

## SEROPREVALENCE AND DISTRIBUTION OF *FLAVIVIRIDAE*, *TOGAVIRIDAE*, AND *BUNYAVIRIDAE* ARBOVIRAL INFECTIONS IN RURAL CAMEROONIAN ADULTS

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**Abstract.** Arboviruses from the families *Flaviviridae*, *Togaviridae*, and *Bunyaviridae* are suspected to cause widespread morbidity in sub-Saharan African populations, but little research has been done to document the burden and distribution of these pathogens. We tested serum samples from 256 Cameroonian adults from nine rural villages for the presence of Dengue-2 (DEN-2), West Nile (WN), Yellow fever (YF), Chikungunya (CHIK), O'nyong-nyong (ONN), Sindbis (SIN), and Tahyna (TAH) infection using standard plaque-reduction neutralization tests (PRNT). Of these samples, 12.5% were DEN-2 positive, 6.6% were WN positive, 26.9% were YF positive, 46.5% were CHIK seropositive, 47.7% were ONN positive, 7.8% were SIN positive, and 36.3% were TAH positive. DEN-2, YF, and CHIK seroprevalence rates were lower among individuals living in dwellings with grass or thatched roofs versus corrugated tin and in villages isolated from urban centers. Seroprevalence rates of YF and CHIK increased with age. These results suggest that inter-epidemic arboviral infection is common in central African populations.

### INTRODUCTION

Despite their significant and increasing<sup>1</sup> public health impact on individuals worldwide, arboviruses from the families *Flaviviridae*, *Togaviridae*, and *Bunyaviridae* remain poorly understood and even less well controlled. Once confined to limited geographic areas, several of these viruses have spread well beyond their historically endemic regions to become pathogens of global importance.<sup>1–6</sup> The World Health Organization (WHO) estimates that dengue virus alone produces more than 50 million clinical cases each year,<sup>7</sup> and the burden of all arboviral infections combined is likely to be many times higher. While increasingly well characterized in industrialized countries, arbovirus epidemiology in developing countries remains an imperfect science and is often characterized in the form of epidemic reporting<sup>8–10</sup> rather than as a part of ongoing population health surveillance programs.

Tropical Africa is the likely site of origin of many of the *Flaviviridae*, *Togaviridae*, and *Bunyaviridae* arboviruses of modern medical importance<sup>11–14</sup> and remains one of the most affected regions in the world today. Despite relatively common arboviral epidemics on the continent,<sup>8,9,15–17</sup> and a long history of viral isolation, the burden of inter-epidemic disease, the epidemiology of infection, and principle reservoirs of viral maintenance are not well understood. Much of this confusion stems from the difficulty of clinical discrimination between different arboviral infections,<sup>18</sup> between arboviral infections and those caused by often hyperendemic *P. falciparum*, and additionally the lack of adequate laboratories to diagnose these viruses.

Cameroon is located in central Africa (Figure 1) and has a population of about 16 million people, most of who work in the agricultural sector. The central and southern part of the country is largely lowland rain forest and is characterized by high-intensity malaria transmission<sup>19</sup> and a high burden a

parasitic infections. Entomological surveys have discovered significant *Aedes*, *Anopheles*, and *Culex* species populations in Cameroon,<sup>20–22</sup> suggesting the presence of competent vector systems for many of the arboviruses in the *Flaviviridae*, *Togaviridae*, and *Bunyaviridae* families. Additionally, a recent survey has discovered *Aedes albopictus* in Cameroon,<sup>21</sup> a species with worrisome potential as a vector for dengue and other emerging arboviruses.<sup>23</sup> This said, the impact of dengue has thus far been limited in Africa, and dengue hemorrhagic fever and shock syndrome are rarely seen. Although all four dengue serotypes have been isolated on the continent,<sup>24–26</sup> most dengue infections in Africa are thought to be caused by the dengue-2 serotype.<sup>24,27,28</sup>

Cameroon and central Africa are considered to be endemic for many of the arboviruses from the *Flaviviridae*, *Togaviridae*, and *Bunyaviridae* families,<sup>13,15,17,29,30</sup> but only one clinic-based survey of febrile patients has documented the burden of these pathogens among the Cameroonian people.<sup>31</sup> In this report, we present the results of a population-based cross-sectional survey of antibody to Dengue-2 (DEN-2), West Nile (WN), Yellow fever (YF), Chikungunya (CHIK), O'nyong-nyong (ONN), Sindbis (SIN), and Tahyna (TAH) viruses in healthy rural Cameroonian adults.

### MATERIALS AND METHODS

**Study participants and sites.** In this study, we evaluated serum samples from individuals in nine rural villages in Cameroon (Figure 1). The serum samples had been collected from individuals participating in a large epidemiologic survey of HIV and other infectious diseases conducted by the Johns Hopkins Cameroon Program and the Cameroonian ministries of health and defense between 2000 and 2003. This epidemiologic survey had been conducted in conjunction with a community-based HIV prevention campaign that offered targeted risk reduction messages to small rural villages otherwise missed by HIV/AIDS prevention campaigns. During the conduct of the HIV/AIDS prevention campaign, adults were approached and told about the epidemiologic survey, and the survey was described to those who were interested. All adults

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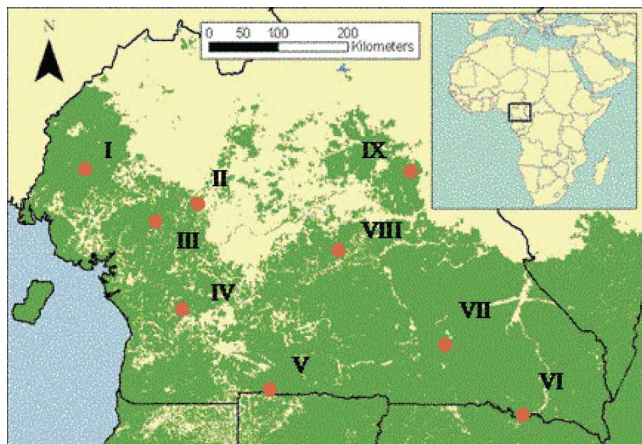


FIGURE 1. Map of study sites in southern Cameroon in relation to the distribution of lowland tropical forest in central Africa (in green). This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

over the age of 16 were eligible to be enrolled in the study. Study description and the informed consent procedure were done orally in English or French, which are widely spoken as second languages in the study villages. Informed consent included a clause permitting the use of participants' biologic specimens for future research studies. Each participant who agreed to participate in this study was administered an anonymous questionnaire that included assessment of demographics, sexual behaviors, occupational behaviors, and dietary patterns. Each participant underwent venipuncture. Study participants were reimbursed for their participation with a small monetary award and a box of sweetened condensed milk as dictated by Cameroonian governmental policy. HIV testing results were provided to participants who requested them along with appropriate counseling. The study protocol was approved by the Johns Hopkins Bloomberg School of Public Health Committee for Human Research, the Cameroon National Ethical Review Board, and the HIV Tri-Services Secondary Review Board.

For this study of arboviral epidemiology, we randomly selected a subset of roughly 30 serum samples from individuals who had undergone venipuncture at 9 of the 17 sites covered by the large epidemiologic survey. We chose the nine sites for their proximity to large intact lowland forest habitat, their rural nature, and their representation of multiple elevations.

**Laboratory methods.** Serum samples were screened for neutralizing antibodies to each of the seven viruses by 90% plaque-reduction neutralization tests (PRNT) using standard methods.<sup>32–34</sup> Virus strains used in these PRNTs were Yellow Fever 17D; West Nile NY99-35262; DEN-2 16681; Sindbis EgAr 339; Chikungunya S27; O'nyong Nyong 1854; and Tahyna Leiv 8545Kar. The human sera were heat inactivated at 56°C for 30 minutes and diluted at 1:10 and 1:20 with phosphate-buffered saline (PBS). Diluted serum samples were mixed with an equal volume of the tested virus containing exogenous human complement and incubated for overnight at 4°C. The exogenous human complement was provided by adding a normal human serum, which previously tested negative for antibodies against several arboviruses, to the final concentration of 4% in the serum–virus mixture. Six-well plates of confluent Vero cells were inoculated with the serum–virus mixtures and incubated at 37°C in a 5% CO<sub>2</sub> in-

cubator for 1.5 hours. Plates were overlaid with 4 mL of the medium containing 0.8% agarose and again with 2 mL of second overlay medium containing neutral red vital stain as described previously.<sup>35</sup> Depending on the viral growth rate the second agarose overlay was added at 1–7 days after inoculation. Each assay was performed in triplicate, and back titrations of the input viruses were included in sextuplicate in each assay. The neutralizing antibody titer was identified as the highest serum dilution that reduced the number of virus plaques in the test by 90% or greater. Endpoint titrations were conducted for some samples with titer greater than 1:40 in the screening test for YF, WN, and DEN-2 viruses. All serological testing was conducted at the Division of Vector-Borne Infectious Diseases, CDC (Fort Collins, CO).

**Statistical methods.** For each of the seven viruses we calculated overall seropositivity rates, as well as village-specific seropositivity rates. We used  $\chi^2$  and Fisher exact tests to evaluate heterogeneity of rates among the different villages. We calculated descriptive statistics for demographic and occupational questionnaire variables. We used unadjusted logistic regression models to examine the effects of sex and age on the odds of seropositivity to each evaluated virus more formally. We subsequently evaluated number of years of formal education, number of individuals in the surveyed individual's household, house roofing material, and occupation on odds of seropositivity, again using logistic regression models, although this time adjusting by age strata and sex. All analyses were conducted using SAS v. 8.02 (SAS Institute, Cary, NC).

## RESULTS

Demographic information for the 256 individuals included in this study is shown in Table 1. As seen in this table, study participants were drawn from both sexes and from a wide range of adult ages. Study participants also reflected a diversity of formal educational attainment and household sizes, but most participants worked in either subsistence or market agriculture and had corrugated tin roofs with unfinished ceilings.

Of these individuals, 32 (12.5%) were DEN-2 positive, 17 (6.6%) were WN positive, 69 (26.9%) were YF positive, 119 (46.5%) were CHIK positive, 122 (47.7%) were ONN positive, 20 (7.8%) were SIN positive, and 93 (36.3%) were TAH positive. Table 2 shows village-specific seropositivity rates for each of the nine villages. Seropositivity rates were significantly different among the different villages for all of the evaluated viruses except for TAH. DEN-2, YF, CHIK, and ONN followed roughly similar patterns of seropositivity with higher rates seen in the more geographically central study sites II, III, IV, and VIII, and low rates seen in sites VI and VII. In contrast, WN and SIN seropositivity was high at site VI.

Table 3 shows the odds of seropositivity for each of the evaluated viruses according to demographic classifications. Seropositivity to each of the evaluated viruses was similar among men and women and across different age strata with the exception of rates of YF, CHIK, and ONN, which increased significantly with age ( $P < 0.01$ , 0.01, and  $< 0.01$ , respectively).

In multivariable analysis, increasing years of formal educa-

TABLE 1  
Characteristics of the study participants ( $n = 256$ )

Characteristic	Number	Percent*
Sex		
Male	143	55.9
Female	113	44.1
Age group		
16-25	73	28.5
26-35	51	19.9
36-45	40	15.6
46-55	28	10.9
> 55	64	25.0
Formal education		
0-4	74	28.9
4-6 years	81	31.6
7-9 years	77	30.1
> 9 years	22	8.6
Missing	2	0.8
Number of people in household		
1-4	71	28.3
5-7	79	30.9
8-10	52	20.3
> 10	48	18.8
Missing	6	2.3
House roofing material		
Corrugated tin, unfinished	160	62.5
Grass or thatched	53	20.7
Corrugated tin, finished	12	4.7
Missing	31	12.1
Primary occupation		
Agriculture, subsistence	98	38.3
Agriculture, market	91	35.6
General housework	33	12.9
Other†	33	12.9
Missing	1	0.4

\* Percentages are rounded to one decimal place.

† Other group includes; hunting, fishing, student, market vendor, bar/pub worker, craft maker, truck driver, home brewing, retired, bricklayer, hair stylist.

tion had no significant effect on serpositivity to any of the evaluated viruses. Having a household with greater than 10 members conferred significant protection against DEN-2, but no other household sizes significantly predicted seropositivity rates. Individuals having a grass or thatched roof had significantly lower odds of DEN-2, YF, CHIK, and ONN versus individuals with a corrugated tin roof with an unfinished ceiling. Different primary occupations had no significant effects on seropositivity rates, with the exception of significantly elevated rates of YF among individuals reporting general housework versus subsistence agriculture as their primary occupation.

TABLE 2  
Village-specific seropositivity rates (%) for each evaluated virus

Site no.	Flaviviridae			Togaviridae			Bunyaviridae
	DEN-2	WN	YF	CHIK*	ONN*	SIN	TAH
I	10	0	13	87	87	0	30
II	7	3	38	90	90	0	34
III	58	17	33	67	75	13	42
IV	23	15	19	27	27	8	25
V	7	3	30	17	17	3	27
VI	0	17	7	10	10	33	33
VII	0	4	21	7	7	0	39
VIII	17	3	63	77	80	10	37
IX	0	0	17	38	38	3	52

\* Cross-reactivity may occur.

## DISCUSSION

This survey found high rates of seropositivity to all seven evaluated arboviruses in rural Cameroonian adults, but as in the evaluation of all pathogens with highly variable clinical-to-subclinical ratios, translation of seropositivity rates into actual measures of morbidity can be difficult. There was a high degree of cross-reactivity between the CHIK and ONN assays, but this result was not entirely unexpected. The similarity of these two viruses and their antigens has been described previously,<sup>11,36-38</sup> and serological studies have shown a one-way cross-reactivity between the two viruses.<sup>36,39,40</sup> Because antibody to CHIK virus reacts almost equally with both CHIK and ONN viruses, and ONN virus antibodies react only weakly against CHIK virus, most of the CHIK/ONN antibody seen in this survey could be attributable to CHIK virus. Previously, Ndip and others also reported similar observations in their complement fixation tests of CHIK and ONN in serum samples obtained from Cameroonian patients.<sup>31</sup> However, from our PRNT tests, there were some samples from sites III and VIII that reacted to ONN but not to CHIK (Table 2), and some sera from various sites had higher PRNT titers to ONN ( $\geq 1:40$ ) than titers to CHIK (1:20) (data not shown), indicating possible ONN circulation in these areas. Most of the positive samples had PRNT titers  $\geq 1:40$  for both CHIK and ONN (data not shown), and without endpoint PRNT titers against CHIK and ONN, we could not determine the infecting virus(es). It is likely that both viruses were circulating in the populations evaluated for this study.

There is also cross-reactivity among the four serotypes of DEN virus in serological tests.<sup>31</sup> Endpoint titration of PRNT tests against all four serotypes may differentiate these serotypes in some samples from primary infection, but not from secondary or multiple DEN infections. Although only DEN-2 virus was tested in this work, DEN-1 and DEN-2 as single antigens pick up approximately 95% of primary infections of the other serotypes and 100% of other serotype secondary infections in hemagglutination-inhibition (HI) and ELISA assays.<sup>41</sup> To this end, the results presented herein likely reflect the cumulative burden of all dengue serotypes. A definitive characterization of the serotypes circulating in the populations evaluated for this study would require both clinical and entomologic surveillance, which was not possible given the cross-sectional nature of the parent survey.

Seropositivity rates for DEN-2, YF, and CHIK, each of which is carried by common vectors *Ae. aegypti* and *Ae. albopictus*, were higher in the geographically central sites, which are among the closest of those surveyed to major urban centers, than in the two most isolated of the surveyed villages. As has been seen elsewhere,<sup>42,43</sup> these data suggest that urban cycles play an important role in the maintenance of these pathogens in Cameroonian populations, but the relative contribution of urban versus sylvatic cycles to the maintenance of these viruses in African populations remains to be clarified.

The similarity of WN and SIN seropositivity rates was additionally expected given their common *Culex* species vectors and the fact that birds serve as major amplifying hosts for both of these viruses.<sup>44-47</sup> However, the distribution of the WN and SIN seropositivity rates was more difficult to interpret, because both central sites as well as a highly isolated site in the extreme south of the country (site VI) had substantial WN and SIN seroprevalence rates. Most likely these sero-

TABLE 3  
Odds of seropositivity for each evaluated virus according to demographic variables

Characteristic	DEN-2	WN	YF	CHIK†	ONN‡	SIN	TAH
Sex	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Male	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Female	1.1 (0.5, 2.4)	1.1 (0.4, 3.0)	1.0 (0.6, 1.7)	0.7 (0.4, 1.2)	0.7 (0.4, 1.1)	0.5 (0.2, 1.4)	1.2 (0.7, 2.0)
Age group							
16–25	1.0	1.0	1.0	1.0	1.0	1.0	1.0
26–35	0.8 (0.3, 2.5)	2.0 (0.4, 9.3)	2.0 (0.4, 9.3)	1.0 (0.5, 2.1)	1.1 (0.5, 2.2)	0.6 (0.1, 3.0)	1.0 (0.5, 2.2)
36–45	1.1 (0.4, 3.3)	1.9 (0.4, 9.8)	6.8 (1.7, 26.8)	0.9 (0.4, 1.9)	1.0 (0.4, 2.1)	1.9 (0.5, 7.2)	1.7 (0.8, 3.7)
46–55	1.1 (0.3, 3.7)	1.8 (0.3, 11.4)	23.3 (5.9, 92.1)	1.9 (0.8, 4.6)	1.9 (0.8, 4.6)	1.1 (0.2, 5.7)	2.7 (1.1, 6.7)
> 55	0.7 (0.2, 1.9)	2.0 (0.5, 8.6)	36.4 (10.3, 128.3)	2.0 (1.0, 3.9)	2.1 (1.1, 4.2)	1.4 (0.4, 4.8)	0.8 (0.4, 1.7)
Formal education*							
0–4 years	1.0	1.0	1.0	1.0	1.0	1.0	1.0
4–6	2.5 (0.8, 7.9)	0.8 (0.2, 3.2)	1.2 (0.5, 2.9)	2.0 (0.9, 4.2)	2.0 (1.0, 4.3)	0.4 (0.1, 1.4)	1.0 (0.5, 2.2)
7–9 years	1.6 (0.5, 5.5)	1.0 (0.3, 4.3)	1.0 (0.4, 2.6)	1.9 (0.9, 4.2)	1.8 (0.8, 3.8)	0.4 (0.1, 1.3)	1.2 (0.6, 2.7)
> 9 years	2.0 (0.4, 10.4)	1.4 (0.2, 9.7)	0.8 (0.1, 4.5)	1.3 (0.4, 3.9)	1.2 (0.4, 3.6)	0.4 (0.1, 3.6)	1.9 (0.6, 5.6)
Number of people in household*							
1–4	1.0	1.0	1.0	1.0	1.0	1.0	1.0
5–7	0.5 (0.2, 1.9)	0.5 (0.1, 2.0)	0.7 (0.3, 1.6)	1.0 (0.5, 2.0)	1.0 (0.5, 1.8)	0.3 (0.1, 1.3)	0.9 (0.5, 1.7)
8–10	0.4 (0.1, 1.1)	0.8 (0.2, 3.3)	1.3 (0.5, 3.3)	1.1 (0.5, 2.3)	1.0 (0.5, 2.1)	0.5 (0.1, 2.1)	1.0 (0.5, 2.1)
> 10	0.3 (0.1, 0.9)	1.4 (0.4, 5.1)	0.7 (0.3, 1.8)	0.8 (0.4, 1.8)	0.8 (0.4, 1.6)	1.1 (0.3, 3.6)	0.6 (0.3, 1.4)
House roofing material*							
Corrugated tin, unfinished	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Grass or thatched	0.3 (0.1, 1.0)	0.7 (0.1, 3.6)	0.4 (0.1, 0.9)	0.4 (0.2, 0.7)	0.4 (0.2, 0.7)	‡	1.1 (0.5, 2.1)
Corrugated tin, finished	1.9 (0.5, 8.0)	2.0 (0.2, 18.7)	0.4 (0.1, 2.1)	1.0 (0.3, 3.4)	0.9 (0.3, 3.2)	1.4 (0.2, 13.1)	1.1 (0.3, 3.9)
Primary occupation*							
Agriculture, subsistence	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Agriculture, market	0.7 (0.3, 1.8)	3.7 (0.8, 16.4)	0.6 (0.3, 1.6)	0.6 (0.3, 1.2)	0.6 (0.3, 1.2)	0.5 (0.2, 1.8)	0.6 (0.3, 1.3)
General housework	0.5 (0.1, 2.1)	1.3 (0.2, 7.5)	6.3 (2.1, 19.5)	1.6 (0.7, 3.5)	1.6 (0.7, 3.7)	0.4 (0.0, 3.2)	0.4 (0.2, 1.0)
Other	0.5 (0.1, 2.1)	3.7 (0.7, 19.9)	1.6 (0.5, 5.7)	0.6 (0.2, 1.3)	0.7 (0.3, 1.7)	0.9 (0.2, 3.8)	1.3 (0.6, 3.4)

\* Adjusted for sex and age strata.

† Cross-reactivity may occur.

‡ No estimate.

prevalence patterns reflect the ecology of preferred amplifying avian species, especially that of the order *Passeriformes*,<sup>45</sup> but at present, the ecology of these viruses in central Africa remain poorly understood.

In this study, seroprevalence of YF was low among young adults (data not shown), but odds of infection climbed sharply in individuals beginning in their late thirties. This sharp change in slope suggests a combination endemic-epidemic etiology for the YF infections seen in this survey. Although prior YF vaccination campaigns could have produced serological patterns similar to those seen in this study, our discussions with Cameroonian health officials suggested a low penetrance of YF vaccination campaigns among the populations evaluated for this study. Although we believe the age trend for YF is quite clear, the cross-sectional nature of this survey is unable to assess and account for secular trends in disease ecology that could attenuate or invalidate the trend.

Additionally of interest, odds of DEN-2, YF, and CHIK seropositivity were significantly lower among individuals with grass or thatched roofs versus those individuals with corrugated tin roofs with unfinished ceilings. This demographic variable correlated roughly with proximity to central Cameroon and therefore with proximity to major urban centers (data not shown), but the fact that grass or thatched roofs proved a statistically sensitive marker itself for contact with the *Aedes* species-associated viruses may be of significance in the design and conduct of preventive interventions.

The results of this survey provide important insights into the possible disease burden and distribution of viruses in the *Flaviviridae*, *Togaviridae*, and *Bunyaviridae* families. The

variability of the seroprevalence rates for most of the evaluated viruses underscore the complexities of arbovirus epidemiology in a region of high biodiversity.

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