Household-Based Sero-Epidemiologic Survey after a Yellow Fever Epidemic, Sudan, 2005

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Abstract. From September through early December 2005, an outbreak of yellow fever (YF) occurred in South Kordofan, Sudan, resulting in a mass YF vaccination campaign. In late December 2005, we conducted a serosurvey to assess YF vaccine coverage and to better define the epidemiology of the outbreak in an index village. Of 552 persons enrolled, 95% reported recent YF vaccination, and 25% reported febrile illness during the outbreak period: 13% reported YF-like illness, 4% reported severe YF-like illness, and 12% reported chikungunya-like illness. Of 87 persons who provided blood samples, all had positive YF serologic results, including three who had never been vaccinated. There was also serologic evidence of recent or prior chikungunya virus, dengue virus, West Nile virus, and Sindbis virus infections. These results indicate that YF virus and chikungunya virus contributed to the outbreak. The high prevalence of YF antibody among vaccinees indicates that vaccination was effectively implemented in this remotely located population.

INTRODUCTION

Yellow fever virus (YFV) is a mosquito-borne flavivirus endemic in the tropics of Africa and South America. It causes hemorrhagic fever (HF) with a case-fatality rate of 20%–50%, and a milder febrile illness and asymptomatic infection.¹ This virus is maintained in enzootic cycles, in which mosquitoes (*Aedes* spp. in Africa and *Haemagogus* spp. in South America) infect non-human primates. Humans can be accidentally infected by these jungle YF cycles. In Africa, the forest savanna and surrounding moist savanna represent the zone of emergence where tree hole–breeding sylvatic *Aedes* spp. mosquitoes transmit YFV to humans and cause endemic and epidemic YF.² Viremic humans entering more populous areas can begin an urban YF cycle in which peri-domestic *Aedes aegypti* mosquitoes transmit YFV among humans and cause epidemics in dry savanna and urban areas.² Yellow fever outbreaks may be prevented and controlled through vaccination and mosquito control.

From September through December, 2005, an outbreak of HF occurred in the state of South Kordofan, Sudan, beginning in the Nuba Mountains region.^{3,4} Testing at the U.S. Naval Medical Research Unit 3 in Cairo, Egypt found IgM against YFV in 13 of 38 serum samples collected from acutely ill patients at the start of the outbreak.⁵ The Arboviral Diagnostic Laboratory at the U.S. Centers for Disease Control and Prevention (CDC) in Fort Collins, Colorado received 32 of the 38 samples and found YFV IgM by enzyme-linked immunosorbent assay (ELISA) in 14. Of these 14, three also had dengue virus (DENV) IgM by quadrivalent ELISA. Because a plaque-reduction neutralization test (PRNT) was not performed on these initial samples, a precise interpretation of flavivirus serologic results was not possible. One of the 32 samples tested had chikungunya virus (CHIKV) IgM in addition to DENV and YFV IgM. Chikungunya virus was isolated in BHK-21 cells from the serum of two patients who lived in Kortalla, one of two villages in South Kordofan that first reported cases of HF in late October 2005.⁴ This virus is an alphavirus that, like YFV, is transmitted by *Aedes* spp.

mosquitoes. These results suggested that YFV and CHIKV had caused acute febrile illness in South Kordofan.

A description of the epidemiologic characteristics of this outbreak has been published.⁴ A total of 605 suspect YF cases were reported to the Sudanese Federal Ministry of Health (FMOH); there were 163 deaths (case-fatality rate = 27%).^{3,4} The last YF outbreak documented in this area occurred in 1940 when more than 15,000 cases with a case-fatality rate of approximately 10% were reported.⁵ During the 2005 YF outbreak, the FMOH launched a mass YF vaccination campaign, targeting the entire population of South Kordofan greater than nine months of age. Within two weeks from late November through early December, more than 1.5 million persons were vaccinated.⁶ The FMOH invited a World Health Organization Global Outbreak Alert and Response Network (GOARN) team to investigate the outbreak.

At the time the GOARN team began its investigation, the dry season had begun and the outbreak was waning. As part of the epidemiologic investigation, we conducted a household survey to better describe clinical manifestations of illness, further evaluate the etiology of the outbreak, and determine YF vaccine coverage in the village of Kortalla, located in the Dilling region. This village was selected for the survey because it was well-demarcated and accessible, and because a plurality (48%) of suspected YF cases had been reported from the Dilling region (the proportion actually from Kortalla could not be determined because data regarding village of residence was not captured by the surveillance system).⁴ The Shenabla nomadic tribe had traveled through Kortalla early in the outbreak and many of them had become ill, which suggested that intense transmission had occurred there. Furthermore, Kortalla abuts forested mountains inhabited by monkeys, which could support sylvatic YF cycles. An entomologic investigation had revealed *Aedes aegypti* and *Ae. luteocephalus* mosquitoes in a neighboring village; however, possibly because of the onset of the dry season and mosquito control efforts, few mosquitoes were found in Kortalla at the time of the survey.⁴

MATERIALS AND METHODS

Description of Kortalla. Kortalla is located in the savanna immediately abutting the Nuba Mountains. Residents lead a

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primarily agricultural lifestyle, growing crops and raising cattle, donkeys, sheep, and poultry. They venture into the mountain forests mainly during the rainy season (June through September) to obtain water from small ponds that dry out completely during the dry season. Potential mosquito-breeding sites include baobab trees with tree holes, rock holes, and a water reservoir on the outskirts of the village. Monkeys dwell in the mountain forests and enter the outskirts of the village during the rainy season.

Survey design. During December 21–25, 2005, we conducted a household serosurvey in Kortalla. Village leaders compiled a list of the number of residents living in each household. Six hundred seventy-eight households were listed comprising 2,874 residents, for an estimated average household size of 4.2 residents per household. Using single-stage cluster sampling, we selected a random sample of households and enrolled all residents who had been living in selected households from the start of Ramadan (October 4, 2005) until the date of the survey. We interviewed the head of each selected household to collect demographic and clinical information (age, sex, illness, death, clinical signs and symptoms, and vaccination status) about each household member, including those who had died during the epidemic. Questionnaires were translated into Arabic from English, and administered by team members in Arabic or another local language by an interpreter. We attempted to randomly select one household member from those present to have blood drawn for arboviral serologic testing. If the selected individual (or parent/guardian) refused, another household member was selected at random or occasionally the head of the household would suggest a household member for the blood draw. This process continued until one household member consented or all available household members refused. Households with no members present were visited twice before being determined to be vacant.

Approval for the survey was obtained in advance from Habila Hospital and Dilling Hospital, the South Kordofan Ministry of Health, the Sudan FMOH, World Health Organization-Sudan, GOARN, and the CDC Human Research Protection Office. Because the survey constituted part of the public health response to the outbreak, it was determined to be exempt from Institutional Review Board review at CDC. Informed consent was obtained verbally from the head of each household prior to enrollment.

Serologic testing. Blood samples were stored on ice packs in the field and subsequently refrigerated (range = 4°C to ambient temperature). After settling overnight, serum was separated and kept refrigerated until transport to Khartoum on ice packs. The samples were subsequently shipped to the Arboviral Diagnostic Laboratory at CDC in Fort Collins, Colorado in a liquid nitrogen dry shipper and then stored at –70°C until testing.

All samples were tested for YFV IgM, DENV 1–4 IgM, and CHIKV IgM and IgG by ELISA;^{7,8} West Nile virus (WNV) and Saint Louis encephalitis virus IgM by microsphere immunoassay;⁹ and Sindbis virus neutralizing antibodies by 90% plaque reduction neutralization test (PRNT).¹⁰ The PRNT for YFV, DENV 1–4, and WNV was performed on all samples with negative or equivocal YFV IgM results and on all samples from unvaccinated volunteers, and those with positive or equivocal DENV 1–4 or WNV IgM results. In addition, PRNT was conducted on a subset of samples that were YFV IgM positive from participants who were thought to be unvaccinated at the time of testing.

The ELISA optical density ratios (sample/negative control) of 3.0 or more were considered positive, 2.0–2.9 equivocal, and <2.0 negative. The PRNT titers $\geq 1:10$ were considered reactive. Because secondary flavivirus infection results in broadly reactive neutralizing antibodies, serologic tests showing negative results for YFV IgM but with PRNT results at similar titers to more than one flavivirus were interpreted as flavivirus infection, timing unknown, unable to discriminate the infecting virus(es). Those samples with one increased flavivirus PRNT titer and samples with PRNT titers ≤ 10 against the other flaviviruses were interpreted as showing evidence of exposure at unknown time to that virus with the increased titer. With one exception, all of these persons also had exposure to YF vaccine.

Statistical analysis. Data were entered into Microsoft (Redmond, WA) Access 2003 and analyzed by using SAS Version 9.1 (SAS Institute, Cary, NC). Bleeding was defined as one or more of the following: epistaxis, gingival bleeding, hematemesis (bright red blood), coffee-ground emesis, hematochezia, or melena. A YF-like illness was defined as fever with jaundice (icteric sclerae) or bleeding. Severe YF-like illness was defined as fever, jaundice, and bleeding. Chikungunya-like illness was defined as fever and arthralgias, without jaundice or bleeding. Trends of reported illness by age and associations of illness with sex were assessed using chi-square tests.

RESULTS

Household enrollment and demographic characteristics.

Of 113 selected households, one was mistakenly listed and interviewed twice, but counted as one household, for an actual total of 112 households. All were visited at least once; 89 (79%) had members present who agreed to participate; 23 (21%) households were vacant. There were 552 household members in the 89 households enrolled, resulting in an average household size of 6.2 persons. Blood samples were obtained from one person at each of 86 (97%) of the enrolled households. An additional blood sample was obtained at one of these households from an unvaccinated person, for a total of 87 blood samples.

The demographic characteristics of the surveyed population are shown in Table 1. We could not obtain demographic

TABLE 1
Demographic characteristics (serosurvey versus overall survey), Sudan*

Characteristic	Serosurvey (N = 87), no. (%)	Overall survey (N = 552), no. (%)	Two-tailed P†
Sex			
F	50 (57)	253 (46)	0.0002
Median age, years	32	18	
Age group, years			
0–4	0 (0)	84 (15)	< 0.0001
5–14	7 (8)	156 (28)	< 0.0001
15–29	29 (33)	135 (24)	0.036
30–44	24 (28)	80 (14)	0.002
> 44	27 (31)	97 (18)	0.003
Rates			
Any illness	44 (51)	144 (26)	< 0.0001
Hospitalization	16 (18)	52 (9)	0.002
Mortality	–	5 (1)‡	0.332
YF vaccination	84 (97)	518 (94)	0.252

*YF = yellow fever.

†Chi-square statistic comparing serosurvey participants (n = 87) with non-serosurvey participants (n = 465).

‡Four 4–8 years of age and one 40 years of age.

TABLE 2
Attack rates of type of illness stratified by sex and age group, Sudan*

Illness syndrome**	Sex			Age group, years					
	M, no. (%)	F, no. (%)	Two-tailed <i>P</i> †	0–4, no. (%)	5–14, no. (%)	15–29, no. (%)	30–44, no. (%)	> 44, no. (%)	Two-tailed <i>P</i> ‡
Febrile (n = 139)	71 (24)	68 (27)	0.40	14 (17)	31 (20)	30 (22)	32 (40)	32 (33)	0.0002
YF-like§ (n = 66)	39 (13)	27 (11)	0.39	8 (10)	22 (14)	11 (8)	14 (18)	11 (11)	0.69
Severe YF-like¶ (n = 22)	10 (3)	12 (5)	0.40	4 (5)	8 (5)	1 (1)	4 (5)	5 (5)	1.00
CHIK-like# (n = 64)	28 (9)	36 (14)	0.08	5 (6)	8 (5)	17 (13)	15 (19)	19 (20)	< 0.0001
Total	299	253		84	156	135	80	97	

* YF = yellow fever; CHIK = chikungunya; attack rates are expressed as a percentage of the total number of respondents in each sex and age group.

† Chi-square statistic.

‡ Trend test for age.

§ Fever and jaundice/hemorrhage.

¶ Fever and jaundice and hemorrhage.

Fever and arthralgias, no jaundice/hemorrhage.

** Illness syndromes are not mutually exclusive.

data for all of Kortalla in sufficient detail to compare with our sample. When compared with the total survey population, the subgroup that provided blood samples to the serosurvey was older, had more females, and was more likely to report illness and hospitalization (Table 1).

Characteristics of reported illness. Of the 552 household members enrolled, 144 (26%) reported illness at some point between October 4, 2005 and the day of the survey. One hundred thirty-nine (25%) reported febrile illness, 66 (12%) reported YF-like illness (as defined above), 22 (4%) reported severe YF-like illness, and 64 (12%) reported CHIK-like illness. Fifty-two (9%) reported having been hospitalized and five (1%) were reported by family members to have died. Of those with reported illness, the most commonly reported symptom was fever (97%), followed by headache (92%) and arthralgias (88%). The attack rates of reported illness types were similar among males and females (Table 2). Although CHIK-like illness was reported for more females than males, this difference was not statistically significant. Attack rates for reported febrile illness and CHIK-like illness increased significantly with age (Table 2).

The ages of the five persons reported to have died were 4, 4, 5, 8, and 40 years. All reportedly had severe YF-like illness, with fever, jaundice, and bleeding. Thus, the case-fatality rates were 4% (5 of 139) for febrile illness, 8% (5 of 66) for YF-like illness, and 23% (5 of 22) for severe YF-like illness. No one with reported CHIK-like illness died.

Vaccination coverage. Of the 547 household members who were alive at the time of the vaccination campaign, 518 (95%) reported YF vaccination during December 1–4, 2006, when the YF vaccination campaign was held in Kortalla. Most families presented their vaccination cards as evidence of recent vaccination. Those persons who were unvaccinated reported traveling during the dates the vaccine campaign was conducted in Kortalla.

Serologic results. Eighty-four (97%) of the 87 persons who provided blood samples had received YF vaccine approximately three weeks before the survey; three reported no history of YF vaccination. Overall results of serologic testing are shown in Table 3, and individual results are shown in Tables 4 and 5. Two of the three unvaccinated persons had serologic evidence of recent YF infection with a positive result for YFV IgM and a YFV PRNT titer $\geq 1:20$. One of these persons was an 18-year-old man who reported a CHIK-like illness starting in mid-October, with fever, headache, arthralgias, and abdominal pain (participant #64, Table 4); he was evaluated in a local clinic and treated presumptively for malaria. The second person was an 18-year-old man who had been asymptomatic (participant #58,

Table 4). The third person was an unvaccinated 25-year-old woman who had evidence of prior YFV infection by PRNT but no detectable YFV IgM; she had also been asymptomatic (participant #46, Table 5).

Of the 84 YF vaccine recipients tested, 83 (99%) had evidence of protective immunity to YFV by either IgM ELISA or PRNT (Tables 3–5). Results for YFV IgM were positive in 43; of the 41 that were YFV IgM negative or equivocal, 40 had YFV PRNT titers $\geq 1:20$, and one had a titer of 1:10. Of these 41 vaccinees, all had evidence of another flavivirus infection sometime in the past by PRNT: five (12%) had evidence of YFV vaccination and/or infection alone, and one (2%) had evidence of DENV infection with YFV vaccination, and the specific flavivirus exposure could not be determined in the remaining 35 (85%) because of broadly reactive flavivirus antibody responses.

Among the 87 volunteers tested, none had DENV IgM; one had WNV IgM, which appeared to be cross-reactive given the broad flavivirus PRNT response; one (1%) had CHIKV IgM, confirmed by PRNT; and 37 (43%) had CHIKV IgG (Tables 3–5). Of the 38 with CHIKV IgM or IgG, 14 (37%) had CHIK-like illness, 9 (24%) had YF-like illness, 1 had a gastrointestinal illness, and 13 (34%) reported no illness. Three (3%) of the 87 volunteers had neutralizing antibody to Sindbis virus by PRNT.

TABLE 3
Interpretation of 87 serologic results, Kortalla serosurvey, Sudan*

Serologic interpretation	No.
Flavivirus serologic results†	
Recent YFV infection (not vaccinated, YFV IgM+, YFV PRNT+)	2
YFV infection, timing unknown (not vaccinated, YFV IgM–, YFV PRNT+)	1
Recent YFV vaccination or infection (vaccinated, YFV IgM+) Flavivirus infection, timing unknown, and/or YFV vaccination (vaccinated, YFV IgM–)	43
(YFV infection or vaccination only = 5) (YFV vaccination with DENV infection, timing unknown = 1) (Undetermined flavivirus infection, timing unknown = 35)	41
Total	87
Alphavirus serologic results	
Recent CHIKV infection	1
CHIKV infection, timing unknown	34
CHIKV + SINV infection, timing unknown	3
No evidence of alphavirus infection	49
Total	87

* YFV = yellow fever virus; PRNT = plaque-reduction neutralization test; CHIKV, chikungunya virus; SINV = Sindbis virus.

† Flavivirus (YFV, dengue virus, West Nile virus) PRNT was performed for three unvaccinated participants; 41 YF vaccine recipients with negative, equivocal, or indeterminate YFV IgM; and 4 YF vaccine recipients who were thought to be unvaccinated at the time of testing.

TABLE 4
Serologic results for 45 YFV IgM-positive participants, Sudan*

No.	YFV IgM	YFV NT	DENV IgM	DENV NT	WNV MIA	WNV NT	CHIKV IgM	CHIKV IgG	CHIKV NT	SINV NT	Recent illness	YF vaccine	Flavivirus exposure	Alphavirus exposure
1	6.2	5,120	1.5	> 320	Neg	40	1.5	0.72	ND	< 10	No	Yes	Recent YFV†‡	None
2	3.8	ND	1.2	ND	Neg	ND	1.8	5.6	ND	< 10	No	Yes	Recent YFV†	Past CHIKV
4	3.7	ND	1.2	ND	Neg	ND	1.9	5.3	ND	< 10	No	Yes	Recent YFV†	Past CHIKV
5	10.7	ND	1.1	ND	Neg	ND	0.76	1.5	ND	< 10	Yes	Yes	Recent YFV†	None
6	3.8	ND	1.3	ND	Neg	ND	1.3	8.6	ND	< 10	No	Yes	Recent YFV†	Past CHIKV
7	3.5	ND	1.1	ND	Neg	ND	1.4	3.3	ND	< 10	Yes	Yes	Recent YFV†	Past CHIKV
8	3.5	ND	1.2	ND	Nonspecific	ND	1.3	4.7	ND	< 10	Yes	Yes	Recent YFV†	Past CHIKV
9	4.4	ND	1.2	ND	Neg	ND	1.6	0.51	ND	< 10	Yes	Yes	Recent YFV†	None
12	4.6	ND	1.1	ND	Neg	ND	1.7	0.74	ND	< 10	No	Yes	Recent YFV†	None
19	4.9	320	1.4	> 320	Neg	> 320	1.9	4.48	ND	< 10	Yes	Yes	Recent YFV†	Past CHIKV
21	3.2	40	1.2	10	Neg	40	1.2	0.64	ND	< 10	No	Yes	Recent YFV†	None
22	3.5	ND	1	ND	Neg	ND	0.95	1.14	ND	< 10	No	Yes	Recent YFV†	None
24	4.5	ND	1.5	ND	Neg	ND	0.99	1.1	ND	< 10	Yes	Yes	Recent YFV†	None
26	6.3	ND	1	ND	Neg	ND	1.4	0.73	ND	< 10	Yes	Yes	Recent YFV†	None
27	3.4	ND	1.3	ND	Neg	ND	1.5	0.61	ND	< 10	No	Yes	Recent YFV†	None
29	3.7	ND	1.1	ND	Neg	ND	1.2	3.96	ND	< 10	No	Yes	Recent YFV†	Past CHIKV
31	7.7	ND	1.1	ND	Neg	ND	1.8	4.26	ND	< 10	Yes	Yes	Recent YFV†	Past CHIKV
32	7.7	2,560	2.3	2,560	Neg	< 10	1.3	0.73	ND	< 10	No	Yes	Recent YFV†	None
33	6	ND	1.2	ND	Neg	ND	1.3	5.33	ND	< 10	No	Yes	Recent YFV†	Past CHIKV
34	3.6	ND	1.2	ND	Neg	ND	1.3	0.96	ND	< 10	No	Yes	Recent YFV†	None
37	4.6	ND	1.2	ND	Neg	ND	1.3	0.77	ND	< 10	No	Yes	Recent YFV†	None
38	4.7	ND	1.2	ND	Neg	ND	1.2	0.75	ND	< 10	No	Yes	Recent YFV†	Past CHIKV
41	4.4	ND	1.1	ND	Neg	ND	1.5	3.9	ND	< 10	No	Yes	Recent YFV†	None
42	8	ND	1.1	ND	Neg	ND	1.3	0.7	ND	< 10	Yes	Yes	Recent YFV†	Past CHIKV
43	3.7	ND	1.5	ND	Neg	ND	§	3.65	ND	< 10	Yes	Yes	Recent YFV†	None
44	3.4	ND	1.2	ND	Neg	ND	§	4.85	ND	< 10	Yes	Yes	Recent YFV†	Past CHIKV
45	7.2	ND	1.1	ND	Neg	ND	§	3.3	ND	< 10	Yes	Yes	Recent YFV†	Past CHIKV
53	3.1	ND	1	ND	Neg	ND	1.7	4.8	ND	< 10	Yes	Yes	Recent YFV†	Past CHIKV
55	4.5	ND	1	ND	Neg	ND	1.1	0.73	ND	< 10	No	Yes	Recent YFV†	None
56	4.5	ND	1.2	ND	Neg	ND	1.3	4.02	ND	< 10	No	Yes	Recent YFV†	Past CHIKV
57	3.4	ND	1.2	ND	Neg	ND	1.2	5.09	ND	< 10	Yes	Yes	Recent YFV†	Past CHIKV
58	4	320	1.1	80	Neg	320	1.8	4.09	ND	< 10	No	No	Recent YFV infection‡	Past CHIKV
59	4.6	ND	1.2	ND	Nonspecific	ND	0.94	3.6	ND	40	Yes	Yes	Recent YFV†	Past CHIKV + SINV
60	4.8	ND	1.4	ND	Neg	ND	1.2	0.76	ND	< 10	Yes	Yes	Recent YFV†	None
61	5.4	ND	1.1	ND	Neg	ND	1	0.75	ND	< 10	No	Yes	Recent YFV†	None
63	6.9	ND	1.3	ND	Neg	ND	1	§	ND	< 10	No	Yes	Recent YFV†	None
64	3.9	1,280	1.1	40	Neg	< 10	1.1	0.85	ND	< 10	Yes	No	Recent YFV infection‡	None
66	4.5	ND	1.3	ND	Neg	ND	1.3	0.8	ND	< 10	No	Yes	Recent YFV†	None
67	4.6	ND	1.1	ND	Neg	ND	1.9	3.96	ND	< 10	Yes	Yes	Recent YFV†	Past CHIKV
73	8.1	ND	1.3	ND	Neg	ND	§	3.78	ND	< 10	Yes	Yes	Recent YFV†	Past CHIKV
76	9.1	ND	1.9	ND	Neg	ND	1.2	0.79	ND	< 10	No	Yes	Recent YFV†	None
77	5.6	ND	1.1	ND	Neg	ND	1.9	5.7	ND	< 10	Yes	Yes	Recent YFV†	Past CHIKV
81	3.4	ND	1.2	ND	Neg	ND	§	5.47	ND	< 10	No	Yes	Recent YFV†	Past CHIKV
86	7.4	ND	1.2	ND	Neg	ND	1.7	4	ND	10	Yes	Yes	Recent YFV†	Past CHIKV + SINV
87	5.4	ND	1.1	ND	Neg	ND	1	0.74	ND	< 10	Yes	Yes	Recent YFV†	None

*YFV = yellow fever virus; NT = neutralization titer; DENV = dengue virus; WNV = West Nile virus; MIA = microsphere immunoassay; CHIKV = chikungunya virus; SINV = Sindbis virus; Neg = negative; ND = not done; IgM and IgG enzyme-linked immunosorbent assay values are reported as P/N, where P = optical density of patient serum dilution with viral antigen, and N = mean optical density of normal human serum. Neutralizing antibody titers represent 90% plaque-reduction neutralizing antibody titers. Shaded areas are participants who did not report receiving YF vaccine. **Bold** values are positive/significant results.
† Recent YFV vaccination or infection.
‡ Recent YFV infection or evidence of cross-reactive flavivirus response.
§ Indeterminate.

TABLE 5
Serologic results for 42 YFV IgM-negative participants, Sudan*

No.	YFV IgM	YFV NT	DENV IgM	DENV NT	WNV MIA	WNV NT	CHIKV IgM	CHIKV IgG	CHIKV NT	SINV NT	Recent illness	YFV vaccine	Flavivirus exposure†	Alphavirus exposure
3	1.5	10	1.1	160	Nonspecific	< 10	1.9	0.35	ND	< 10	No	Yes	DENV‡	None
10	1.7	640	1.3	10	Neg	160	1.3	3.6	ND	< 10	Yes	Yes	Undetermined flavivirus§	CHIKV
11	1.7	1,280	1.2	160	Neg	160	1.4	2	ND	< 10	Yes	Yes	Undetermined flavivirus§	None
13	1.7	640	1.2	640	Neg	160	1.4	3.05	ND	< 10	No	Yes	Undetermined flavivirus§	CHIKV
14	2.9	160	1.1	160	Neg	640	1.5	5	ND	< 10	Yes	Yes	Undetermined flavivirus§	CHIKV
15	2.6	640	1.3	320	Neg	320	1.9	2.6	ND	< 10	Yes	Yes	Undetermined flavivirus§	None
16	1.8	80	1.2	80	Neg	40	1.2	3.2	ND	< 10	Yes	Yes	Undetermined flavivirus§	CHIKV
17	1.3	640	1.1	20	Neg	160	1.9	7.1	ND	< 10	Yes	Yes	Undetermined flavivirus§	CHIKV
18	1.1	80	1.1	320	Neg	< 10	1	3.5	ND	10	Yes	Yes	Undetermined flavivirus§	CHIKV + SINV
20	1.1	160	1.1	80	Neg	80	0.99	0.85	ND	< 10	No	Yes	Undetermined flavivirus§	None
23	2.5	1,280	1.1	10	Neg	< 10	1	1.02	ND	< 10	No	Yes	YFV‡	None
25	1.8	320	1.1	10	Neg	10	¶	4.38	ND	< 10	Yes	Yes	YFV‡	CHIKV
28	2.2	80	1.1	10	Neg	80	1.3	0.69	ND	< 10	No	Yes	Undetermined flavivirus§	None
30	1.6	80	1	10	Neg	80	1.3	4.93	ND	< 10	No	Yes	Undetermined flavivirus§	CHIKV
35	1.5	80	1.2	640	Neg	80	1.3	0.63	ND	< 10	Yes	Yes	Undetermined flavivirus§	None
36	1.6	80	1.2	20	Neg	40	1.4	0.7	ND	< 10	Yes	Yes	Undetermined flavivirus§	None
39	2.7	2,560	1.2	320	Neg	640	1.5	5.9	ND	< 10	Yes	Yes	Undetermined flavivirus§	CHIKV
40	¶	320	¶	20	Neg	80	¶	1.1	ND	< 10	No	Yes	Undetermined flavivirus§	None
46	0.96	80	1	< 10	Neg	< 10	1.2	1.09	ND	< 10	No	No	YFV infection†	None
47	2.3	160	1.1	20	Neg	80	1.3	0.65	ND	< 10	No	Yes	Undetermined flavivirus§	None
48	0.83	320	2.7	20	Neg	40	1.3	2.1	ND	< 10	No	Yes	Undetermined flavivirus§	None
49	1.5	320	0.99	80	Neg	320	1.4	2.57	ND	< 10	Yes	Yes	Undetermined flavivirus§	None
50	1.4	160	1.2	160	WNV	10	¶	3.15	ND	< 10	Yes	Yes	Undetermined flavivirus§	CHIKV
51	1.2	40	1.2	< 10	Neg	160	1.1	0.8	ND	< 10	No	Yes	Undetermined flavivirus§	None
52	¶	320	1.2	10	Neg	< 10	1.3	0.88	ND	< 10	No	Yes	YFV‡	None
54	1.1	80	1.1	20	Neg	< 10	1.2	2.3	ND	< 10	Yes	Yes	YFV‡	None
62	¶	160	1.5	80	Neg	160	1.6	0.84	ND	< 10	No	Yes	Undetermined flavivirus§	None
65	2.8	320	1.1	160	Neg	640	1.3	0.8	ND	< 10	Yes	Yes	Undetermined flavivirus§	None
68	1.9	160	1.1	80	Neg	160	1.9	1.34	ND	< 10	Yes	Yes	Undetermined flavivirus§	None
69	0.94	20	1.1	40	Neg	160	1.2	1.7	ND	< 10	No	Yes	Undetermined flavivirus§	None
70	2.6	1,280	1.2	1280	Neg	320	1.3	2.26	ND	< 10	Yes	Yes	Undetermined flavivirus§	None
71	1.2	160	1.1	160	Neg	80	1.1	1.88	ND	< 10	No	Yes	Undetermined flavivirus§	None
72	1.3	80	1.2	80	Neg	80	1.2	0.7	ND	< 10	No	Yes	Undetermined flavivirus§	None
74	2.4	160	1.1	1280	Nonspecific	320	1.6	7.1	ND	< 10	Yes	Yes	Undetermined flavivirus§	CHIKV
75	2.8	320	1.1	< 10	Neg	< 10	3.2	¶	1280	< 10	Yes	Yes	YFV‡	Recent CHIKV
78	0.97	80	1.1	160	Neg	20	1.2	6.8	ND	< 10	Yes	Yes	Undetermined flavivirus§	CHIKV
79	1.5	80	0.98	640	Neg	160	1.5	3.1	ND	< 10	No	Yes	Undetermined flavivirus§	CHIKV
80	2.5	160	0.98	80	Neg	160	¶	5.09	ND	< 10	No	Yes	Undetermined flavivirus§	CHIKV
82	¶	160	1.1	80	Neg	20	1.3	0.74	ND	< 10	Yes	Yes	Undetermined flavivirus†	None
83	¶	320	1.3	80	Neg	80	1.6	0.86	ND	< 10	Yes	Yes	Undetermined flavivirus§	None
84	1.2	160	1.1	10	Nonspecific	160	1.1	1.3	ND	< 10	No	Yes	Undetermined flavivirus§	None
85	1.7	320	1.2	160	Nonspecific	20	1.3	¶	ND	< 10	Yes	Yes	Undetermined flavivirus§	None

*YFV = yellow fever virus; NT = neutralization titer; DENV = dengue virus; WNV = West Nile virus; MIA = microsphere immunoassay; CHIKV = chikungunya virus; SINV = Sindbis virus; ND = not done; Neg = negative; IgM and IgG enzyme-linked immunosorbent assay values are reported as PN, where P = optical density of patient serum dilution with viral antigen, and N = mean optical density of normal human serum. Neutralizing antibody titers represent 90% plaque-reduction neutralizing antibody titers. Shaded areas are participants who did not report receiving YF vaccine. **Bold** values are positive/significant results.

†Timing unknown.

‡Or YFV vaccination, timing unknown.

§Flavivirus infection, timing unknown, unable to discriminate the infecting virus(es); and/or YF vaccination.

¶Indeterminate.

DISCUSSION

Sporadic YF epidemics have been reported in eastern Africa since 1940, when the first documented YF epidemic in the region occurred also in the Nuba Mountains of Sudan.^{5,11} A smaller YF epidemic in Sudan occurred in 1959 in the Blue Nile and Upper Nile states bordering Ethiopia, followed by a large outbreak in Ethiopia during 1960–1962.¹² In recent years, sylvatic YF outbreaks have occurred in Kenya in 1992–1993,^{13–15} and in the Imatong Mountains of southern Sudan in 2003.^{16,17} Entomologic investigations performed during these epidemics have demonstrated the importance of sylvatic *Aedes* vectors in addition to the urban YF vector, *Ae. aegypti*, in Sudan. These vectors also transmit CHIKV, which is endemic in Sudan.^{18–20}

Our survey indicates that YFV and CHIKV were recently transmitted in Kortalla, and contributed to the outbreak. The survey results provide a more detailed clinical and laboratory evaluation of the illnesses that would have been reported as suspected YF, confirming that multiple etiologies likely contributed to the 605 suspected YF cases reported from South Kordofan state to the FMOH.⁴ The history of recent YF vaccination in most of the persons who provided blood samples limits the interpretation of serologic results because we cannot differentiate wild-type YFV infection from vaccination serologically by using ELISA and PRNT. However, the serologic evidence of acute YFV infection in two reportedly unvaccinated persons provides evidence of recent YFV transmission, and the positive result for CHIKV IgM in one person (together with the isolation of CHIKV from two ill persons who lived in Kortalla noted in the introduction to this paper) suggests recent CHIKV transmission. The case-fatality rates in Kortalla of 4% for febrile illness, 8% for YF-like illness, and 23% for severe YF-like illness are consistent with mortality rates reported in prior YF outbreaks in Africa.^{14,17,21,22}

Twelve percent of household members reported an illness suggestive of chikungunya fever. However, one of these persons was a participant with serologic evidence of recent YF, reinforcing the concept that clinical manifestations of arboviral infection overlap and specific diagnosis of arboviral illness requires laboratory evidence of infection with a specific virus. Although antibodies against CHIKV cross-react with the related alphavirus o'nyong-nyong virus, antibodies to o'nyong-nyong virus react only weakly against CHIKV, and neither flavivirus antibody nor alphavirus antibody typically cross-react outside their respective families.²³ Serosurvey participants with CHIKV IgG but not IgM may have been infected with CHIKV early during the outbreak, or may have been infected at an undetermined time in the past.

The YF vaccination campaign appeared to be effectively implemented in Kortalla. Recent YF vaccination was reported in 95% of household members, and 99% of vaccinated household members tested had evidence of protective immunity to YFV. Serosurvey participants with no detectable YF IgM may have had a suppressed IgM response because of prior flavivirus exposure.²⁴ Because Sudan has not incorporated YF vaccine into routine immunization programs, and there have been no prior YF vaccination campaigns in South Kordofan, the residents of Kortalla probably had not received any YF vaccination prior to the 2005 campaign.

The results of our survey may be limited by recall bias as well as cultural and language barriers. Such information bias could result in overestimation or underestimation of illness

attack rates. The accuracy of our estimates would also be limited if other undetected etiologies, such as malaria, contributed to the symptoms reported in the outbreak. To reduce selection bias, we attempted to choose serosurvey participants randomly. However, all household members were rarely present, and parents often did not want small children to be bled. Residual selection bias is likely manifested in the higher rates of illness reported by serosurvey participants compared with all participants in the household survey (Table 1). In addition, because we performed the survey during mid-day, the serosurvey probably underrepresented people who work in the fields and might have greater exposure to the sylvatic YF cycle. This finding is reflected in the demographic differences between the serosurvey group and the reported household demographics. These possible selection biases limit inferences regarding the prevalence of antibody and illness in the community. Finally, estimation of the true attack rates of YF through interpretation of the serologic results is limited by the high coverage of recent YF vaccination.

Despite these limitations, the results of this investigation suggest that CHIKV and YFV contributed to febrile illness in Kortalla during this outbreak. Evidence that both viruses contributed to the wider outbreak in South Kordofan has been previously published.⁴ Finally, the high reported vaccination rates and serologic evidence of YF immunity indicate that the intensive vaccination campaign conducted in South Kordofan had high coverage and resulted in protective humoral immunity. These results indicate that it is possible to achieve good vaccine coverage in remote areas at high risk for outbreaks of sylvatic YF.

Received February 25, 2009. Accepted for publication January 22, 2010.

Acknowledgments: We thank the people of Kortalla, Sudan for generously giving their time to participate in this survey, the residents of Kortalla and Dilling for acting as guides and translators, the staff at Habila Hospital for invaluable descriptions of hospitalized cases from the Kortalla area, and the Dilling Locality and South Kordofan State Ministries of Health for facilitating this survey.

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