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High Seroprevalence of Chikungunya Virus Antibodies Among Pregnant Women Living in an Urban Area in Benin, West Africa

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Abstract. The aim of this study was to investigate the seroprevalence of antichikungunya virus (anti-CHIKV) antibodies in pregnant women living in an urban area of Benin (West Africa). Results were obtained by screening sera collected in 2006 and 2007 with enzyme-linked immunosorbent assay (ELISA) for anti-CHIKV immunoglobulin G (IgG) and IgM. Positive results were confirmed by indirect immunofluorescence test and microneutralization assay. We found that a large proportion (36.1%) of pregnant women living in Cotonou had specific IgG against CHIKV, indicating a high seroprevalence of the infection in urban southern Benin, whereas no active cases of CHIKV infection were detected.

Arboviruses that are transmitted by *Aedes* mosquitoes, such as dengue virus and chikungunya virus (CHIKV) infections, are a global threat because of their rapid and abrupt spread worldwide and the infection-related disease burden.[1–3] Although the increasing rate is well-characterized in industrialized countries, the distribution of these arboviruses remains quite obscure in Africa and other tropical regions, with no ongoing surveillance programs.

Among these viruses, CHIKV, an RNA virus belonging to the *Togaviridae* family, has been identified as the cause of outbreaks of febrile illness in sub-Saharan Africa since the 1950s. After a period of quiescence, numerous African countries faced the re-emergence of CHIKV infection at the end of 1990s, with outbreaks in Senegal in 1996 and 1997,[4] the Democratic Republic of Congo in 2000,[5] and Kenya and islands of the Indian Ocean in 2004–2006[6]; cases in Sudan in 2005[7] and Tanzania in 2007 and 2008[8]; and outbreaks in Gabon in 2007 and 2010[9], Cameroon in 2006 and Republic of Congo in 2011.[10] Furthermore, a number of studies indicates CHIKV circulation in Kenya and Cameroon during interepidemic periods.[11–13]

Circulation of CHIKV has also been reported in a few West African countries: it has been described in a cluster of travelers returning from Senegal with active CHIKV infection[14] and acute cases of CHIKV-related disease were detected in Guinea[15] and likely, Sierra Leone.[16] Furthermore, a recent study reported a seroprevalence of 46% for CHIKV-specific immunoglobulin G (IgG) in hospitalized patients in Nigeria during 2008.[17]

To our knowledge, no studies have so far analyzed the circulation of CHIKV in other West African countries, such as the Republic of Benin. The aim of this study was to fill this gap by investigating the seroprevalence of CHIKV infection in pregnant women living in an urban area of Benin.

Serum samples were previously collected for a study about malaria in pregnancy.[18] For this study, 352 pregnant women were enrolled at delivery after informed consent was obtained from July of 2006 to January of 2007 from the Hospital Mother and Child Lagune, the main obstetrical referring hospital in Cotonou, Benin and the Houenoussou Health Center in Cotonou, Benin. Women who underwent delivery at the above-mentioned clinics between July of 2006 and January of 2007 were enrolled in the study, regardless of presence or absence of fever or other symptoms. The main objective of the study was to identify the prevalence of pregnancy malaria at delivery and the proportion of *Plasmodium falciparum* transmission to the offspring, whereas the secondary objective of the study was to investigate whether the innate immunity of the newborn was influenced by maternal malaria at delivery. This study was approved by the Science and Health Faculty Ethics Committee of Benin. Malaria transmission in this area is hyperendemic (i.e., intense and perennial), with two peaks during the rainy seasons (April to July and September to November).[19] Demographic information for 352 women included in this study is shown in Table 1. *P. falciparum* status during pregnancy was determined by microscopic examination of thin and thick smears that were prepared from maternal peripheral blood at each antenatal visit, and it is also reported in Table 1.

Serum samples were examined for CHIKV IgM and IgG antibodies using as the screening test a commercial enzyme-linked immunosorbent assay (ELISA; Enzywell; DIESSE, Siena, Italy). Samples exhibiting weak positivity for IgG by ELISA (Optical Density [OD] values in the range of 0.4–0.7) were further tested by a more specific indirect immunofluorescence assay (IIFA) to detect IgG (anti-CHIKV IgG FI 293a-1005G; Euroimmun AG, Lübeck, Germany); all samples that tested positive for IgM by ELISA were further tested by IIFA to specifically detect IgM (anti-CHIKV virus IgM FI 293a-1005M; Euroimmun AG, Lübeck, Germany). All of the anti-CHIKV IgG- and IgM-positive samples identified in the previous steps were confirmed by microneutralization assay (MNTA). MNTA against CHIKV was performed by using a viral strain that was isolated from a patient during the CHIKV outbreak in Italy in 2007.[20] Briefly, serum samples were inactivated at 56°C for 30 minutes and serially diluted starting at 1:5. Diluted sera were incubated with 150 $TCID_{50}$ (tissue culture infectious dose 50%) units of CHIKV in DMEM (Dulbecco's Modified Eagle Medium) supplemented with antibiotics and fetal calf serum for 1 hour

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TABLE 1
Characteristics of the study participants (N = 352)

Characteristic	Number (%)
Age group (years)	
Undefined*	
14–20	26 (7.4)
21–30	185 (52.5)
31–42	131 (37.2)
Malaria presence	
Undefined*	3 (0.8)
Positive	56 (15.9)
Negative	293 (83.2)

*Missing information.

at 37°C in 5% CO_2 atmosphere. The mixture was added to 10^4 Vero E6 cells per well in 96-well plates, incubated for 5 days, and then evaluated for the presence of virus-induced cytopathic effect.

Three hundred fifty-two serum samples collected from pregnant women were analyzed for the presence of anti-CHIKV IgM and IgG antibodies; 136 of 352 samples tested positive for IgG by ELISA. Because 9 of 136 positive samples exhibited weak positivity by ELISA, they were further tested by IIFA, and only 6 of 9 samples were confirmed as positive. Thus, 133 samples among 352 tested (37.8%) were positive for anti-CHIKV IgG by ELISA and IIFA.

In total, 18 of 352 samples tested positive for anti-CHIKV IgM by ELISA and were further analyzed by IgM IIFA; using this assay, only one sample was confirmed as positive. Samples testing positive for anti-CHIKV IgG (N = 133) or IgM (N = 1) by the combination of ELISA and IIFA were further analyzed by MNTA; 127 samples were confirmed as having neutralizing antibodies against CHIKV with antibody titers in the range of 1:40 to > 1:1,280. All confirmed samples were IgG-positive, and no specific IgM-positive samples could be identified. Thus, CHIKV seropositivity was 36.1% (127 of 352) among pregnant women in Cotonou, whereas no specific anti-CHIKV IgM was found (Table 2). The highest seropositivity was observed in the 14- to 20-years-old age group (57.7%) (Table 3). Furthermore, high (> 1:160) and low (≤ 1:160) titers of neutralizing antibodies against CHIKV were evenly distributed among the different age groups.

This study is the first to investigate the circulation of CHIKV in the Beninese population. To our knowledge, the only proof for the presence and circulation of CHIKV in Benin before this report comes from a study in the 1990s showing a moderate positivity (5.7%) of anti-CHIKV IgG in German expatriates returning from Benin.[21] We found that 127 of 352 samples (36.1%) from Beninese pregnant women had anti-CHIKV IgG, whereas no sample exhibited specific IgM, thus indicating a high prevalence of immune response against CHIKV in this Beninese population. Seroprevalence studies for arbovirosis in endemic countries are often limited by the lack of specificity of classical serological methods.[13] By using the MNTA as a confirmatory test for samples testing positive for anti-CHIKV IgG by ELISA and IIFA, we reduced the risk for cross-reactivity with other alphaviruses.

TABLE 2
Presence of anti-CHIKV IgM and IgG in Beninese pregnant women from 2006 to 2007

	CHIKV IgM n positive/n tested (%)	CHIKV IgG n positive/n tested (%)
ELISA/IIFA	1/352 (0.3)	133/352 (37.8)
MNTA	0/1 (–)	127/133 (94.8)
Total	0/352 (–)	127/352 (36.1)

TABLE 3
CHIKV seropositivity and IgG titers among different age groups

Age group (years)	n	CHIKV seropositivity n positive/n tested (%)	CHIKV IgG neutralizing titer ≤ 1:160 n positive/n tested (%)	CHIKV IgG neutralizing titer > 1:160 n positive/n tested (%)
Undefined*	10	6/10 (60.0)	2/6 (33.3)	4/6 (66.6)
14–20	26	15/26 (57.7)	8/15 (53.3)	7/15 (46.6)
21–30	185	60/185 (32.4)	31/60 (51.6)	29/60 (48.3)
31–42	131	46/131 (35.1)	22/46 (47.8)	24/46 (52.2)
Total	352	127/352 (36.1)	63/127 (49.6)	64/127 (50.4)

*Missing information.

No acute cases of CHIKV infection were detected among Beninese pregnant women included in our study, and all positive cases were judged as past infections. Furthermore, high seroprevalence rates were observed in young women, suggesting that these individuals became infected with CHIKV during early life. This is in line with recent findings from Tanzania indicating that CHIKV mainly circulates in children during endemicity periods.[8]

Although our study was not designed to validate diagnostic kits for the detection of specific antibodies against CHIKV, we observed that, in 94.5% of samples, a positive result by CHIKV IgG ELISA and CHIKV IgG IIFA was confirmed by MNTA, indicating a good specificity of the combination of these commercial methods. Conversely, all 18 samples that tested IgM-positive against CHIKV by ELISA were judged as false positive when evaluated by CHIKV IgM IIFA and MNTA, suggesting a poor performance in terms of specificity for the ELISA IgM kit.

Mother-to-child transmission of CHIKV was reported during the 2005 and 2006 outbreak on Réunion Island[22]; vertical transmission was observed almost exclusively in the context of intrapartum viremia, and it was associated with a high rate of neonatal morbidity.[23,24] Because pregnancy-associated malaria is an important cause of maternal and fetal morbidity and mortality,[25] in a malaria high-risk area such as Cotonou, acute CHIKV infection of a malaria-infected woman close to delivery could worsen pregnancy outcome. We observed that, among 56 women who had *P. falciparum* infection during pregnancy, 17 (30.3%) women were positive for CHIKV IgG, but none exhibited anti-CHIKV IgM. Because no cases of acute/recent CHIKV infection were detected at delivery, we believe that there would have been no chance of mother-to-child transmission of CHIKV in our study group.

There were several limitations in our study. We cannot exclude that other alphaviruses, such as o'nyong-nyong virus (ONNV), could be responsible for some of the IgG-positive results for CHIKV that we observed in this study, because CHIKV and ONNV are cross-reactive, even in MNTA. Furthermore, we used serum samples that had been previously collected, which limited our geographic approach. Although our data showed the presence of IgG against CHIKV in the economic capital of Benin, they do not provide seroprevalence information on distribution of this infection in the rest of the country. Additionally, our study population consisted exclusively of females, which could lead to sex confounding, limiting the value of our findings.

In conclusion, despite no reported CHIKV acute cases or outbreaks in this country, our results indicate a high seroprevalence of CHIKV antibodies in pregnant women living in urban Benin in 2006 and 2007. After our accurate identification of the serostatus for this arboviral infection, Benin should be considered endemic for CHIKV infection. Because this arbovirosis causes febrile illness that would be clinically undistinguishable from malaria, more tailored patient care with improvement of diagnostic methods to specifically detect CHIKV would be desirable to avoid underdiagnosis and misdiagnosis of CHIKV in this West African malaria-endemic area.

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