was extracted from the plasma samples using the QIAamp Viral RNA Mini Kit (Qiagen N.V., Venlo, The Netherlands) followed by detection of specific DENV serotypes 1–4 (DENV-1 to -4) using the AgPath-ID One-Step Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Kit (Applied Biosystems, Foster City, CA) on an ABI 7300 Fast Dx Real-Time PCR Instrument (Applied Biosystems) as described previously.[15]

## RESULTS

Demographic characteristics of the study population are presented in Table 1. Of 218 plasma samples tested, 7 (3.2%) were positive for dengue-specific IgM, whereas 47 (21.6%) were positive for dengue-specific IgG. The IgG response differed significantly across study sites ($\chi^2 = 8.29$, $P = 0.016$), with a lower rate observed in Kintampo compared with Accra

TABLE 1

Individual sociodemographic, behavioral, and clinical characteristics of 218 children stratified by three study sites in Ghana: Accra, Kintampo, and Navrongo

| | Total sample | | Accra | | Kintampo | | Navrongo | |
|---|---|---|---|---|---|---|---|---|
| Characteristic | Frequency | Percentage or mean (SE) | Frequency | Percentage or mean (SE) | Frequency | Percentage or mean (SE) | Frequency | Percentage or mean (SE) |
| Dependent measures | 218 | 100 | 42 | 19.3 | 94 | 43.1 | 82 | 37.6 |
| IgM response | 7 | 3.2 | 1 | 2.4 | 1 | 1.1 | 5 | 6.1 |
| IgG response* | 47 | 21.6 | 10 | 23.8 | 12 | 12.8 | 25 | 30.5 |
| Independent measures | 218 | 100 | 42 | 19.3 | 94 | 43.1 | 82 | 37.6 |
| Sociodemographic | | | | | | | | |
| Sex is male | 114 | 52.3 | 25 | 59.5 | 51 | 54.3 | 38 | 46.3 |
| Age (years) | | 5.7 (0.20) | | 6.4 (0.51) | | 5.5 (0.31) | | 5.4 (0.28) |
| Mother has no education* | 62 | 28.4 | 6 | 14.3 | 49 | 52.1 | 7 | 8.5 |
| Owned by parent or guardian | | | | | | | | |
| Cement block house* | 90 | 41.3 | 17 | 40.5 | 54 | 57.4 | 19 | 23.2 |
| Thatched house* | 120 | 55.0 | 2 | 4.8 | 54 | 57.4 | 64 | 78.0 |
| Car* | 24 | 11.0 | 9 | 21.4 | 9 | 9.6 | 6 | 7.3 |
| Television* | 132 | 60.6 | 38 | 90.5 | 46 | 48.9 | 48 | 58.5 |
| Radio | 189 | 86.7 | 38 | 90.5 | 81 | 86.2 | 70 | 85.4 |
| Farm* | 155 | 71.1 | 12 | 28.6 | 83 | 88.3 | 60 | 73.2 |
| Refrigerator* | 79 | 36.2 | 36 | 85.7 | 15 | 16.0 | 28 | 34.1 |
| Behavioral | | | | | | | | |
| Antimalarial drug taken in last 2 weeks | 32 | 14.7 | 9 | 21.4 | 10 | 10.6 | 13 | 15.9 |
| Traditional/herbal medicine used | 23 | 10.6 | 3 | 7.1 | 6 | 6.4 | 14 | 17.1 |
| Daily bed net use* | 155 | 71.1 | 16 | 38.1 | 68 | 72.3 | 71 | 86.6 |
| Clinical | | | | | | | | |
| Symptoms | | | | | | | | |
| Fever* | 206 | 94.5 | 36 | 85.7 | 88 | 93.6 | 82 | 100 |
| Headache | 175 | 80.3 | 34 | 81.0 | 76 | 80.9 | 65 | 79.3 |
| Nausea* | 125 | 57.3 | 19 | 45.2 | 46 | 48.9 | 60 | 73.2 |
| Chills* | 80 | 36.7 | 34 | 81.0 | 46 | 48.9 | 0 | 0 |
| Joint pain | 39 | 17.9 | 8 | 19.0 | 22 | 23.4 | 9 | 11.0 |
| Diarrhea | 39 | 17.9 | 6 | 14.3 | 21 | 22.3 | 12 | 14.6 |
| Convulsions | 8 | 3.7 | 4 | 9.5 | 2 | 2.1 | 2 | 2.4 |
| Jaundice | 8 | 3.7 | 2 | 4.8 | 5 | 5.3 | 1 | 1.2 |
| Other: cough* | 23 | 10.6 | 0 | 0 | 12 | 12.8 | 11 | 13.4 |
| Other: abdominal pain* | 49 | 22.5 | 2 | 4.8 | 8 | 8.5 | 39 | 47.6 |
| Parasite density (/μL; $N = 201$)* | | 76,298 (8,059) | | 20,725 (3,409) | | 139,458 (18,111) | | 44,005 (3,853) |
| Hemoglobin (g/dL; $N = 214$) | | 10.2 (0.12) | | 10.7 (0.28) | | 10.1 (0.17) | | 10.0 (0.18) |

*Statistically significant ($P \leq 0.05$) differences between study sites from $\chi^2$ (for categorical measures) or $F$ (for continuous measures) tests.

and Navrongo. Kintampo's geographic location results in fewer travelers from neighboring countries, which may be important if local dengue exposure is associated with imported cases. No dengue viral RNA was detected by real-time PCR, suggesting that none of the study participants had an acute dengue infection. In dengue infections, IgM is detected about 5 days after the onset of illness and may persist for about 90 days, whereas IgG is detected after 10–15 days and may persist for years. The observed IgM prevalence is, thus, likely caused by exposure toward the latter end of the 90-day window, at which point most children would have already cleared the virus. The observed IgG prevalence may be attributable to the same dengue wave or some other previous exposure. Because all PCR results were negative and the plaque reduction neutralization test (PRNT) was not available at the time in Ghana, our seroprevalence results are limited by the sensitivity and specificity of the ELISAs and the potential for cross-reactivity with antibodies for other flaviviruses in the plasma samples. Although antibodies from yellow fever and influenza vaccinations may present cross-reactivity issues for the IgG ELISA,[14] yellow fever vaccination coverage is high among Ghana's youth (although no reliable data exist), and we would have expected much higher rates of IgG response if vaccinations were significantly skewing our results.

To evaluate predictors of positive IgM and IgG antibody responses in the study cohort, we implemented multilevel logistic regression models using the GLIMMIX procedure in SAS 9.3 (Cary, NC) to control for three study sites in Ghana. Independent measures from Table 1 were introduced in separate blocks of sociodemographic, behavioral, and biomedical measures; any model terms that presented convergence issues caused by lack of variation were removed. In the full model presented in Table 2, only two measures were statistically significantly associated with higher odds of an IgM response: lower daily bed net use ($P = 0.018$) and higher parasite density ($P = 0.030$). This result may be spurious, caused by the low number of positive IgM responses detected per study site, and reflected in higher –2 residual log pseudolikelihood for this model. Daily bed net use and parasite density were negatively related, with daily bed net users averaging two times the parasite density as non-users (88,497/μL versus 43,914/μL; $F = 6.24$, $P = 0.013$). Mean parasite density was higher for seven IgM-positive children relative to the rest of the sample, but the difference was not statistically significant.

We only assessed sociodemographic predictors of 47 IgG responses because current symptoms and health-seeking behaviors would not be indicative of previous exposure to DENV (i.e., more than 90 days ago). As presented in Table 2, these cases were less likely to come from parents who owned a cement house ($P = 0.019$) or farm ($P = 0.008$). This result is more intuitive, because DENV transmission is typically driven by the ubiquitous presence of water-holding containers in the

TABLE 2

β-Coefficients and SEs for random intercept models assessing the association between demographics, symptoms, and parasitemia on prevalence of dengue-specific IgM and IgG antibodies among Ghanaian children

| | IgM | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Sociodemographic factors ($N = 218$) | | Clinical and behavioral factors ($N = 199$) | | Full model ($N = 199$) | | IgG sociodemographic factors ($N = 218$) | |
| Characteristics | β | SE | β | SE | β | SE | β | SE |
| Intercept | –3.32 | 1.67 | –6.55 | 4.01 | –6.58 | 5.20 | –1.48 | 0.83 |
| Sociodemographic measures | | | | | | | | |
| Sex is male | –1.06 | 0.89 | | | –2.28 | 1.43 | 0.54 | 0.37 |
| Age (years) | –0.15 | 0.17 | | | –0.39 | 0.31 | 0.11 | 0.06 |
| Mother has no education | –0.72 | 1.13 | | | –1.12 | 2.08 | 0.51 | 0.45 |
| Owned by parent or guardian | | | | | | | | |
| Cement block house | –0.96 | 1.19 | | | –3.61 | 2.46 | –1.12* | 0.47 |
| Thatched house | 1.72 | 1.25 | | | 0.10 | 2.23 | 0.30 | 0.52 |
| Car | | | | | | | –1.14 | 0.74 |
| Television | –0.01 | 1.06 | | | –1.08 | 1.71 | 0.24 | 0.47 |
| Radio | | | | | | | –0.08 | 0.59 |
| Farm | –0.20 | 0.99 | | | 0.96 | 1.92 | –1.25* | 0.47 |
| Refrigerator | 1.10 | 1.14 | | | 0.70 | 1.97 | 0.27 | 0.51 |
| Behavioral/biomedical measures | | | | | | | | |
| Antimalarial drug taken in last 2 weeks | | | 1.41 | 1.19 | 1.59 | 1.60 | | |
| Traditional/herbal medicine used | | | 0.46 | 1.13 | 0.27 | 1.84 | | |
| Daily bed net use | | | –2.42* | 1.06 | –4.27* | 1.79 | | |
| Symptoms | | | | | | | | |
| Headache | | | –0.20 | 1.05 | 0.72 | 1.39 | | |
| Nausea | | | –0.42 | 0.99 | –0.33 | 1.26 | | |
| Chills | | | –0.98 | 1.49 | –0.99 | 1.97 | | |
| Joint pain | | | 0.67 | 1.25 | 0.91 | 1.49 | | |
| Diarrhea | | | 0.22 | 1.20 | 0.41 | 1.60 | | |
| Convulsions | | | | | | | | |
| Jaundice | | | | | | | | |
| Other: cough | | | 1.53 | 1.13 | 1.19 | 1.88 | | |
| Other: abdominal pain | | | 1.82 | 1.17 | 1.98 | 1.70 | | |
| Parasite density (/μL; $N = 201$) | | | 8.23 E–6* | 3.49 E–6 | 1.10 E–5* | 5.10 E–6 | | |
| Hemoglobin (g/dL; $N = 214$) | | | 0.28 | 0.31 | 0.60 | 0.44 | | |
| Model diagnostics | | | | | | | | |
| –2 Res log pseudolikelihood | 1,518.72 | | 1,444.32 | | 1,765.41 | | 1,070.89 | |
| Generalized $\chi^2$ | 212.48 | | 133.29 | | 100.59 | | 211.41 | |

*Statistical significance ($P < 0.05$).

urban environment (as both household objects and refuse); higher-quality housing would be expected to protect against dengue transmission by limiting the *Aedes* vectors' access to residents in the home. The vast majority of these children have lived in a single home and typically spend little time outside of their community, thus implicating local exposure.

## CONCLUSION

Our findings highlight the complexity of febrile illnesses in Ghana by revealing previous dengue exposure in 21.6% of 218 children and recent exposure in 3.2% of children. This may be the first seroprevalence survey in Ghana for dengue exposure, which is increasingly being recognized as a neglected tropical disease in west Africa.[16,17] We observed dengue exposure in an unlikely population—confirmed malaria cases—which is probably the tip of the proverbial iceberg for local dengue fever epidemiology. Our study sample is precisely the opposite population of febrile illness patients who we would normally screen for active dengue infection, because we would assume that laboratory-confirmed malaria patients have been properly diagnosed. Given that the local dengue fever burden probably remains obscured by misdiagnosed malaria cases, the prevalence of DENV (and potentially, other flaviviruses) in malaria-negative febrile illness patients is likely to be higher.

The epidemiological profile of infectious diseases remains cloudy in west Africa and demands a more detailed assessment of the local viral fever baseline. Improved clinical awareness, surveillance, and diagnostic testing of febrile illness present a substantial opportunity to improve population health and deploy healthcare resources more efficiently throughout the region.

Received October 27, 2014. Accepted for publication December 4, 2014.

Published online January 12, 2015.

Acknowledgments: We are grateful to Mr. Nicholas Amoako of the Kintampo Health Research Centre and Mr. James Abugri of the University of Development Studies, Navrongo Campus, for their contributions to sample collection. The authors thank two anonymous reviewers for suggestions that helped us improve this report.

Financial support: This study used samples collected as part of projects supported by Royal Society–Leverhulme Africa Award AA110050 (to G.A.A.) and National Institutes of Health Grant 1R01AI102848 (to G.A.A.). Additional funding was provided by the Office of the Provost and the College of Arts and Sciences at the University of Miami.

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