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Ubydul Haque

Johns Hopkins Bloomberg School of Public Health

Gregory E. Glass

Johns Hopkins Bloomberg School of Public Health

Arne Bomblies

University of Vermont

Masahiro Hashizume

Nagasaki University

Dipak Mitra

Johns Hopkins Bloomberg School of Public Health

See next page for additional authors

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Authors

Ubydul Haque, Gregory E. Glass, Arne Bomblies, Masahiro Hashizume, Dipak Mitra, Nawajish Noman, Waziul Haque, M. Moktadir Kabir, Taro Yamamoto, and Hans J. Overgaard

Risk Factors Associated with Clinical Malaria Episodes in Bangladesh: A Longitudinal Study

Ubydul Haque, Gregory E. Glass, Arne Bombli, Masahiro Hashizume, Dipak Mitra, Nawajish Noman, Waziul Haque, M. Moktadir Kabir, Taro Yamamoto, and Hans J. Overgaard*

Department of Mathematical Sciences and Technology, Norwegian University of Life Sciences, Ås, Norway; Department of Molecular Microbiology and Immunology, and Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; Department of Civil and Environmental Engineering, University of Vermont, Burlington, Vermont; Institute of Tropical Medicine and Global Center of Excellence Program, Nagasaki University, Nagasaki, Japan; Esri, Redlands, California; Oslo and Akershus University College of Applied Sciences, Oslo, Norway; BRAC Health, Nutrition and Population Programme, Dhaka, Bangladesh

Abstract. Malaria is endemic to Bangladesh. In this longitudinal study, we used hydrologic, topographic, and socio-economic risk factors to explain single and multiple malaria infections at individual and household levels. Malaria incidence was determined for 1,634 households in 54 villages in 2009 and 2010. During the entire study period 21.8% of households accounted for all ($n = 497$) malaria cases detected; 15.4% of households had 1 case and 6.4% had ≥ 2 cases. The greatest risk factors for malaria infection were low bed net ratio per household, house construction materials (wall), and high density of houses. Hydrologic and topographic factors were not significantly associated with malaria risk. This study identifies stable malaria hotspots and risk factors that should be considered for cost-effective targeting of malaria interventions that may contribute to potential elimination of malaria in Bangladesh.

INTRODUCTION

In Bangladesh, malaria remains endemic to 13 of the 64 administrative districts. In 2007, there were 59,857 cases of laboratory-confirmed malaria and 228 deaths,¹ yielding a crude prevalence of 4.0%.² Almost 90% of the cases were caused by *Plasmodium falciparum*.³ By 2011, the numbers were reduced to 51,773 cases and 36 deaths,⁴ corresponding to a 13.5% reduction in morbidity and 84% reduction in mortality. Approximately 80% of all malaria cases in Bangladesh continue to be reported in three districts in the Chittagong Hill Tracts (CHT) located in the southeastern part of the country. Rajasthali, a single sub-district in the CHT, had a prevalence of 36% in 2007.² In 2011, the prevalence of malaria in this sub-district was reduced to 11.5%.⁵ The main reasons for this reduction were the implementation of the national malaria control program supported by the Global Fund,⁶ which included insecticide-treated bed nets, long-lasting insecticide-treated nets (LLINs), rapid diagnosis tests (RDTs), and new drug regimens (artemisinin-based combination therapy).⁷ We have shown that the level of knowledge of malaria transmission,⁸ treatment-seeking behavior,^{7,9} ownership and use of ITNs/LLINs, and household and individual level risk factors have changed over the last five years (2007–2011)^{7–9} and contributed to the decreasing trend of malaria transmission.^{4,9} Coverage of LLINs and insecticide-treated nets has already exceeded targeted goals in Bangladesh.^{8,10}

Cross-sectional studies have shown that malaria is heterogeneously distributed in Bangladesh,^{3,9} and national malaria risk maps have been created from these analyses.^{11,12} A household-based risk map also has been created within Rajasthali by using cross-sectional data,⁹ and risk factors related to higher exposure to malaria have been identified.^{2,9,13} Identifying malaria risk factors and transmission hotspots create opportunities for targeted and more cost-effective malaria control.¹⁴ However, these studies did not evaluate the potential impacts of topo-

graphic and hydrologic features that influence the locations and abundance of vector breeding sites. Several variables such as elevation, slope, aspect, topographic convergence and wetness index (an approximate measure of predicted water accumulation), stream order, and stream network have been used to predict malaria risk at finer spatial scales in other countries.^{15–18}

We have used longitudinal data collected during January 2009–2010 for malaria incidence in 54 villages in the Gilachari Union in the Rajasthali Sub-district. This study investigates bed net ratio per household, housing construction materials (wall), hydrologic and topographic risk factors of malaria, and spatial patterns of the disease over two years in the same population.

MATERIALS AND METHODS

Study area. The study was conducted in Gilachari Union, Rajasthali Sub-district, Rangamati District in the CHT (22°20′–27′ N, 92°14′–22′ E). The Bangladeshi geographic administrative units are described in more detail elsewhere.³ Gilachari Union contains 54 villages in an area of 113.83 km² that is covered with forested hilly mountains, lakes, agricultural land, and streams, and is representative of much of the CHT. The total population of Gilachari was 7,922 persons living in 1,634 households in 2009. The population contains several ethnic groups. Malaria is hyperendemic and occurs throughout the year with a peak in the rainy season (April–October).

Malaria data. The Bangladeshi Ministry of Health, in collaboration with the BRAC (a national non-governmental development organization) are implementing the National Malaria Control Program supported by the Global Fund (2007–2014).^{5,19} Malaria diagnosis and treatment is free in Bangladesh. Under the National Malaria Control Program, health workers provide malaria diagnosis and treatment at the community level,^{5,8} and trained laboratory technicians perform microscopic tests in hospitals. Rapid diagnostic tests (*Paracheck-Pf*[®]; Orchid Biomedical Systems, Goa, India) are used in the field to confirm cases. Any villager with a febrile illness can obtain treatment. Name, sex, age, diagnosis method, date, diagnosis result, and village name were recorded for every person tested by community health workers or at the hospital.

*Address correspondence to Hans J. Overgaard, Department of Mathematical Sciences and Technology, Norwegian University of Life Sciences, Ås, Norway. E-mail: hans.overgaard@umb.no

We collated monthly malaria diagnosis reports during January 2009–December 2010 from the BRAC local office in Rajasthali and from the Rajasthali Hospital. We excluded 13 confirmed cases reported from BRAC because their names and households did not match with our records. We might have missed some malaria-positive cases, e.g., if persons bought anti-malarial drugs directly from drug vendors and/or preferred self-treatment. Furthermore, there could have been false-positive or false-negative results in microscopic tests or RDTs, which was a fact that we could not control. These factors were the major limitations of this data set. However, we attempted to check the accuracy of the reports by quarterly visits to each of the 1,634 households by health workers after cases were reported. The independent ethics committee of Nagasaki University reviewed and approved this sampling protocol.

Socioeconomic and geographic data. A socioeconomic survey of all households in the study area was conducted in 2009 as part of a previous study.⁸ All households were georeferenced by using global positioning systems. Geographic coordinates and sociodemographic information were entered into a geographic information system (GIS) (ArcGIS version 10; ESRI, Redlands, CA) as described elsewhere.^{5–9} Bed net ratio (number of nets present in the household/persons), age, sex, and individual education was updated and calculated from our previous study.⁸ We also determined local house density by using the global positioning system location of all houses and calculated the distance from every single house to all other houses. We then counted the number of houses within a 2-km circular buffer area surrounding each house to determine the density. The house density was categorized into

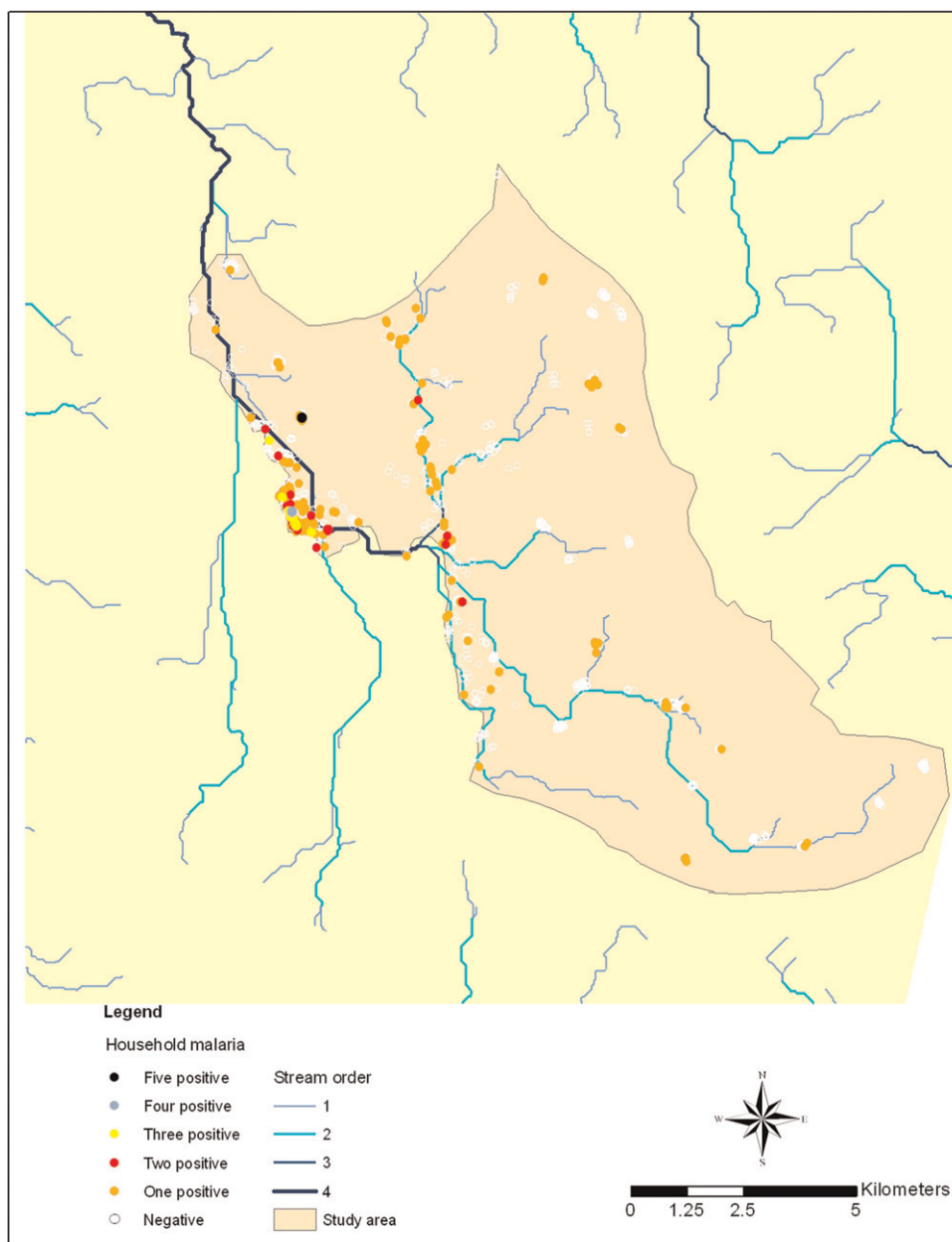


FIGURE 1. Stream order network in the study area, Bangladesh.

1–200, 201–500, 501–1,000, and > 1,000 houses within a 2-km radius from each house.

Remote sensing data. We obtained Advanced Spaceborne Thermal Emission and Reflection Radiometer digital elevation models (DEM) with 30-meter resolution over 60 × 60 km from Glovis, the U.S. Geological Survey global visualization viewer (path 136, row 44). The DEM was converted to 15-meter resolution by using bilinear interpolation, entered in ArcGIS using the spatial analyst extension, and clipped using PCI Geomatica (Arlington, VA) software. We used the open-source GIS program SAGA (www.saga-gis.org/en/index.html) to extract the topographic wetness index, aspect, elevation, and topographic convergence index for all houses in the Gilachari Union. The topographic convergence index quantifies the likelihood of saturation, and is defined as the logarithm of the ratio of the flow accumulation and the local slope in a specific pixel.

Statistical analysis. We performed individual and household level analyses with malaria incidence. Individual level bivariate negative binomial regression analysis was performed to explore the associations between clinical malaria episodes and age and sex. Household level analysis was performed

separately. To identify potential risk factors, bivariate analyses were first performed to evaluate the unadjusted effects of covariates. All variables with a *P* value < 0.05 for the likelihood ratio test in the bivariate analysis were entered into multivariate negative binomial regression models. Statistical analyses were performed with STATA version 11 (StataCorp LP, College Station, TX).

Spatial analysis. We used the model builder in ArcGIS 10 to create water flow direction, accumulation, watershed, stream network, and stream link layers. We then created stream order networks (first through fourth), and used the ArcGIS proximity tool to calculate the distance from every household to different stream orders (Figure 1).

We used SaTScan version 8.2.1²⁰ to detect spatial clusters in episodes of malaria, and explored the statistical significance of the clusters using 999 Monte Carlo replications to ensure adequate power to define the clusters. For this analysis, we sampled a Poisson distribution (after adjusting for population) in which the null hypothesis assumed that the expected number of cases in each area was proportional to the person-years of follow-up in that area. We used a maximum window of 10% of the population for scanning clusters of malaria

TABLE 1
Risk factors for malaria, Bangladesh*

Factor	No. households (%)	No. positive households (%)	Unadjusted IRR (95% CI)	<i>P</i>	Adjusted IRR (95% CI)	<i>P</i>
Bed net ratio (per person/per household)						
0–0.5	469 (28.7)	107 (22.81)	1		1	
> 0.5–1	971 (59.42)	211 (21.73)	0.99 (0.78–1.26)	0.951	0.71 (0.56–0.91)	0.006
> 1	194 (11.87)	38 (19.59)	0.84 (0.58–1.24)	0.388	0.42 (0.28–0.62)	0.0001
Wall						
Jute stick/bamboo	1,534 (93.88)	317 (89.04)	1		1	
Tin/concrete	40 (2.45)	13 (3.65)	2.07 (1.18–3.63)	0.011	1.63 (0.94–2.82)	0.081
Mud	60 (3.67)	26 (7.30)	2.88 (1.88–4.42)	0.0001	2.17 (1.45–3.26)	0.0001
House density (no.)						
1–200	821 (50.24)	115 (32.30)	1.00		1	
201–500	222 (13.59)	61 (17.13)	2.078 (1.51–2.87)	0.0001	1.80 (1.09–3.00)	0.022
501–1,000	60 (3.67)	11 (3.09)	1.697 (0.95–3.02)	0.072	1.43 (0.70–2.92)	0.326
> 1,000	531 (32.50)	169 (47.47)	3.00 (2.37–3.80)	0.0001	2.79 (1.70–4.56)	0.0001
First-order streams (km)						
< 2	1,527 (93.45)	326 (21.35)	1.00			
≥ 2	107 (6.55)	30 (28.04)	1.22 (0.93–2.23)	0.353		
Second-order streams (km)						
< 2	1,451 (88.80)	333 (22.95)	1.00		1.00	
≥ 2	183 (11.20)	23 (12.57)	0.46 (0.30–0.70)	0.0001	0.73 (0.32–1.71)	0.475
Third-order streams (km)						
< 2	240 (14.69)	60 (25.00)	1.00			
≥ 2	1,394 (85.31)	296 (21.23)	1.02 (0.75–1.38)	0.917		
Fourth-order streams (km)						
< 2	890 (54.47)	252 (28.31)	1.00		1.00	
≥ 2	744 (45.53)	104 (13.98)	0.40 (0.32–0.51)	0.0001	0.82 (0.49–1.37)	0.446
Aspect						
Eastern or southeastern	467 (28.58)	261 (22.37)	0.98 (0.77–1.24)	0.868		
Western or northwestern	1,022 (62.55)	95 (20.34)	1.04 (0.84–1.31)	0.696		
Elevation (meters)						
			0.996 (0.995–0.998)	0.0001	1.001 (0.997–1.003)	0.597
Convergence						
1 (wettest)	327 (20.01)	80 (22.47)	1.00			
2	327 (20.01)	78 (21.91)	0.96 (0.69–1.34)	0.826		
3	327 (20.01)	63 (17.70)	0.75 (0.53–1.07)	0.110		
4	327 (20.01)	62 (17.42)	0.90 (0.64–1.26)	0.536		
5 (driest)	326 (19.95)	73 (20.51)	0.90 (0.65–1.26)	0.548		
Wetness index						
1 (driest)	328 (20.07)	62 (17.42)	1.00			
2	328 (20.07)	69 (19.38)	1.16 (0.81–1.66)	0.406		
3	325 (19.89)	84 (23.60)	1.58 (1.12–2.22)	0.009		
4	329 (20.13)	68 (19.10)	1.15 (0.80–1.64)	0.451		
5 (wettest)	324 (19.83)	73 (20.51)	1.35 (0.96–1.92)	0.088		

*IRR = incidence rate ratio; CI = confidence interval. IRR for the reference category is 1.00

TABLE 2
Geographic cluster of malaria cases (adjusted Poisson model for 2009 and 2010), Bangladesh*

Year and cluster	Population	No. observed cases	No. expected cases	Relative risk	P
2009					
1	378	44	12.08	4.20	0.00001
2	535	44	17.10	2.90	0.00001
2010					
1	118	20	3.63	5.90	0.00001
2	151	22	4.65	5.10	0.00001
3	76	14	2.34	6.28	0.0007
4	48	11	1.48	7.74	0.0019
5	619	41	19.07	2.38	0.0170

*Only significant clusters are shown.

episodes. Finally, we generated all maps by using the ArcGIS 10 software.

RESULTS

Malaria incidence. There were 497 (6.3%) episodes of malaria recorded for 7,922 persons during the study period. Approximately 80% were identified by RDT and the rest by microscopy. There were 253 cases recorded in 2009 and 244 cases recorded in 2010. The incidence rate was 31.37 cases/1,000 person-years. During the study period, 1,278 households (78.2%) did not experience any malaria, 252 households (15.4%) had one malaria case, and 104 households (6.4%) had multiple infections (i.e., 2–5 infections). Among households with a documented case, approximately half (252 of 497) had one infection during the study. Among the remaining malaria episodes with multiple infections, 90.6% (222 of 245) occurred

among different household members. Eleven households had more than one person within the same month who became infected. The temporal distribution of second cases in households ranged from the first month to 22 months.

Only 23 (0.3%) persons had two infections in the two years of study. The shortest time interval to a second episode among persons was 6 months and the longest interval was 21 months.

Individual level risk factors. Using children < 5 years of age as a reference (incidence rate ratio [IRR] = 1), we found that the risk for clinical malaria episode decreased with age (5–14 years, IRR = 0.70, 95% confidence interval [CI] = 0.54–0.90; 14–49 years, IRR = 0.46, 95% CI = 0.36–0.58; > 49 years, IRR = 0.27, 95% CI = 0.18–0.41). Malaria was equally distributed among males and females in the population.

Household level risk factors. Factors significantly related to clinical malaria episodes were house density, housing construction material (wall), second-order stream, fourth-order stream, and elevation in bivariate analysis (Table 1). However, multivariate analysis showed that malaria was associated only with having > 0.5 bed nets per person/household, living in a house made of mud walls, and with an area with a house density of either 201–500 or > 1,000 houses/km² (Table 1).

Spatial clusters. Spatial clusters of malaria incidence occurred in the western and northern part of the study area. In 2009, two statistically significant malaria clusters were observed; in 2010, there were five such clusters (Table 2). Malaria hotspots persisted during the two-year study period and 120 particular households appeared in the significant clusters in both consecutive years. Malaria incidence was highly heterogeneous between villages, but a higher incidence tended to be found in northeastern part of the study area (Figure 2A) in 2009 and 2010 (Figure 2B).

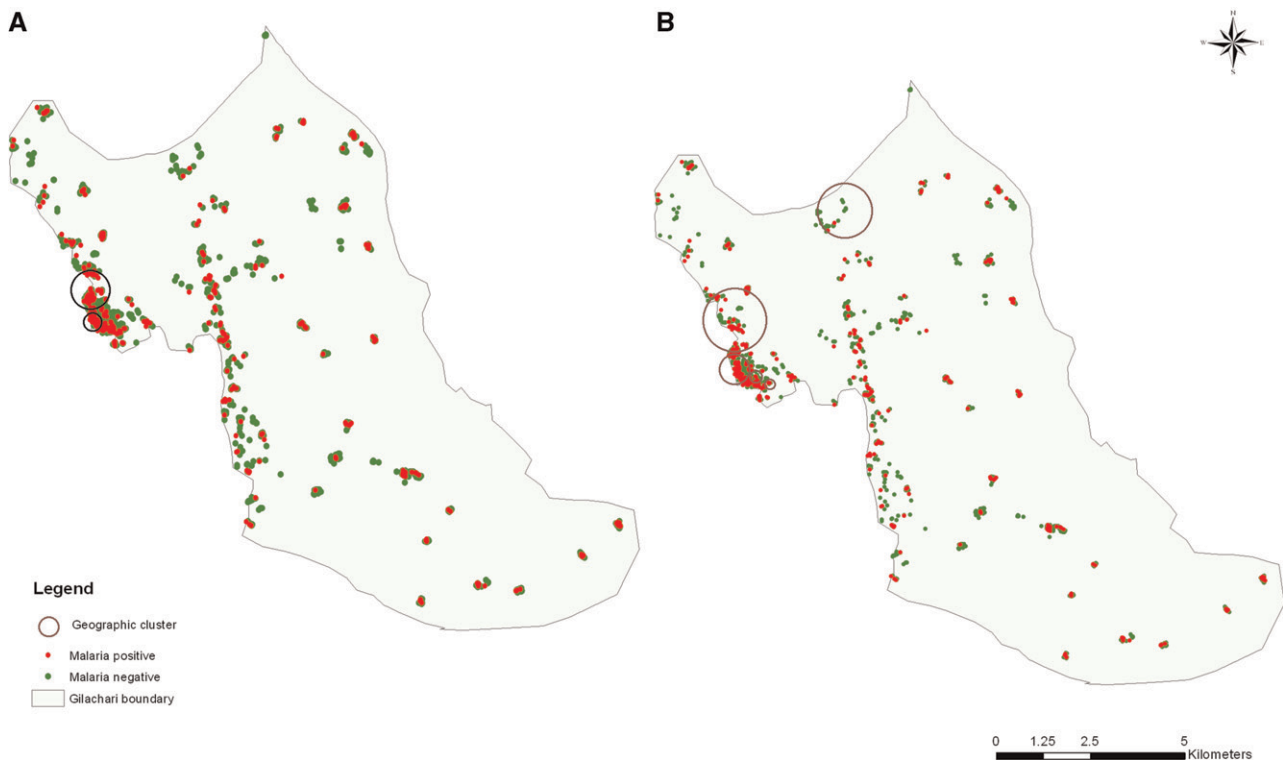


FIGURE 2. A, Spatial distribution of malaria clusters in 2009. B, Spatial distribution of malaria clusters in 2010, Bangladesh.

DISCUSSION

The results of this study show that three years after the initiation of a national malaria control program, malaria continues to persist but has become focal. Even in the highest risk region only a small fraction of households had cases of malaria over the two-year study period and even fewer had multiple cases. The relative rarity of persons with repeated infections suggests that household or neighborhood environmental factors could be primary predictors of malaria in the CHT. More longitudinal studies in the near future can provide further insight to this suggestion.

We identified significant malaria clusters in two consecutive years (Figure 2). The clusters were defined as hotspots according to the definition by Bousema and others²¹ because transmission exceeded the average level of malaria in the area. Furthermore, the fact that the hotspots were present in both years is evidence for stable hotspots. These stable hotspots were located mostly in a densely populated area in the northwestern region. It is surprising that 120 households remained in the hotspot clusters in both years. We were not able to identify any particular features common for these households. Most households did not experience malaria over the two-year study period, but many households ($n = 104$) reported multiple infections.

The results show clear clustering of malaria in this area. However, the reasons for the spread of more but smaller clusters in 2010 are unknown. Successful strategies to eliminate malaria in the CHT should first focus on identifying such malaria hotspots, then implementing context-specific interventions, such as targeted integrated vector control activities²² and targeted reduction of the human infectious reservoir.²¹ The affected households and nearby households of malaria cases diagnosed in hospitals and clinics should be given treatment with antimalarial drugs.²³ Reactive screening for asymptomatic and symptomatic infections can be done periodically in such hotspots²³ and cases be treated individually or the whole community provided with mass drug administration.²⁴

We examined various household and neighborhood effects by characterizing patterns of hydrologic, topographic, and socioeconomic variables. Many of the variables have been associated with changes in malaria risk in Bangladesh and in other countries in cross-sectional studies.⁹ In our study, none of the physical environmental factors, including hydrologic variables, showed a positive relationship with malaria risk. These findings are different to those from other published studies, e.g., in Zambia, where third-order streams were a significant risk factor for malaria.¹⁷

Our study area consists of forested hills and mountains. The main malaria vectors in these areas are *Anopheles baimai*, *An. minimus* s.l., and *An. annularis*.^{25–27} A recent entomologic study near our study site showed that these species were the most likely malaria vectors in the CHT because of their anthropophilic nature.²⁸ *Anopheles baimai* (formerly *An. dirus* species D) and *An. minimus* s.l., species are also important malaria vectors in Southeast Asia and are generally associated with forested and forest fringe areas.^{29,30} The breeding sites of *An. minimus* are at the edges of grassy and shaded banks of stable clear and slow-moving streams³¹ and those of *An. baimai* are small heavily shaded puddles and pools in the deep forests.³² The resolution of our data was probably not fine enough to detect hydrologic and topographic risk factors of malaria.

We found that the topographic parameters, including the topographic convergence index, wetness index (spatial distribution of soil moisture), aspect, and slope, were not significant risk factors in contrast to those in other studies.^{16,17} Microtopography can determine the formation of suitable larval developmental habitat in some transmission environments and exert a strong control on mosquito population dynamics.^{33,34} When rainfall recurrence interval is less than the persistence time of pools, the malaria vectors can thrive.³³ This finding was not the case for the study site at Rajasthali. Pools may be contributing to the mosquito population, but they do not explain variability in malaria incidence.

Self-medication is rare in these villages because free diagnosis and treatment is available at the community level. Households included in this longitudinal study preferred proper diagnosis and treatment either from BRAC health workers or from the Rajasthali Thana health complex.⁸ Demographic and socioeconomic risk factors still influence malaria risk in Bangladesh.⁹ Age remained one of the main risk factors as reported.^{3,9} With the support of rounds six and nine of the global fund project, 100% of households were supposed to be supplied with two LLINs.¹⁹ In this study, increased bed net coverage was associated with lower incidence as expected, but coverage was low (0.5 bed net/per-person/household) in a small portion (0.92%) of households. Most of the houses were poorly constructed and inhabitants living in houses made with mud walls are vulnerable to malaria infection. This finding is intuitive but has not been published for Bangladesh. Areas with high density housing (from 201–500 and $> 1,000$ houses/km²) were also at increased risk.

This longitudinal study in a small geographic area provided some novel findings about stability of hot spots and multiple infections among households and persons. Furthermore, areas that require expanded malaria control efforts based on malaria foci have been identified. The cluster method that was used could be a useful research tool for investigation of other temporally and spatially clustered stable malaria hotspots in the CHT. The location of households in villages is an independent and important factor for variation in malaria incidence. Supplying LLIN, impregnating bed nets with insecticides, and providing treatment at the community level may not be enough to eliminate malaria from Bangladesh. Mass screening can be conducted in stable hotspots to identify asymptomatic reservoirs, and providing treatment to these populations can reduce further malaria burden in the region. This study also confirmed the existence of some malaria risk factors (bed net ratio, poor housing construction materials, and house density) and geographic clusters identified in previous studies. For targeted interventions, detail risk mapping using GIS, remote sensing, and DEM may be important for policy implementation.

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Authors' addresses: Ubydul Haque and Gregory E. Glass, Department of Molecular Microbiology and Immunology, John Hopkins Bloomberg School of Public Health, Baltimore, MD, E-mails: uhaque@jhsph.edu and gglass@jhsph.edu. Arnie Bomblics, Department of Civil and Environmental Engineering, University of Vermont, Burlington, VT, E-mail: abomblic@uvm.edu. Masahiro Hashizume, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan, E-mail: hashizum@nagasaki-u.ac.jp. Depak Mitra, Department of International Health, John Hopkins Bloomberg School of Public Health, Baltimore, MD, E-mail: dmitra@jhsph.edu. Nawajish Noman, Esri, Redlands, CA, E-mail: nman@esri.com. Waziul Haque, Oslo and Akershus University College of Applied Sciences, Oslo, Norway, E-mail: whicddrb@gmail.com. M. Moktadir Kabir, BRAC Health, Nutrition and Population Programme, Dhaka, Bangladesh, E-mail: moktadir.k@brac.net. Taro Yamamoto, Department of International Health, Nagasaki University, Nagasaki, Japan, E-mail: y-taro@nagasaki-u.ac.jp. Hans J. Overgaard, Department of Mathematical Sciences and Technology, Norwegian University of Life Sciences, Ås, Norway, E-mail: hans.overgaard@umb.no.

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