Surveillance of Vector-Borne Infections (Chikungunya, Dengue, and Malaria) in Bo, Sierra Leone, 2012–2013

Donald F. Dariano III,[1] Chris R. Taitt,[2] Kathryn H. Jacobsen,[3] Umaru Bangura,[4] Alfred S. Bockarie,[4,5] Moses J. Bockarie,[6] Joseph Lahai,[4] Joseph M. Lamin,[4] Tomasz A. Leski,[2] Chadwick Yasuda,[2] David A. Stenger,[2] and Rashid Ansumana[4,5,6]*

[1]*College of Science, George Mason University, Fairfax, Virginia;* [2]*Center for Biomolecular Science and Engineering, U.S. Naval Research Laboratory, Washington, District of Columbia;* [3]*Department of Global and Community Health, George Mason University, Fairfax, Virginia;* [4]*Mercy Hospital Research Laboratory, Bo, Sierra Leone;* [5]*Njala University, Bo Campus, Sierra Leone;* [6]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

* Address correspondence to Rashid Ansumana, Mercy Hospital Research Laboratory, Bo, Sierra Leone. E-mail: r.ansumana@liverpool.ac.uk

Abstract.

Malaria remains a significant cause of morbidity and mortality in West Africa, but the contribution of other vector-borne infections (VBIs) to the burden of disease has been understudied. We used rapid diagnostic tests (RDTs) for three VBIs to test blood samples from 1,795 febrile residents of Bo City, Sierra Leone, over a 1-year period in 2012–2013. In total, 24% of the tests were positive for malaria, fewer than 5% were positive for markers of dengue virus infection, and 39% were positive for IgM directed against chikungunya virus (CHIKV) or a related alphavirus. In total, more than half (55%) of these febrile individuals tested positive for at least one of the three VBIs, which highlights the very high burden of vector-borne diseases in this population. The prevalence of positives on the Chikungunya IgM and dengue tests did not vary significantly with age ($P > 0.36$), but higher rates of malaria were observed in children < 15 years of age ($P < 0.001$). Positive results on the Chikungunya IgM RDTs were moderately correlated with rainfall ($r^2 = 0.599$). Based on the high prevalence of positive results on the Chikungunya IgM RDTs from individuals Bo and its environs, there is a need to examine whether an ecological shift toward a greater burden from CHIKV or related alphaviruses is occurring in other parts of Sierra Leone or the West African region.

Malaria and other vector-borne infections (VBIs) are responsible for nearly 10% of the disability-adjusted life years lost globally each year to infectious diseases,[1] and they cause nearly 1 million deaths worldwide annually.[2] Sierra Leone, in West Africa, has one of the highest rates of malaria transmission among African countries,[3] with about half of all outpatient clinic consultations and a sizeable proportion of inpatient hospitalizations due to malaria.[4,5] However, many patients with febrile illnesses have unknown infections. For example, 27.3% of hospitalized febrile patients in Kenema, Sierra Leone, in 2011–2012 had malaria, but more than half (53.4%) of the patients did not have a known diagnosis at discharge.[5] Little is known about the epidemiological burden from nonmalarial VBIs in Sierra Leone, but preliminary reports of circulation of other VBIs such as chikungunya virus (CHIKV) and dengue virus have been reported.[6–9]

As part of a larger study of the etiology of febrile illnesses in and around Bo, Sierra Leone, Mercy Hospital Research Laboratory personnel tested 1,795 blood samples from adults and children ages 6 and older who were residents of Bo city and presented to the laboratory with self-reported or clinically confirmed fever with onset within the previous 7 days. Detailed symptomatic information beyond febrile status was obtained for about one in three participants (N = 565). Informed consent from patients (or, for minor children, consent from their parents) was obtained and documented before collection of the survey data and biological specimens. The research protocol was approved by the institutional review boards of Njala University, George Mason University, the Liverpool School of Tropical Medicine, the U.S. Naval Research Laboratory, and the Sierra Leone Ethics and Scientific Review Committee.

Participants' blood samples were tested with at least one of four commercial rapid diagnostic tests (RDTs) for VBIs according to manufacturer's instructions: malaria was tested with Paracheck™ (Orchid Biomedical Systems, Goa, India) and Malaria Ag Pf/Pan (SD Bioline, Gyeonggi-do, Republic of Korea); chikungunya with Chikungunya IgM (SD Bioline); and dengue with Dengue Duo IgG/IgM/NS1 (SD Bioline). For 1,260 patients, the sample volume was sufficient to allow tests for all three VBIs to be performed. Although some of the tests measure multiple markers, a positive result for any of the markers was designated as a positive test.

Malarial antigens were detected in 23% of the samples tested using one or both of the malaria RDTs (Table 1). There were no differences in malarial detection by gender (P = 0.898) but rates were higher in children than in adults (P < 0.001). There was no significant correlation between rainfall and the percentage of tests that were positive for malaria by month (r^2 = 0.034; Table 2).[10] The malaria detection rates determined here are significantly lower than those documented elsewhere.[3,8] This disparity may reflect an actual decrease in the malaria burden in Bo, or they could be due to the cohort tested, the age distribution of participants, or the generally poorer sensitivity of the RDTs as compared with gold standard microscopic tests, enzyme-linked immunosorbent assays (ELISAs), and polymerase chain reaction.[11] However, the two malaria tests used here meet World Health Organization (WHO) procurement criteria for use in resource-limited environments,[12] and in spite of sensitivity issues, RDTs may improve management of malaria in settings with inadequate diagnostic facilities.[13,14]

In total, 4.5% of the participants tested positive for at least one marker of dengue virus (DENV) infection. No significant difference was observed by gender (P = 0.311) or by age (P = 0.368). The overwhelming majority of these participants (95%) were positive for the presence of anti-DENV IgG, but not anti-DENV IgM or NS1 antigen (Supplemental Table 1), suggesting that a high proportion of DENV-positive participants had antibodies from previous infections rather than suffering from an acute dengue infection at the time of testing. Although two-thirds of the DENV-positive samples were collected in June, the prevalence of DENV was not significantly correlated with rainfall (r^2 = 0.042), but this may be due to the low number of positive samples. The low rates of DENV IgG/IgM seropositivity observed here agree with a recent study in neighboring Kenema,[9] but several other papers report significantly higher seroprevalence of anti-DENV antibodies.[7,8]

A high percentage (39%) of participants had positive results on the Chikungunya IgM RDT. Participants who tested positive were significantly more likely than those with negative results to report joint pain and/or backaches (P < 0.001), both characteristic symptoms of CHIKV infections (Supplemental Table 2). We observed no significant difference in results for the

Chikungunya IgM tests by gender ($P = 0.934$) or age ($P = 0.391$). Rates of positive test results showed a moderate correlation with monthly rainfall amounts ($r^2 = 0.599$). The high proportion of positive test results on the Chikungunya IgM RDT agrees with our previous report from Bo[6] and with another study performed with samples collected in the same time (35%).[8] A separate study using samples from 2006 to 2008 documented much lower seroprevalence.[9]

The manufacturer of the Chikungunya IgM RDT reports a sensitivity of 97.1% and specificity of 91.1% compared with ELISAs.[15] However, other studies have documented lower sensitivity and specificities,[16–18] suggesting that at least some of the positive samples may be false positive or may be due to cross-reactivity with other uncharacterized or unidentified alphaviruses. Indeed, a recent study at the Kenema Government Hospital documented a 55.8% seroprevalence rate for pan-alphavirus antibodies, indicating widespread alphaviral exposure.[19] Although the test used here claims to target anti-CHIKV antibodies, we cannot rule out the possibility of cross-reaction with other related alphaviruses; to date, we have been unable to confirm the presence of CHIKV via molecular assays or culture. These results suggest that a significant proportion of population of Bo and its environs has been exposed to chikungunya or an as yet unidentified alphaviral species. It would be beneficial to retest residents of Bo using more definitive tests for CHIKV and related alphaviruses to more precisely identify the alphaviral agents causing so many febrile infections.

Among the 1,260 participants tested for all three VBIs, approximately 11% had positive results on more than one test, suggesting possible coinfections (Figure 1). About 45% of participants did not test positive for any of the three VBIs, and the etiological agent of fever remains unknown. However, it is remarkable that half of the febrile individuals in our study tested positive for malaria or chikungunya (or a similar alphavirus), which suggests a heavy burden from vector-borne disease in this city.

One of the limitations of this study is the poor overall sensitivity of RDT technology and single-timepoint testing of samples collected from febrile patients reporting to the clinic. Although WHO recognizes the role of RDTs in management of malaria in resource-limited settings,[12,13] the sensitivity or selectivity of RDTs may limit their effectiveness in diagnostic applications. Nevertheless, the VBI tests used here can serve as valuable tools to identify potential public health issues. Furthermore, the concentrations of both antigens and antibodies are greatly affected by the time of testing, a better option would be to test each subject at multiple timepoints to distinguish acute and convalescent phases. While the Chikungunya IgM test likely missed many actual CHIKV infections or detected other alphaviral infections, the high numbers of positives observed here point to the possibility of a significant alphaviral problem in the tested population, which requires further investigation. A larger set of VBI tests for various alphaviruses (such as O'nyong-nyong virus), bunyaviruses (such as Tahyna virus), and flaviviruses (such as West Nile, Zika, and yellow fever viruses)[20,21] should be used in Bo and its environs to identify the true burden of nonmalarial VBIs in this area.

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Authors' addresses: Donald F. Dariano 3rd, George Mason University, College of Science, Fairfax, VA, E-mail: dariano9933@gmail.com. Chris R. Taitt, Tomasz A. Leski, Chadwick Yasuda, and David A. Stenger, Center for Biomolecular Science and Engineering, U.S. Naval Research Laboratory, Washington, DC, E-mail: chris.taitt@nrl.navy.mil, tomasz.leski@nrl.navy.mil, chadwick.yasuda@nrl.navy.mil, and david.stenger@nrl.navy.mil. Kathryn H. Jacobsen, Global and Community Health, George Mason University, Fairfax, VA, E-mail: kjacobse@gmu.edu. Umaru Bangura, Alfred S. Bockarie, Joseph Lahai, Joseph M. Lamin, and Rashid Ansumana, Mercy Hospital Research Laboratory, Bo, Sierra Leone, E-mails: umarbans@yahoo.co.uk, asbock2@gmail.com, josephlahaiphlebo@gmail.com, jmlamin6076@gmail.com, and r.ansumana@liverpool.ac.uk. Moses J. Bockarie, Centre for Neglected Tropical Diseases, Liverpool School of Tropical Medicine, Liverpool, United Kingdom, E-mail: moses.bockarie@lstmed.ac.uk.

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FIGURE 1. Overlap in vector-borne infection (VBI) rapid diagnostic test results in 1,260 samples tested for all three VBIs.

TABLE 1

Test results by gender and age, July 2012 to June 2013, Bo, Sierra Leone

Infection	Population	No. of tested	No. of positive	% Positive	*P* value
Malaria	All	1,576	361	22.9	–
	Male	611	141	23.1	0.898
	Female	965	220	22.8	
	Age 6–14	88	39	44.3	< 0.001
	Age 15–29	444	105	23.6	
	Age 30–44	271	56	20.7	
	Age 45+	400	74	18.5	
Dengue	All	1,392	62	4.5	–
	Male	534	20	3.7	0.311
	Female	858	42	4.9	
	Age 6–14	8	0	0.0	0.368
	Age 15–29	376	21	5.6	
	Age 30–44	238	19	8.0	
	Age 45+	326	16	4.9	
Chikungunya	All	1,668	645	38.7	–
	Male	648	249	38.4	0.934
	Female	1,020	394	38.6	
	Age 6–14	93	29	31.2	0.391
	Age 15–29	490	192	39.2	
	Age 30–44	283	102	36.0	
	Age 45+	427	149	34.9	

TABLE 2

Percentage of positive tests each month for malaria, dengue, and chikungunya

Month	Malaria		DENV		CHIKV		Average monthly rainfall (cm, 1990–2012)[10]
	No. of positive/ No. of tested	% Positive	No. of positive/ No. of tested	% Positive	No. of positive/ No. of tested	% Positive	
July 2012	73/148	37.1	0/148	0	73/148	49.3	46.9
August 2012	28/134	20.9	0/135	0	80/135	59.3	53.7
September 2012	43/131	32.8	2/131	1.5	69/131	52.7	42.1
October 2012	8/69	11.6	0/65	0	21/68	30.9	28.5
November 2012	29/104	27.9	0/104	0	23/66	34.8	8.6
December 2012	17/124	13.7	0/122	0	46/124	27.1	1.8
January 2013	39/160	24.4	14/157	8.9	48/138	34.8	0.6
February 2013	35/137	25.5	5/42	11.9	45/137	32.8	1.3
March 2013	28/115	24.3	0/75	0	51/174	29.3	3.5
April 2013	23/91	25.3	0/67	0	47/130	36.2	9.5
May 2013	27/124	21.8	0/28	0	63/137	37.7	19.7
June 2013	29/239	12.1	41/288	14.2	77/250	30.8	30.4
Correlation (r^2) with rainfall	–	0.034	–	0.042	–	0.599	1.00

CHIKV = chikungunya virus; DENV = dengue virus.

SUPPLEMENTAL TABLE 1

Positive results for individual markers of VBI using RDTs to test blood samples from febrile individuals, July 2012 to June 2013

Test		Analyte	Number tested	Positive results	% Positive
Chikungunya		IgM	1,700	655	38.5
Dengue		NS1	1,149	1	0.1
		IgM	1,421	2	0.1
		IgG	1,421	60	4.2
Malaria	Paracheck™	*Plasmodium falciparum* (HRP2)	723	148	20.5
	Pf/Pan	*P. falciparum* (HRP2)	882	226	25.6
		Malaria Pan (pLDH)	882	113	12.8

RDT =rapid diagnostic test; VBI = vector-borne infection.

SUPPLEMENTAL TABLE 2

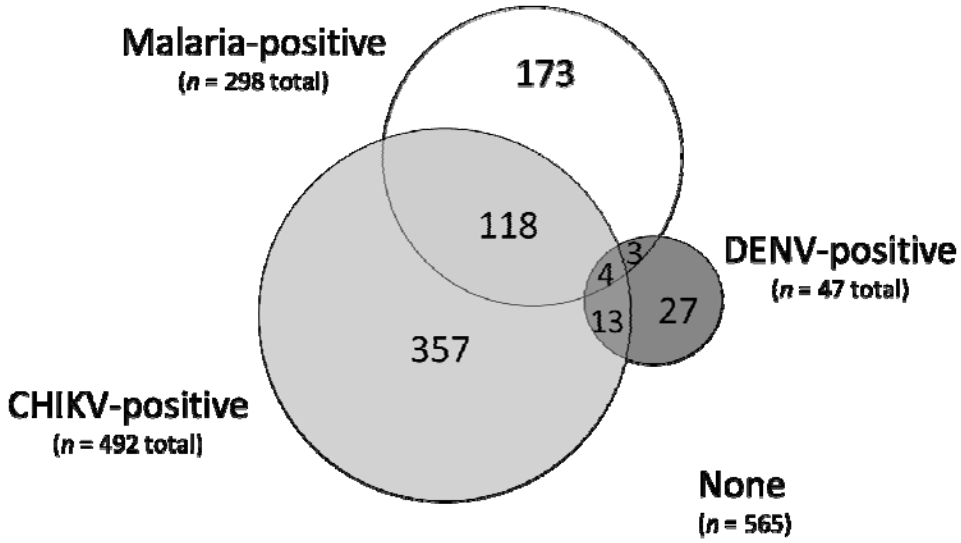
Prevalence of symptoms other than fever for 565 participants for whom detailed symptomatic descriptions were recorded

Symptom	All febrile individuals m (N = 565)	Individuals with positive malaria tests (N = 153)	Individuals with positive CHIKV tests (N = 250)
Headache	97/565 (17.3%)	35 (22.9%)*	52 (20.8%)
Body aches	90 (15.9%)	23 (15.0%)	44 (17.6%)
Cough	36 (6.4%)	6 (3.9%)	22 (8.8%)
Abdominal discomfort and/or diarrhea	34 (6.0%)	14 (9.2%)	15 (6.0%)
Backache	31 (4.6%)	11 (7.2%)	25 (10.0%)*
Nausea and/or vomiting	25 (4.4%)	9 (5.9%)	11 (4.4%)
Side pain	20 (3.5%)	4 (2.6%)	18 (7.2%)*
Joint pain	15 (2.7%)	6 (3.9%)	13 (5.2%)*
Dizziness	15 (2.7%)	5 (3.3%)	8 (3.2%)
Chest pain	8 (1.4%)	3 (2.0%)	5 (2.0%)
Neck pain	4 (0.7%)	2 (1.3%)	4 (1.6%)
Hypertension	4 (0.7%)	1 (0.7%)	0 (0.0%)

CHIKV = chikungunya virus; DENV = dengue virus. The number of DENV-positive individuals was too small to provide meaningful analysis.

* Prevalence of symptom is significantly higher in participants with positive tests rather than negative tests ($P < 0.05$, χ^2 test, corrected for continuity).

Figure 1


Malaria-positive
(n = 298 total)
173
118
3
4
DENV-positive
(n = 47 total)
27
13
357
CHIKV-positive
(n = 492 total)
None
(n = 565)