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Chikungunya and Dengue Fever among Hospitalized Febrile Patients in Northern Tanzania

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Abstract. Consecutive febrile admissions were enrolled at two hospitals in Moshi, Tanzania. Confirmed acute Chikungunya virus (CHIKV), Dengue virus (DENV), and flavivirus infection were defined as a positive polymerase chain reaction (PCR) result. Presumptive acute DENV infection was defined as a positive anti-DENV immunoglobulin M (IgM) enzyme-linked immunsorbent assay (ELISA) result, and prior flavivirus exposure was defined as a positive anti-DENV IgG ELISA result. Among 870 participants, PCR testing was performed on 700 (80.5%). Of these, 55 (7.9%) had confirmed acute CHIKV infection, whereas no participants had confirmed acute DENV or flavivirus infection. Anti-DENV IgM serologic testing was performed for 747 (85.9%) participants, and of these 71 (9.5%) had presumptive acute DENV infection. Anti-DENV IgG serologic testing was performed for 751 (86.3%) participants, and of these 80 (10.7%) had prior flavivirus exposure. CHIKV infection was more common among infants and children than adults and adolescents (odds ratio [OR] 1.9, $P = 0.026$) and among HIV-infected patients with severe immunosuppression (OR 10.5, $P = 0.007$). CHIKV infection is an important but unrecognized cause of febrile illness in northern Tanzania. DENV or other closely related flaviviruses are likely also circulating.

INTRODUCTION

Little work has been done to characterize the role of the arboviruses Chikungunya (genus *Alphavirus*, family *Togaviridae*) and Dengue (genus *Flavivirus*, family *Flaviviridae*) as causes of febrile illnesses in sub-Saharan Africa. Human infection with either virus is associated with fever, arthralgia, malaise, headache, and rash. *Aedes aegypti* and, to a lesser extent, *Aedes albopictus* are the primary vectors in Chikungunya virus (CHIKV) and Dengue virus (DENV) epidemics in sub-Saharan Africa,[1–3] but the viruses are also maintained in some parts of Africa in sylvatic cycles involving primates and forest dwelling *Aedes* species.[1,4–6]

The epidemiology of CHIKV infection in sub-Saharan Africa is poorly understood. CHIKV was first isolated and described during an epidemic in present-day Tanzania in 1952.[7] Since that time, periodic CHIKV outbreaks have been reported across the African continent,[3,8,9] but we are aware of no published reports of CHIKV infection in Tanzania since its discovery. Moreover, because most CHIKV studies in Africa have been conducted during epidemics, little is known about virus circulation among humans between outbreaks. A study in West Africa with 47 participants found four with serologically confirmed acute CHIKV infection during a non-epidemic period,[10] suggesting that the virus may continue to be transmitted to humans between outbreaks. To our knowledge no such study has been conducted in East Africa.

Many questions remain about the epidemiology of DENV infection in sub-Saharan Africa as well. Although several DENV epidemics have been reported in sub-Saharan Africa,[11–13] DENV infection is likely to be considerably underreported, because of limited diagnostic capacity and misclassification as malaria.[14] In East Africa, little is known about the prevalence of DENV infection and how the virus is maintained between epidemics. Although DENV outbreaks have not been reported in Tanzania,[13] DENV infection has been confirmed in travelers returning from the country,[15,16] suggesting that the virus is circulating in Tanzania. However, DENV infection, like CHIKV infection, is a diagnosis rarely considered by local clinicians, presumably due in part to a lack of information about disease prevalence.

To understand the role of CHIKV and DENV as causes of febrile illness in northern Tanzania during a non-epidemic period, we investigated the prevalence, characteristics, and correlates of febrile inpatients with these infections.

MATERIALS AND METHODS

Setting. Moshi (population > 144,000) is situated in the Kilimanjaro Region (population > 1.4 million) of northern Tanzania at 890 m above sea level. This study was conducted at two hospitals in Moshi: Kilimanjaro Christian Medical Centre, a 458-bed referral hospital serving several regions in northern Tanzania, and Mawenzi Regional Hospital, a 300-bed hospital serving the Kilimanjaro Region.

Study participants and procedures. Febrile inpatients were prospectively enrolled from 17 September 2007 through 31 August 2008. Study procedures are described in detail elsewhere.[17,18] Briefly, adult and adolescent inpatients (≥ 13 years of age) with oral temperature ≥ 38.0°C at admission were eligible for enrollment. For pediatric inpatients (age ≥ 2 months to < 13 years of age), inclusion criteria were history of fever in the past 48 hours, axillary temperature ≥ 37.5°C at admission, or rectal temperature ≥ 38.0°C at admission. For each enrollee, a clinical officer who was a member of the study team obtained a standardized clinical history, performed a physical exam, and collected demographic information. Provisional clinical diagnoses and treatment were also recorded. Within 24 hours of admission and before the initiation of antimicrobial therapy, blood was collected for complete blood count,

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examination for parasites, human immunodeficiency virus (HIV) antibody testing for those ≥ 18 months of age,[19] or HIV-1 RNA polymerase chain reaction (PCR) for those < 18 months of age,[20,21] aerobic and mycobacterial blood cultures, and serologic investigation. Results of all study investigations were provided immediately after testing was complete to the clinical team to inform patient management.

Laboratory methods. Serum was stored at –80°C in Moshi and was transported on dry ice to the Duke-National University of Singapore Graduate Medical School Program in Emerging Infections laboratory in Singapore. Serum samples were tested for anti-DENV immunoglobulin M (IgM) and IgG antibodies using an IgM capture enzyme-linked immunosorbent assay (ELISA) and Indirect IgG ELISA (both PanBio, Brisbane, Australia), according to manufacturer's instructions.

RNA was extracted from serum samples using the QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions.

Reverse transcription was performed using Invitrogen Superscript III First Strand Synthesis System (Life Technologies, Carlsbad, CA), according to manufacturer's instructions.

Real-time PCR for flavivirus, DENV, and CHIKV was carried out with the LightCycler 480 SYBR Green I Master kit (Roche Diagnostics, Penzberg, Germany) in a total reaction volume of 20 μL containing 2 μL of cDNA using primers published elsewhere.[22–24] Forty-five PCR cycles were done at 95°C for 10 seconds, 55°C for 5 seconds, and 72°C for 10 seconds, followed by melt curve for analysis purposes.

Study definitions. Confirmed acute CHIKV, DENV, and flavivirus infections were defined as a positive PCR result for CHIKV, DENV, and flavivirus viral RNA, respectively. Presumptive acute DENV infection was defined as a positive anti-DENV IgM ELISA result. Prior flavivirus exposure was defined as a positive anti-DENV IgG ELISA result.[25] Severe immunosuppression was defined as a CD4-positive T-lymphocyte count (CD4 count) < 100 cells/mm^3 for adults and adolescents or CD4% < 25% for patients < 12 months of age; < 20% for patients < 3 years of age; and < 15% for children ≥ 3 years of age.[26]

Participants' village of residence was classified as rural or urban based on the 2002 Tanzania Population and Housing Census for all those whose village of origin was known.[27] Locally collected temperature and rainfall data (Noel HP, personal communication) were used to calculate the average monthly rainfall and the average daily minimum temperature for the study period. A dry month was defined as any month with cumulative rainfall less than the monthly average for the study period. A cold month was defined as any month with an average minimum temperature below the average minimum temperature for the study period.

Statistical analysis. Data were entered using Cardiff Teleform 9.0 (Cardiff Inc., Vista, CA) and analyzed using JMP 8.0 (SAS, Cary, NC). Descriptive statistics are presented as medians, ranges, and interquartile ranges (IQR) for continuous variables and as proportions for categorical variables. Mann-Whitney *U* tests were used to compare differences in medians for continuous data. Pearson's χ^2 was used to compare categorical data; Fisher's exact test was used in cases when expected frequencies were < 5. All *P* values are two-sided. Locally validated[28] and established[29] age-specific reference ranges were used for analysis of participants' hematologic findings.

Research ethics. This study was approved by the Kilimanjaro Christian Medical Centre (KCMC) Research Ethics Committee, the Tanzania National Institutes for Medical Research National Research Ethics Coordinating Committee, and institutional review boards of Duke University Health System and the National University of Singapore.

RESULTS

There were 870 patients enrolled in the study, including 403 (46.3%) adults and adolescents and 467 (53.7%) infants and children.

Demographic descriptions of this study population have been previously reported.[17,18]

Chikungunya. Sera were available for PCR testing for 700 (80.5%) participants, including 368 (91.3%) adults and adolescents and 332 (71.1%) infants and children. Of these, 55 (7.9%) met the definition for confirmed acute CHIKV infection, including 21 (5.7%) adults and adolescents and 34 (10.2%) infants and children (Table 1). CHIKV infection was more common among infants and children than older participants (odds ratio [OR] 1.9, $P = 0.026$).

The demographic and clinical features of patients with and without CHIKV infection are presented in Table 2. The CHIKV-infected patients were significantly more likely to present during a study-defined dry month (OR 3.2, $P = 0.001$) and during a study-defined cold month (OR 3.9, $P < 0.001$) than other febrile inpatients.

Hepatomegaly (OR 2.3, $P = 0.043$) and an absence of vomiting (OR 0.49, $P = 0.043$) were the only assessed signs or symptoms associated with CHIKV infection. There were no significant differences between the hematologic and radiographic findings of those with and without CHIKV infection.

Of CHIKV-infected patients, 36 (65.5%) had no evidence of acute co-infections. HIV infection was not more common among participants with CHIKV infection (22.9%) than other febrile inpatients (26.1%) (OR 0.84, $P = 0.628$). However, among HIV-infected participants > 5 years of age, the median (range) CD4 count was 38 (2, 264) cells/mm^3 for those with CHIKV co-infection, compared with 112 (1, 1,631) cells/mm^3 for those without CHIKV infection ($P = 0.010$). Among all HIV-infected patients, lymphopenia was significantly more common among those with CHIKV co-infection (9 of 11 patients, 81.8%) than those without CHIKV co-infection (70 of 157 patients, 44.6%) (OR 5.6, $P = 0.017$). Furthermore, 9 (90%) of the CHIKV and HIV co-infected patients met the study definition for severe immunosuppression, compared with 71 (43.3%) of the CHIKV-uninfected HIV-infected patients (OR 10.5, $P = 0.007$).

No participant received a clinical diagnosis of CHIKV infection during their hospital stay. Among those with confirmed CHIKV infection, the most common provisional diagnosis was malaria in 23 (41.8%). Forty-eight (87.3%) of those with CHIKV infection were treated with an antibacterial or antimalarial, compared with 462 (71.6%) of other febrile inpatients (OR 2.7, $P = 0.012$).

Five (9.1%) CHIKV-infected patients died before discharge from the hospital. One patient had a 1-year history of fever with a large unexplained right-sided pleural effusion. Two patients had advanced HIV infection with chest radiographs showing bilateral macronodules and multilobar consolidation, respectively. A fourth patient was admitted with diabetic

TABLE 1
Prevalence of CHIKV and DENV infections among febrile inpatients, northern Tanzania, 2007–2008

	All patients		Pediatric		Adult and adolescent			
	n/N	(%)	n/N	(%)	n/N	(%)	OR (95% CI)	*P* value
Acute CHIKV infection	55/700	(7.9)	34/332	(10.2)	21/368	(5.7)	1.9 (1.1–3.3)	0.026*
Presumptive acute DENV infection	71/747	(9.5)	35/380	(9.2)	36/367	(9.8)	0.93 (0.57–1.5)	0.780
Prior flavivirus exposure	80/751	(10.7)	19/384	(5.0)	61/367	(16.6)	0.26 (0.15–0.45)	< 0.001*

* $P < 0.05$.
CHIKV = Chikungunya virus; DENV = Dengue virus; OR = odds ratio; CI = confidence interval.

ketoacidosis and a bloodstream infection with *Escherichia coli*. The final patient had no evidence of any co-infections and presented with headache, neck stiffness, convulsions, and loss of consciousness with a normal peripheral white blood cell count.

Dengue. None of the 700 participants for whom PCR analysis was performed had confirmed acute DENV or flavivirus infection. Serum was available for DENV serology for 747 (85.9%) of participants. Among these, 71 (9.5%) met the definition for presumptive acute DENV infection, including 36 (9.8%) adults and adolescents and 35 (9.2%) infants and children (Table 1).

The demographic and clinical features of patients with presumptive acute DENV infection are shown in Table 3. No demographic characteristics, presenting symptoms, physical examination findings, hematologic results, or radiographic features distinguished patients with presumptive DENV infection from other febrile inpatients.

Forty (56.3%) participants with presumptive DENV infection had no evidence of acute co-infections. HIV infection was

TABLE 2
Presenting features of febrile inpatients with and without CHIKV infection ($N = 700$), northern Tanzania, 2007–2008

	Acute CHIKV infection ($N = 55$)		No CHIKV infection ($N = 645$)			
	n/*N*	(%)	n/*N*	(%)	OR (95% CI)	*P* value
Demographics						
Age, median (range) years	5.5 (0.2, 63.5)		19.4 (0.2, 95.8)		–	0.088
Female	21/55	(38.2)	321/645	(49.8)	0.62 (0.35–1.1)	0.099
Rural residence	22/43	(51.1)	290/559	(51.2)	0.97 (0.52–1.8)	0.928
Presentation in dry month	45/55	(81.8)	375/645	(58.1)	3.2 (1.6–6.5)	0.001*
Presentation in cold month	26/55	(47.3)	120/645	(18.6)	3.9 (2.2–6.9)	< 0.001*
Clinical Signs and symptoms						
Days ill, median (IQR)	4 (3, 14)		6 (3, 14)		–	0.596
Temperature, median (IQR) °C	38.1 (38.0, 39.0)		38.5 (38.0, 39.0)		–	0.173
Headache†	13/21	(61.9)	245/344	(71.2)	0.66 (0.26–1.6)	0.363
Lymphadenopathy	4/54	(7.4)	67/634	(10.6)	0.68 (0.24–1.9)	0.464
Cough	33/55	(60.0)	407/644	(63.2)	0.87 (0.50–1.5)	0.637
Difficulty breathing	26/55	(47.3)	221/640	(34.5)	1.7 (0.98–3.0)	0.058
Vomiting	10/55	(18.2)	201/643	(31.3)	0.49 (0.24–0.99)	0.043*
Diarrhea	10/55	(18.2)	141/641	(22.0)	0.78 (0.39–1.6)	0.510
Hepatomegaly	9/52	(17.3)	54/640	(8.4)	2.3 (1.1–4.9)	0.043*
Laboratory and radiographic findings‡						
Anemia	29/55	(52.7)	266/635	(41.9)	1.5 (0.89–2.7)	0.119
Leukopenia	5/55	(9.1)	43/635	(6.8)	1.4 (0.52–3.6)	0.576
Lymphopenia	21/55	(38.2)	222/624	(35.6)	1.1 (0.63–2.0)	0.669
Thrombocytopenia	6/55	(10.9)	122/634	(19.2)	0.51 (0.21–1.2)	0.127
HIV-infected	11/48	(22.9)	161/617	(26.1)	0.84 (0.42–1.7)	0.628
Abnormal chest radiograph	21/31	(67.7)	228/403	(56.6)	1.6 (0.74–3.5)	0.226
Evidence of other acute infection						
Malaria	2/54	(3.7)	20/638	(3.1)	1.1 (0.27–5.2)	0.687
Bloodstream infections	5/55§	(9.1)	76/645	(11.8)	0.75 (0.29–1.9)	0.549
Bacterial zoonoses	5/55¶	(9.1)	63/645	(9.8)	0.92 (0.36–2.4)	0.871
Presumptive DENV	9/55	(16.4)	58/637	(9.1)	2.0 (0.91–4.2)	0.081
Provisional diagnosis						
Malaria	23/55	(41.8)	281/645	(43.6)	0.93 (0.53–1.6)	0.802
Pneumonia	22/55	(40.0)	191/645	(29.6)	1.6 (0.90–2.8)	0.108
Meningitis	3/55	(5.5)	45/645	(7.0)	0.77 (0.23–2.6)	1.000
Other	7/55	(12.7)	128/645	(19.8)	0.59 (0.26–1.3)	0.284
Treatment						
Antibacterial	48/55	(87.3)	427/645	(66.2)	3.5 (1.6–7.9)	0.001*
Antimalarial	6/55	(10.9)	118/645	(18.3)	0.54 (0.23–1.3)	0.169
No antibacterial or antimalarial	7/55	(12.7)	183/645	(28.4)	0.37 (0.16–0.83)	0.012*
Death before discharge	5/55	(9.1)	59/641	(9.2)	0.99 (0.38–2.6)	0.978

* $P < 0.05$.
† Data available for adult and adolescent patients only.
‡ Laboratory reference ranges based on locally established reference ranges [27 and 28].
§ Blood culture positive for *Streptococcus pneumoniae* (2), *Salmonella enterica* serotype Typhi (1), *Escherichia coli* (1), *Cryptococcus neoformans* (1).
¶ Serology positive for leptospirosis (3) and Q fever (2).
CHIKV = Chikungunya virus; OR = odds ratio; CI = confidence interval; IQR = interquartile ratio; DENV = Dengue virus.

TABLE 3
Presenting features of febrile inpatients with and without presumptive acute DENV infection ($N = 747$), northern Tanzania, 2007–2008

	Presumptive DENV infection ($N = 71$)		No DENV infection ($N = 676$)			
	n/N	(%)	n/N	(%)	OR (95% CI)	*P* value
Demographics						
Age, median (range) years	14.4 (0.3, 95.8)		12.1 (0.2, 88.7)		–	0.878
Female	32/71	(45.1)	324/676	(47.9)	0.89 (0.55–1.5)	0.646
Rural residence	34/55	(61.8)	292/585	(49.9)	1.6 (0.92–2.9)	0.091
Presentation in dry month	44/71	(62.0)	417/676	(61.7)	1.0 (0.61–1.7)	0.963
Presentation in cold month	19/71	(26.8)	150/676	(22.2)	1.3 (0.73–2.2)	0.381
Clinical signs and symptoms						
Days ill, median (IQR)	4.5 (3, 14)		5 (3, 14)		–	0.680
Temperature, median (IQR) °C	38.5 (38.0, 39.1)		38.5 (38.0, 39.0)		–	0.497
Headache†	24/36	(66.7)	233/328	(71.0)	0.82 (0.39–1.7)	0.585
Lymphadenopathy	5/68	(7.4)	72/666	(10.8)	0.65 (0.26–1.7)	0.375
Cough	42/71	(59.2)	429/675	(63.6)	0.83 (0.51–1.4)	0.465
Difficulty breathing	31/71	(43.7)	236/671	(35.2)	1.4 (0.87–2.3)	0.156
Vomiting	21/71	(29.6)	204/674	(30.3)	0.97 (0.57–1.7)	0.904
Diarrhea	17/69	(24.6)	140/674	(20.8)	1.2 (0.70–2.2)	0.454
Hepatomegaly	10/69	(14.5)	59/670	(8.8)	1.8 (0.85-3.6)	0.122
Laboratory and radiographic findings‡						
Anemia	33/70	(47.1)	265/666	(39.8)	1.3 (0.82–2.2)	0.233
Leukopenia	4/70	(5.7)	48/666	(7.2)	0.78 (0.27–2.2)	0.809
Lymphopenia	25/68	(36.8)	229/657	(34.9)	1.1 (0.64–1.8)	0.753
Thrombocytopenia	13/70	(18.6)	119/665	(17.9)	1.0 (0.55–2.0)	0.888
HIV-infected	6/68	(8.8)	175/638	(27.4)	0.26 (0.11–0.60)	0.001*
Abnormal chest radiograph	20/40	(50.0)	251/423	(59.3)	0.68 (0.36–1.3)	0.252
Evidence of other acute infection						
Malaria	5/69	(7.3)	17/669	(2.5)	3.0 (1.1–8.4)	0.046*
Bloodstream infections	12/71§	(16.9)	69/676	(10.2)	1.8 (0.92–3.5)	0.084
Bacterial zoonoses	9/71¶	(12.7)	59/676	(8.7)	1.5 (0.72–3.2)	0.270
Chikungunya	9/67	(13.4)	46/625	(7.4)	2.0 (0.91–4.2)	0.081
Provisional diagnosis						
Malaria	32/71	(45.1)	292/676	(43.2)	1.1 (0.66–1.8)	0.762
Pneumonia	21/71	(29.6)	214/676	(31.7)	0.91 (0.53–1.5)	0.720
Meningitis	5/71	(7.0)	46/676	(6.8)	1.0 (0.40–2.7)	0.809
Other	13/71	(18.3)	124/676	(18.3)	1.0 (0.53–1.9)	0.995
Treatment						
Antibacterial	52/71	(73.2)	459/676	(67.9)	1.3 (0.75–2.2)	0.357
Antimalarial	16/71	(22.5)	128/676	(18.9)	1.2 (0.69–2.2)	0.464
No antibacterial or antimalarial	15/71	(21.1)	181/676	(26.8)	0.73 (0.40–1.3)	0.303
Death before discharge	3/71	(4.2)	62/672	(9.2)	0.43 (0.13–1.4)	0.156

* $P < 0.05$.
† Data available for adult and adolescent patients only.
‡ Laboratory reference ranges based on locally established reference ranges [27 and 28].
§ Blood culture positive for *Salmonella enterica* serotype Typhi (7), *Escherichia coli* (2), *Streptococcus aureus* (1), *Mycobacterium tuberculosis* (1), *Klebsiella oxytoca* (1).
¶ Serology positive for leptospirosis (5), brucellosis (4), and Q fever (1). (Note: one patient tested positive for both leptospirosis and brucellosis.)

found in 6 (8.8%) of the patients with presumptive DENV infection, compared with 175 (27.4%) of other febrile inpatients (OR 0.26, $P = 0.001$).

None of the participants received a clinical diagnosis of DENV infection during their hospital stay. Fifty-six (78.9%) patients with presumptive DENV infection were treated with either an antibacterial or an antimalarial. Three (4.2%) patients with presumptive DENV infection died before discharge. Two of these patients had bacterial bloodstream coinfections caused by *Mycobacterium tuberculosis* and *E. coli*, and the third had radiographic evidence of active pulmonary tuberculosis.

Prior flavivirus exposure. Anti-DENV IgG serological testing was performed for 751 (86.3%) participants. Eighty (10.7%) patients met the study definition for prior flavivirus exposure. Prior flavivirus exposure was significantly less common among infants and children (19 patients, 5.0%) than adults and adolescents (61 patients, 16.6%) (OR 0.26, $P < 0.001$).

The median (range) age of those with prior flavivirus exposure was 30.8 (0.3, 78.0) years, compared with 8.4 (0.2, 95.8) years for those without prior flavivirus exposure ($P < 0.001$). Forty-six (63.0%) of participants with prior flavivirus exposure resided in a rural area, compared with 281 (49.3%) of those without prior flavivirus exposure (OR 1.8, $P = 0.027$).

DISCUSSION

To our knowledge, this study is the first to prospectively investigate the role of CHIKV and DENV as causes of febrile illness in East Africa. Our study shows that CHIKV infection and possibly DENV infection are common but unrecognized causes of fever among inpatients in northern Tanzania. Acute CHIKV infection was approximately twice as common among infants and children as among adults and adolescents, consistent with the hypothesis that primary CHIKV infection confers lifelong protection against re-infection.[30] The age distribution of CHIKV cases observed is in marked contrast to the preponderance of cases among older patients observed during recent outbreaks among non-immune populations outside of Africa.[31,32]

CHIKV infection was inevitably misdiagnosed as another infection, usually malaria. However, CHIKV infection was

more than twice as common as malaria infection in this study population. CHIKV-infected patients were more likely to receive antibacterial or antimalarial therapy than other febrile patients; such therapy constitutes wasted health resources and potentially promotes antimicrobial resistance. Clinical signs and symptoms and laboratory findings were largely unhelpful in distinguishing patients with CHIKV infection from other febrile inpatients, a finding consistent with a recent study conducted during a CHIKV outbreak on Reunion Island,[32] underscoring the challenges of diagnosing CHIKV infection in the absence of laboratory diagnostic capacity. Although hepatomegaly and an absence of vomiting were associated with CHIKV infection, these associations may have been identified by chance, given the number of comparisons performed in the analyses presented in Tables 2 and 3.

To our knowledge, we report the first case series of HIV and CHIKV co-infection. Although HIV was not a risk factor for CHIKV infection, among HIV-infected individuals there was an association between CHIKV co-infection and lymphopenia, lower CD4 count, and severe immunosuppression. The basis of these associations is unclear. One possible explanation is that CHIKV infection, which is commonly associated with lymphopenia,[33] transiently exacerbates the lymphopenia of HIV infection. However, there was no overall association between CHIKV infection and lymphopenia in our study. Another possible explanation is that severely immunosuppressed individuals are more susceptible to CHIKV infection or to severe CHIKV infection requiring hospitalization. However, animal models suggest that the innate and humoral immune response, both of which are relatively preserved in HIV infection, play a prominent role in the elimination of and subsequent protection against CHIKV infection.[33] A third possible explanation is viral recrudescence. If CHIKV virions remain sequestered in the host after initial infection, they may re-emerge in severely immunocompromised patients to cause symptomatic infection. Although recrudescent CHIKV viremia has never been described,[30] a study in macaques found that CHIKV virions persist in macrophages up to 3 months after initial infection.[34] The observed association between CHIKV infection and severe immunosuppression among HIV-infected patients warrants further investigation.

We report five patients with CHIKV infection who died before discharge from the hospital, and to our knowledge these represent the first report of possible CHIKV-associated deaths in Africa. Four of these patients had evidence of co-infections that could have been causes of death irrespective of CHIKV infection. However, one CHIKV-infected patient who died before discharge was a previously healthy 33 year old with no evidence of co-infection. Her clinical presentation was consistent with meningoencephalitis, and the normal peripheral white blood cell count made bacterial meningitis unlikely. Although a cerebrospinal fluid sample was not obtained, she may have suffered from CHIKV encephalomeningitis, a recently described manifestation of CHIKV infection.[35,36]

CHIKV infection was significantly more likely to occur during cold months and dry months than during warm months and rainy months, respectively. The reason for this observation is unclear, and little work has been done to investigate the relationship between climate and CHIKV infection, especially in Africa. One study found that an outbreak of CHIKV in Kenya in 2004 was preceded by a period of unusually dry, hot weather.[37] Another study found that *Ae. albopictus* larvae incubated at cooler temperatures were more competent and efficient CHIKV vectors.[38] More detailed village-specific temperature and rainfall data and a longer surveillance period will be needed to make more definitive conclusions about the interactions between CHIKV transmission and climate.

Our results also raise questions about vectors of CHIKV transmission in non-epidemic settings. It is possible that the virus is transmitted locally by forest dwelling *Aedes* mosquitoes in a sylvatic cycle, and that the observed human infections were incidental occurrences during an epizootic. This transmission pattern was proposed to explain five cases of human CHIKV infection in Uganda during a 3-month period in 1968 when multiple CHIKV isolations were made from *Aedes africanus* and non-human primates.[5] Sylvatic transmission with spill over to humans may be less likely in our setting, since we observed 55 human CHIKV infections over a 10-month period. It is also possible that CHIKV is transmitted locally by *Ae. aegypti* or *Ae. albopictus*, the vectors traditionally associated with CHIKV outbreaks, but we are not aware of published reports of the presence of either species in northern Tanzania. Vector surveys are clearly needed to elucidate local transmission dynamics.

DENV infection also appeared to be a common cause of fever among inpatients, although we could not confirm the diagnosis with PCR. This was despite the fact that acute serum collection occurred at a median of 4.5 days after illness onset, a period when PCR is considered more sensitive than serology.[39,40] Possible explanations for this observation include cold chain breaks during sample transport that would likely have affected PCR results more than serological results or serological cross-reactivity resulting in false anti-DENV IgM positives. Although a positive anti-DENV IgM result in the setting of an acute febrile illness is often considered diagnostic of acute DENV infection, this approach can be problematic because anti-DENV IgM is often detectable for 2–3 months after initial infection.[41] Moreover, the reported sensitivity and specificity of the DENV serologic assays used in this study have varied widely against conventional standard tests[42–45] and little is known about the performance of any DENV serologic assay in East Africa. Consequently, positive anti-DENV serology has obvious shortcomings for confirming acute symptomatic DENV infection in our study. Despite these concerns, two findings of our study support the conclusion that DENV or a closely related flavivirus is indeed circulating in northern Tanzania. First, the majority of participants who tested positive for anti-DENV IgM had no evidence of other infections that could account for their febrile illness despite extensive evaluation; and second, one in six adults and adolescents tested positive for anti-DENV IgG, indicating prior exposure to DENV or to a closely related flavivirus.[25]

In summary, we show that arboviral infections were a common cause of febrile illness but were routinely misdiagnosed, most commonly as malaria. Greater awareness of the prevalence of arboviral infections among clinicians and the availability of a reliable and affordable diagnostic test would improve patient management and contribute to a more robust assessment of disease burden in sub-Saharan Africa. Although our findings show that CHIKV and possibly DENV are important causes of fever between recognized outbreaks, further investigation is needed to identify local vectors, determine risk factors for human disease, and expand our understanding of the epidemiology of these and other arboviral infections in sub-Saharan Africa.

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REFERENCES

1. Jupp P, McIntosh B, 1988. Chikungunya virus disease. Monath T, ed. *The Arboviruses: Epidemiology and Ecology*. Boca Raton, FL: CRC Press, 137–157.
2. Gubler DJ, 1988. Dengue. Monath T, ed. *The Arboviruses: Ecology and Epidemiology*. Boca Raton, FL: CRC Press, 223–260.
3. Leroy EM, Nkoghe D, Ollomo B, Nze-Nkogue C, Becquart P, Grard G, Pourrut X, Charrel R, Moureau G, Ndjoyi-Mbiguino A, De-Lamballerie X, 2009. Concurrent Chikungunya and dengue virus infections during simultaneous outbreaks, Gabon, 2007. *Emerg Infect Dis 15:* 591–593.
4. Diallo M, Sall AA, Moncayo AC, Ba Y, Fernandez Z, Ortiz D, Coffey LL, Mathiot C, Tesh RB, Weaver SC, 2005. Potential role of sylvatic and domestic African mosquito species in dengue emergence. *Am J Trop Med Hyg 73:* 445–449.
5. McCrae AW, Henderson BE, Kirya BG, Sampala SD, 1971. Chikungunya virus in the Entebbe area of Uganda: isolations and epidemiology. *Trans R Soc Trop Med Hyg 65:* 152–168.
6. Vasilakis N, Cardosa J, Hanley KA, Holmes EC, Weaver SC, 2011. Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. *Nat Rev Microbiol 9:* 532–541.
7. Ross RW, 1956. The Newala epidemic. III. The virus: isolation, pathogenic properties, and relationship to the epidemic. *J Hyg (Lond) 54:* 177–191.
8. Pastorino B, Muyembe-Tamfum JJ, Bessaud M, Tock F, Tolou H, Durand JP, Peyrefitte CN, 2004. Epidemic resurgence of Chikungunya virus in Democratic Republic of the Congo: identification of a new central African strain. *J Med Virol 74:* 277–282.
9. Gear J, Reid F, 1957. The occurrence of dengue-like fever in north-eastern Transvaal. *S Afr Med J 31:* 253–257.
10. Jentes ES, Robinson J, Johnson BW, Conde I, Sakouvougui Y, Iverson J, Beecher S, Bah MA, Diakite F, Coulibaly M, Bausch DG, 2010. Acute arboviral infections in Guinea, West Africa, 2006. *Am J Trop Med Hyg 83:* 388–394.
11. Kanesa-thasan N, Chang GJ, Smoak BL, Magill A, Burrous MJ, Hoke CH, 1998. Molecular and epidemiologic analysis of dengue virus isolates from Somalia. *Emerg Infect Dis 4:* 299–303.
12. Gubler DJ, Sather GE, Kuno G, Cabral JR, 1986. Dengue 3 virus transmission in Africa. *Am J Trop Med Hyg 35:* 1280–1284.
13. World Health Organization, 2009. Dengue in Africa: emergency of DENV-3, Cote d'Ivoire, 2008. *Wkly Epidemiol Rec 84:* 85–96.
14. Gubler DJ, 1998. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev 11:* 480–496.
15. Moi ML, Takasaki T, Kotaki A, Tajima S, Lim CK, Sakamoto M, Iwagoe H, Kobayashi K, Kurane I, 2010. Importation of dengue virus type 3 to Japan from Tanzania and Cote d'Ivoire. *Emerg Infect Dis 16:* 1770–1772.
16. Gautret P, Simon F, Hervius Askling H, Bouchaud O, Leparc-Goffart I, Ninove L, Parola P, EuroTravNet, 2010. Dengue type 3 infections in European travellers returning from the Comoros and Zanzibar, February–April 2010. *Euro Surveill 15:* 19541.
17. Crump JA, Ramadhani HO, Morrissey AB, Msuya LJ, Yang L-Y, Chow S-C, Morpeth SC, Reyburn H, Njau BN, Shaw AV, Diefenthal HC, Bartlett JA, Shao JF, Schimana W, Cunningham CK, Kinabo GD, 2011. Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected children and infants in northern Tanzania. *Trop Med Int Health 16:* 830–837.
18. Crump JA, Ramadhani HO, Morrissey AB, Saganda W, Mwako MS, Yang L-Y, Chow S-C, Morpeth SC, Reyburn H, Njau BN, Shaw AV, Diefenthal HC, Shao JF, Bartlett JA, Maro VP, 2011. Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected adults and adolescents in northern Tanzania. *Clin Infect Dis 52:* 341–348.
19. Mayhood MK, Afwamba IA, Odhiambo CO, Ndanu E, Thielman NM, Morrissey AB, Shao JF, Pence BW, Crump JA, 2008. Validation, performance under field conditions, and cost-effectiveness of Capillus HIV-1/HIV-2 and Determine HIV-1/2 rapid human immunodeficiency virus antibody assays using sequential and parallel testing algorithms in Tanzania. *J Clin Microbiol 46:* 3946–3951.
20. Scott LE, Crump JA, Msuya E, Morrissey AB, Venter WF, Stevens WS, 2011. Abbott RealTi*me* HIV-1 *m2000rt* viral load testing: manual extraction versus the automated *m2000sp* extraction. *J Virol Methods 172:* 78–80.
21. Crump JA, Scott LE, Msuya E, Morrissey AB, Kimaro EE, Shao JF, Stevens WS, 2009. Evaluation of the Abbott *m*2000*rt* RealTi*me* HIV-1 assay with manual sample preparation compared with the ROCHE COBAS AmpliPrep/AMPLICOR HIV-1 MONITOR 1.5 using specimens from East Africa. *J Virol Methods 162:* 218–222.
22. Lai YL, Chung YK, Tan HC, Yap HF, Yap G, Ooi EE, Ng LC, 2007. Cost-effective real-time reverse transcriptase PCR (RT-PCR) to screen for dengue virus followed by rapid single-tube multiplex RT-PCT for serotyping the virus. *J Clin Microbiol 45:* 935–941.
23. Pastorino B, Bessaud M, Grandadam M, Murri S, Tolou HJ, Peyrefitte CN, 2005. Development of a TaqMan RT-PCR assay without RNA extraction for the detection and quantification of African Chikungunya viruses. *J Virol Methods 124:* 65–71.
24. Scaramozzino N, Crance JM, Jouan A, DeBriel DA, Stoll F, Garin D, 2001. Comparison of flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcriptase-PCR assay for detection of flaviviruses targeting to a conserved region of the NS5 gene sequences. *J Clin Microbiol 39:* 1922–1927.
25. Figueiredo LT, Simoes MC, Cavalcante SM, 1989. Enzyme immunoassay for the detection of dengue IgG and IgM antibodies using infected mosquito cells as antigen. *Trans R Soc Trop Med Hyg 83:* 702–707.

26. World Health Organization, 2006. *Antiretroviral Therapy of HIV Infection in Infants and Children: Towards Universal Access.* World Health Organization, ed. Geneva: WHO, 144.
27. Tanzania National Bureau of Statistics, 2002. *Village Statistics, 2002 Population and Housing Census*. Dar es Salaam, Tanzania. Available from https://www.nbs.go.tz. Accessed March 25, 2010.
28. Saathoff E, Schneider P, Kleinfeldt V, Geis S, Haule D, Maboko L, Samky E, de Souza M, Robb M, Hoelscher M, 2008. Laboratory reference values for health adults from southern Tanzania. *Trop Med Int Health 13:* 612–625.
29. Buchanan AM, Muro FJ, Gratz J, Crump JA, Musyoka AM, Sichangi MW, Morrissey AB, M'rimberia JK, Njau BN, Msuya LJ, Bartlett JA, Cunningham CK, 2010. Establishment of hematological and immunological reference values for healthy Tanzanian children in the Kilimanjaro Region. *Trop Med Int Health 15:* 1011–1021.
30. Kam YW, Ong EK, Renia L, Tong JC, Ng LF, 2009. Immunobiology of Chikungunya and implications for disease intervention. *Microbes Infect 11:* 1186–1196.
31. Rianthavorn P, Prianantathavorn K, Wuttirattanakowit N, Theamboonlers A, Poovoravorn P, 2010. An outbreak of Chikungunya in southern Thailand from 2008 to 2009 caused by African strains with A226V mutation. *Int J Infect Dis 14 (Suppl 3):* e161–e165.
32. Staikowsky F, Talarim F, Grivard P, Souab A, Schuffenecker I, Le Roux K, Lecuit M, Michault A, 2009. Prospective study of chikungunya virus acute infection in the Island of La Reunion during the 2005–2006 outbreak. *PLoS ONE 4:* e7603.
33. Schwartz O, Albert ML, 2010. Biology and pathogenesis of Chikungunya virus. *Nat Rev Microbiol 8:* 491–500.
34. Labadie K, Larcher T, Joubert C, Mannioui A, Delache B, Brochard P, Guigand L, Dubreil L, Lebon P, Verrier B, de Lamballerie X, Suhrbier A, Cherel Y, Le Grand R, Roques P, 2010. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. *J Clin Invest 120:* 894–906.
35. Rajapakse S, Rodrigo C, Rajapakse A, 2010. Atypical manifestations of Chikungunya infection. *Trans R Soc Trop Med Hyg 104:* 89–96.
36. Das T, Jaffar-Bandjee MC, Hoarau JJ, Krejbich Trotot P, Denizot M, Lee-Pat-Yuen G, Sahoo R, Guirand P, Ramful D, Robin S, Alessandri JL, Gauzere BA, Gasque P, 2010. Chikungunya fever: CNS infection and pathologies of a re-emerging arbovirus. *Prog Neurobiol 91:* 121–129.
37. Chretien J-P, Anyamba AA, Bedno SA, Breiman RF, Sang R, Sergon K, Powers AM, Onyango CO, Small J, Tucker CJ, Linthicum KJ, 2007. Drought-associated Chikungunya emergence along coastal East Africa. *Am J Trop Med Hyg 76:* 405–407.
38. Westbrook CJ, Reiskind MH, Pesko KN, Greene KE, Lounibos LP, 2010. Larval environmental temperature and the susceptibility of *Aedes albopictus* Skuse (Diptera: Culicidae) to Chikungunya virus. *Vector Borne Zoonotic Dis 10:* 241–247.
39. Lindegren G, Vene S, Lundkvist A, Falk KI, 2005. Optimized diagnosis of acute dengue fever in Swedish travelers by a combination of reverse transcription-PCR and immunoglobulin M detection. *J Clin Microbiol 43:* 2850–2855.
40. Raengsakulrach B, Nisalak A, Maneekarn N, Yenchitsomanus PT, Limsomwong C, Jairungsri A, Thirawuth V, Green S, Kalayanarooj S, Suntayakorn S, Sittisombut N, Malasit P, Vaughn D, 2002. Comparison of four reverse transcription-polymerase chain reaction procedures for the detection of dengue virus in clinical specimens. *J Virol Methods 105:* 219–232.
41. Gubler DJ, 1997. Laboratory diagnosis of dengue virus infections. Gubler DJ, Kuno G, eds. *Dengue and Dengue Hemorrhagic Fever*. Oxford: CABI, 313–33.
42. Blacksell SD, Mammen MP, Thongpaseuth S, Gibbons RV, Jarman RG, Jenjaroen K, Nisalak A, Phetsouvanh R, Newton PN, Day NPJ, 2008. Evaluation of the Panbio dengue virus nonstructural 1 antigen detection and immunoglobulin M antibody enzyme-linked immunosorbent assays for the diagnosis of acute dengue infections in Laos. *Diagn Microbiol Infect Dis 60:* 43–49.
43. Vazquez S, Hafner G, Ruiz D, Calzada N, Guzman MG, 2007. Evaluation of immunoglobulin M and G capture enzyme-linked immunosorbent assay Panbio kits for diagnostic dengue infections. *J Clin Virol 39:* 194–198.
44. Watthanaworawit W, Turner P, Turner CL, Tanganuchitcharnchai A, Jarman RG, Blacksell SD, Nosten FH, 2011. A prospective evaluation of diagnostic methodologies for the acute diagnosis of dengue virus infection on the Thailand-Myanmar border. *Trans R Soc Trop Med Hyg 105:* 32–37.
45. McBride WJ, Mullner H, LaBrooy JT, Wronski I, 1998. The 1993 dengue 2 epidemic in north Queensland: a serosurvey and comparison of hemagglutination inhibition with ELISA. *Am J Trop Med Hyg 59:* 457–461.