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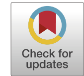
Chikungunya virus antepartum transmission and abnormal infant outcomes in a cohort of pregnant women in Nigeria



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ABSTRACT

Objectives: Chikungunya virus (CHIKV), a reemerging global public health concern, which causes acute febrile illness, rash, and arthralgia and may affect both mothers and infants during pregnancy. Mother-to-child transmission (MTCT) of CHIKV in Africa remains understudied.
Methods: Our cohort study screened 1006 pregnant women with a Zika/dengue/CHIKV rapid test at two clinics in Nigeria between 2019 and 2022. Women who tested positive for the rapid test were followed through their pregnancy and their infants were observed for 6 months, with a subset tested by reverse transcription-polymerase chain reaction (RT-PCR) and neutralization, to investigate seropositivity rates and MTCT of CHIKV.
Results: Of the 1006, 119 tested positive for CHIKV immunoglobulin (Ig)M, of which 36 underwent detailed laboratory tests. While none of the IgM reactive samples were RT-PCR positive, 14 symptomatic pregnant women were confirmed by CHIKV neutralization test. Twelve babies were followed with eight normal and four abnormal outcomes, including stillbirth, cleft lip/palate with microcephaly, preterm delivery, polydactyly with sepsis, and jaundice. CHIKV IgM testing identified three possible antepartum transmissions.
Conclusion: In Nigeria, we found significant CHIKV infection in pregnancy and possible CHIKV antepartum transmission associated with birth abnormalities.

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Introduction

Chikungunya virus (CHIKV), a member of the genus *Alphavirus* of the family *Togaviridae*, is transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes. Before 2005, there were three distinct genotypes of CHIKV: the East-Central-South African (ECSA), West African, and Asian genotypes in different geographic locations [1]. The Indian Ocean lineage arose from the ECSA in 2004-2007 and Asian genotype expanded in late 2013, spreading to the Americas with over 1.3 million suspected cases by April 2015 [1–3]. It has been reported that autochthonous transmission of CHIKV occurs in 114 countries and territories over the tropical and sub-tropical areas where greater than three quarters of the world's population reside [1].

CHIKV is an old world alphavirus belonging to the Semliki Forest virus complex, despite phylogenetic relatedness to O'nyong-nyong (ONNV) in Africa and Mayaro virus (MAYV) in South America and the Caribbean and difficulty to distinguish by

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immunodiagnostic assays or clinical presentation. However, ONNV is vectored by Anopheles mosquitoes, whereas CHIKV in Africa is maintained in an enzootic cycle involving non-human primates and sylvatic *Aedes* mosquitoes; sporadic cases or outbreaks tend to be small-scaled and associated with spillover from the natural reservoir vectors or environmental conditions such as increased rainfall [2–4]. Recently, an increase in larger-scale outbreaks associated with urban areas of Africa have been reported [1,5,6]. The CHIKV strain isolated in Ibadan in the 1960s is most similar to strains found in Indonesia, the Philippines, the US, and Nicaragua in the 2010s, but more recent phylogenetic analysis is lacking despite continued circulation [7]. CHIKV circulates in Africa alongside other arboviruses, including *Flaviviridae* dengue and Zika viruses, with similar clinical presentations that make differential diagnoses and reporting difficult.

CHIKV infection causes acute febrile illness, rash and arthralgia and may affect both pregnancy and infant outcomes [8]. During the Reunion Island outbreak in 2005, the prospective study of CHIKV infection in pregnancy described that mother-to-child transmission (MTCT) was observed in viremic mothers during the intrapartum period (within 2 days prior to and after delivery) with a transmission rate of 48.7%, leading to neonatal disease with enterocolitis, encephalitis, convulsions, and death [9,10]. The study also described 3 exceptional cases of early fetal deaths (before 22 weeks) out of 678 antepartum CHIKV infections which were attributed to intrauterine CHIKV infection. As CHIKV spread from Asia to the Americas between 2007-2014, perinatal transmission of CHIKV was reported in India, Colombia, Brazil, and Curacao [11–14]. Meta-analysis of cohort studies from Asia and Latin America revealed a MTCT rate of 15.5% with symptomatic neonatal disease attributed to intrapartum infection [8]. However, the MTCT of African strains of CHIKV remains poorly understood.

In this study, we screened 1006 pregnant women at two antenatal clinics in Jos, north-central Nigeria from April 2019 to January 2022. Our objectives were to quantify CHIKV seropositivity among pregnant women, investigate maternal-fetal transmission, and determine any associations with disease outcome in infants in our West African cohort.

Methods

Nigeria cohort and human samples

Between April 1, 2019 and January 31, 2022, pregnant women aged ≥18 years, who attended the antenatal clinics at Jos University Teaching Hospital (JUTH) and Our Lady of Apostles Hospital (OLA) in Jos, north-central Nigeria, and presented with fever (≥37.5°C), arthralgia, headache, myalgia, rash, and/or conjunctivitis in the past 3 days were recruited with informed consent for screening by the Chembio DPP® ZCD immunoglobulin (Ig)M/IgG rapid test (Chembio Diagnostics Inc. Medford, NY). To examine arbovirus seropositivity in asymptomatic infection, for every four symptomatic women, one asymptomatic woman with gestational age (GA) within 2 weeks of any of the four symptomatic women was also recruited for screening. The 4:1 ratio was based on optimizing screening for symptomatic women and the capacity for daily enrollment at the smaller of the two clinics (<5). All Zika virus (ZIKV), dengue virus (DENV) or CHIKV rapid test IgM and IgM/IgG reactive women were recruited with informed consent for the prospective observational study as described [15]. Maternal blood samples were collected for testing at screening and at each subsequent antenatal visit, and infants were observed at delivery and routine follow-up visits at 6, 10, and 14 weeks, and 6 months, with infant follow-up data censored on March 31, 2022. The pregnant women with CHIKV infection (CHIKV IgM+ and/or IgG+) and their infants were the focus of this study. The intrapartum period was defined as within 2 days prior to or after delivery; the remaining antepartum period included the first, second and third trimesters as defined by GA ≤12 weeks, >12 weeks to <28 weeks, and ≥28 weeks, respectively.

To evaluate the performance of the Chembio DPP® ZCD IgM/IgG rapid test, convalescent-phase sera from reverse transcription-polymerase chain reaction (RT-PCR)-confirmed CHIKV cases (n = 22) in Brazil served as CHIKV-positive reference samples [16] and CHIKV-negative reference samples (n = 27) were from a dengue seroprevalence study in Taiwan [17], a CHIKV non-endemic country; both were confirmed by CHIKV pseudovirus neutralization test (NT). Convalescent-phase serum/plasma samples from RT-PCR-confirmed DENV (n = 35) and ZIKV (n = 42) cases were also included in the analysis (Table S1) [18].

The study of coded serum or plasma samples was approved by the Institutional Review Boards (IRB) of the Harvard Longwood Campus (IRB# 17-0654), University of Jos (IRB# 127/XIX/5940), and University of Hawaii at Manoa (IRB# 2021-00044 and CHS#17568).

Isolation of viral RNA and reverse transcription-polymerase chain reaction

Viral RNA was isolated from sera using the QIAamp viral RNA mini kit (Qiagen) and subjected to cDNA synthesis using the SuperScript™ III first-strand synthesis kit (Thermo Scientific); an aliquot of cDNA was subjected to nested PCR targeting a conserved region of the NSP2 gene of CHIKV [19]. The RT-PCR products were electrophoresed through 2% agarose gel, and bands with the predicted size were purified for sequencing. The primers were CHIKV-F (5'-TCAATATGATGCAGATGAAAGT-3', position 2541-2562), CHIKV-inner R (5'-GTCACAGGCA GTGTACACC -3', position 2616-2634) and CHIKV-outer R (5'-GCAACGABGA CACAATGGC-3', position 2636-2654). The size of the 1st round and 2nd round products were 113 bp and 93 bp, respectively. The PCR protocol will be provided upon request.

Dengue virus and Zika virus serologic diagnosis

IgM and IgG enzyme-linked immunosorbent assays (ELISAs) using DENV and ZIKV mutated virus like particles (VLP) was performed and microneutralization provided confirmation, as described [15,17,18].

Cell lines and plasmids

Human hepatoma Huh7 cells were obtained from Health Science Research Resources Bank, Japan Health Sciences Foundation (Osaka, Japan). HEK-293T cells were obtained from the American Type Culture Collection (Manassas, VA). Plasmids pNL4-3 R-E-miRFP, which contains the monomeric infrared fluorescent protein (miRFP) gene replacing the Luc gene of an *env*-defective HIV-1 reporter construct pNL4-3.Luc.R-E-, has been described previously [20]. The E3-E2-6K-E1 genes of CHIKV (2955 bp) and Mayaro virus (MAYV) (2952 bp) were PCR amplified from cDNA derived from RNA of CHIKV (H20235 strain) and MAYV (TRVL strain), respectively, (BEI Resources, Manassas, VA), and cloned into pCB vector (by NotI and XhoI sites) to generate plasmids CHIKV and MAYV. All plasmids were confirmed by sequencing of the entire E3-E2-6K-E1 gene insert and verified for expression by transfection and Western blot analysis [20].

Generation of chikungunya virus and Africa and Mayaro virus pseudoviruses

To generate pseudoviruses, HEK-293T cells were seeded in 10-cm dish 1 day before transfection, co-transfected with pNL4-3 R-E-

miRFP (12 µg) and plasmids CHIKV (12 µg) or MAYV (4 µg) using lipofectamine 2000 and incubated with DMEM media containing 10% FBS [20]. The supernatants were collected at 72 hours post transfection, followed by low-speed centrifugation at 300x g for 10 minutes, aliquoted and stored at −80°C. To titrate each pseudovirus,

Three-fold serially diluted supernatants were inoculated to Huh7 cells by spin infection [20]; miRFP signals were quantitated at 72 hours post-infection, and the amount of pseudovirus that resulted in miRFP signals 10 times higher than the mock-infected wells was used for the NT.

Chikungunya virus and Africa and Mayaro virus pseudovirus neutralization test

Huh7 cells (2×10^4 cells/well) were seeded onto 96-well plates 1 day before infection. Pseudovirus (CHIKV or MAYV) was mixed with 4-fold serial dilutions of plasma at 1:1 ratio, incubated at 37°C for 1 hour, and added to each well for spin infection. At 72 h, the plates were scanned by Li-Cor Odyssey imager [20]. The % of infection at different plasma dilutions (from 1:10 to 1:10,240 dilutions) were calculated by the formula (intensity of serum+pseudovirus – intensity of media only)/(intensity of pseudovirus only – intensity of media only) x 100. The % neutralization=100 – % of infection [20]. NT_{50} titer was the plasma dilution that reached 50% neutralization using 4-parameter nonlinear regression analysis (GraphPad 6.0, Boston, MA) (Figure 1). NT_{50} titer<10 was arbitrarily assigned as 5. CHIKV and MAYV NT_{50} titers were compared for confirmation.

Statistical analysis

Our study was largely descriptive, and we report seropositivity rates as proportion reactive over total tested. Because we analyzed a subset of samples from CHIKV IgM-positive women with detailed testing, we performed a sensitivity analysis to compare the women whose samples were analyzed with those not analyzed. The two-tailed Fisher's exact test was used to compare categorical variables, respectively, for the sensitivity analysis and for the comparison of delivery outcomes (Stata 15.1, College Station, TX). The two-tailed Mann-Whitney test and the two-tailed Wilcoxon signed-rank test were used to compare unpaired and paired NT_{50} titers, respectively, between confirmed CHIKV cases and CHIKV-negative individuals (GraphPad 6.0, Boston, MA). Any *P*-value <0.05 was considered significant.

Results

Detection of chikungunya virus infection

The Chembio DPP® ZCD IgM/IgG rapid test was used to detect CHIKV IgM and IgG in consenting pregnant women at the antenatal clinics of JUTH and OLA. To assess the sensitivity/specificity of the CHIKV IgM and IgG assays, we first tested with a panel of convalescent-phase samples from RT-PCR-confirmed CHIKV, DENV and ZIKV cases and CHIKV-negative samples from a dengue seroprevalence study in Taiwan [16–18]. The sensitivity/specificity of the CHIKV IgM and IgG assays was 90.9/95.2% and 100/100%, respectively (Supplementary Table 1).

Between April 2019 and January 2022, 787 symptomatic and 219 asymptomatic women were screened. A third of women (312/1006, 31.0%) were reactive to ZIKV, DENV, CHIKV or some combination. CHIKV IgG was detected in 120/1006, suggesting a seropositivity rate of 11.9%, and CHIKV IgM in 119/1006 (11.8%), suggesting acute or recent CHIKV infection. Among 119 CHIKV IgM reactive women, 42 (35.3%) also demonstrated IgM reactivity to ZIKV and/or DENV (nine ZIKV-only, 25 DENV-only, and eight ZIKV+DENV). Among symptomatic women, headache was the most common symptom followed by fever and fatigue. Unlike similar studies in the Americas, rash and arthralgia were not commonly reported. The seropositivity rate of CHIKV IgM was higher in asymptomatic women (41/219, 18.7%) compared to symptomatic women (78/787, 9.9%). CHIKV RT-PCR was performed but none of the CHIKV IgM reactive samples tested positive.

Confirmation of chikungunya virus infection by neutralization test

We further generated CHIKV pseudovirus based on a lentivirus vector with an miRFP reporter and developed a CHIKV NT (Figure 1) [20]. When testing with a panel of convalescent-phase sera from RT-PCR-confirmed CHIKV cases in Brazil, we found high NT_{50} titers (289-6867) to CHIKV (Figure 1). As a comparison, a MAYV pseudovirus NT was also performed and lower NT_{50} titers (30-1522) to MAYV were observed; the ratio of CHIKV NT_{50} titers to MAYV NT_{50} titers were higher than 2.5, which was consistent with two recent reports [21,22].

We next used the CHIKV NT to examine 36 samples, collected during the first two years of the study, from 119 pregnant women with acute or recent CHIKV infection (IgM+IgG− or IgM+IgG+, respectively). Of the 36 samples, 14 (38.9%) demonstrated IgM reactivity to ZIKV and/or DENV (2 ZIKV only, 8 DENV only, and 4 ZIKV+DENV). Of the 36 samples, we found 18 with detectable neutralizing antibodies to CHIKV (2/17 from the IgM+IgG− subgroup and 16/19 from the IgM+IgG+ subgroup) (Table 1, Figure 2a). The lack of neutralizing antibodies in participants from the CHIKV IgM+IgG− subgroup (15/17) was unexpected, but was in agreement with the observation of five distinct antibody patterns and the presence of an IgM+IgG− period in some individuals based on the study of 133 samples following acute CHIKV infection [23].

Since confirmatory testing was not possible for all 119 pregnant women with CHIKV IgM+ or IgM+/IgG+ screening, we performed a sensitivity analysis to compare the 36 analyzed with the 83 not analyzed in this study. We found no statistically significant difference between the two groups in site, age, ethnic group, education, occupation status, marital status, trimester at screening, or symptomatic vs inapparent infection status (Fisher's exact test), except that the 36 analyzed women were enrolled exclusively during the first 2 years of the study (Supplementary Table 2).

Infant outcomes of antepartum chikungunya virus infection

Of the 36 pregnant women with CHIKV IgM reactivity (17 IgM+IgG– and 19 IgM+IgG+), 30 were symptomatic at the time of screening (15 IgM+IgG– and 15 IgM+IgG+). Of the 30 symptomatic women, 26 had babies who were followed up; among them, one seropositive woman was detected in her first trimester, 8 in their second trimester, and 17 in their third trimester. Of the 26, 19 had normal (one first trimester, seven second trimester, 11 third trimester) and seven had abnormal (one second trimester, six third trimester) outcomes including three stillbirths, two multiple congenital anomalies, one polydactyly with sepsis and jaundice, and one preterm (Table 1). In the subset of 14 acute or recent symptomatic CHIKV infections confirmed by NT (two IgM+IgG- and 12 IgM+IgG+), 12 babies were followed with eight normal and four abnormal outcomes.

The time course of four mothers with acute CHIKV infection confirmed by NT (Figures 2b-e) and their babies with abnormal outcomes was summarized in Figures 3a-d. Notably, babies with multiple congenital anomalies may have lower odds of survival. Case OL-ZP-0031 was enrolled at 39 weeks with CHIKV IgM+/IgG-

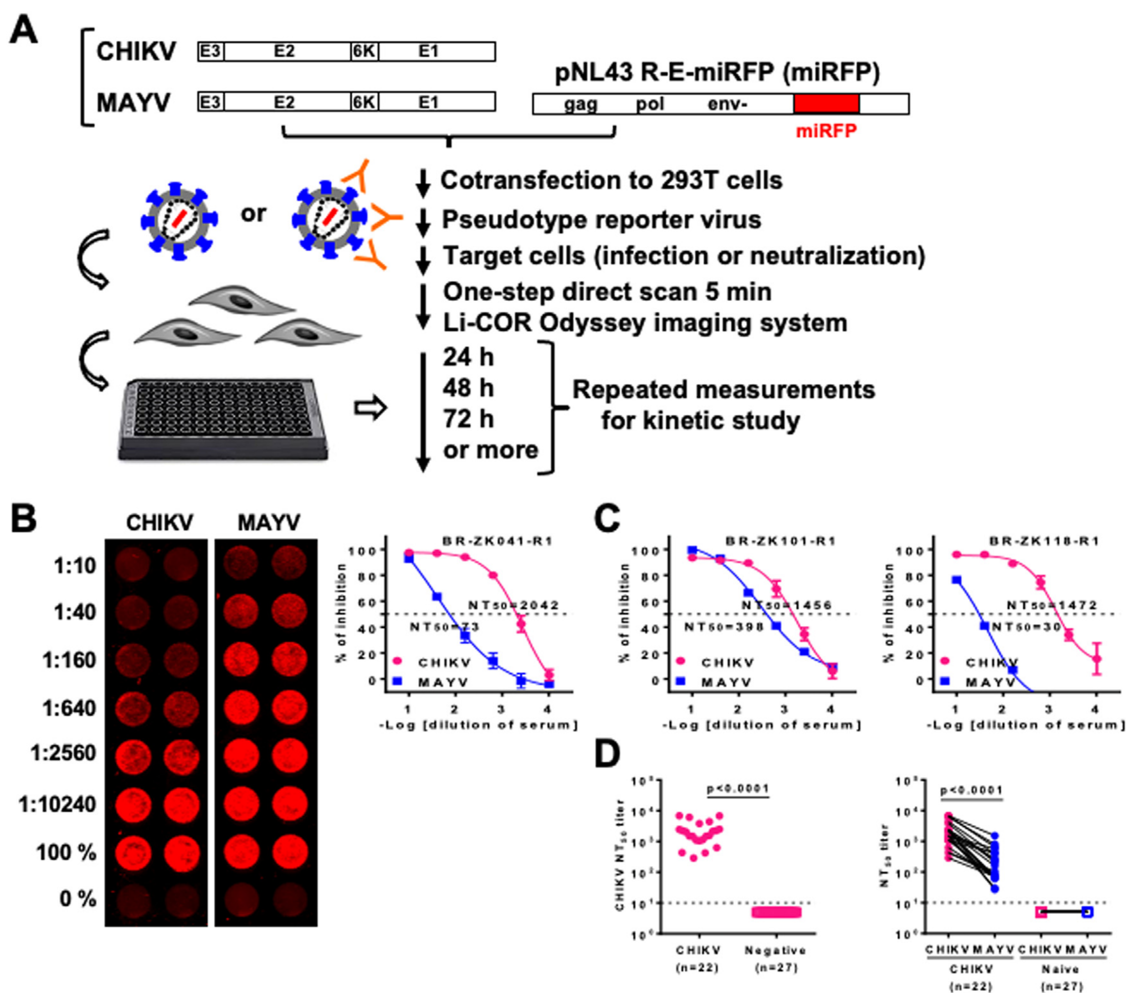

Figure 1. Generation of CHIKV and MAYV pseudoviruses and NT. (a) Schematic drawing of plasmids expressing E2 and E1 proteins (E3-E2-6K-E1 genes) of CHIKV or MAYV and co-transfection with pNL43 R-E-miRFP (miRFP) to generate CHIKV or MAYV pseudoviruses containing miRFP reporter, infection of target cells and one-step imaging. (b) NTs based on CHIKV and MAYV pseudoviruses with miRFP reporter. The miRFP signals (left) and neutralization curves and NT_{50} titers (right) to CHIKV and MAYV pseudoviruses at 72 hours post-infection in Huh7 cells of convalescent-phase serum sample from a reverse transcription-polymerase chain reaction-confirmed CHIKV case from Brazil. (c) Neutralization curves and NT_{50} titers to CHIKV and MAYV pseudoviruses of other two confirmed CHIKV cases. (d) NT_{50} titers to CHIKV and MAYV pseudoviruses of 22 confirmed CHIKV cases and 27 CHIKV-negative individuals. The two-tailed Mann-Whitney (left) and Wilcoxon signed-rank (right) tests (GraphPad 6.0).
CHIKV, chikungunya virus; MAYV, Mayaro virus; miRFP, monomeric infrared fluorescent protein; NT, neutralization test.

and NT_{50} titer of 10 to CHIKV; she gave birth to a baby with multiple congenital anomalies including cleft lip, palate and microcephaly who passed away in 2 days (Figure 3a). Additionally, past flavivirus infection was confirmed by NT. Case OL-ZP-0028 was enrolled at 33 wk with CHIKV IgM+/IgG+ and high NT_{50} titer (of 6657) to CHIKV; due to ZIKV ELISA IgG+, NT was performed and showed NT_{90} titer of 51 to ZIKV and <10 to DENV1-4, suggesting past ZIKV infection (Figure 3d).

We compared birth outcomes for 58 CHIKV rapid test IgM-positive women to 5930 JUTH and OLA delivery records from May 2019 to 2021(Supplementary Table 3). We found potential statistical associations between maternal CHIKV infection during pregnancy and macerated stillbirth ($P = 0.006$), microcephaly ($P < 0.001$), cleft lip/palate ($P = 0.01$), polydactyly ($P = 0.01$), and multiple congenital anomalies ($P < 0.001$) of the infant. We found a significant statistical association between maternal CHIKV infection during pregnancy and any adverse or abnormal gross birth outcome ($P = 0.009$). We also compared birth outcomes for all 15 CHIKV NT confirmed mothers (including asymptomatic) whose babies were observed at birth with the hospital delivery records, and still found significant statistical associations between confirmed infection and any adverse or abnormal gross birth outcome ($P = 0.009$).

Antepartum transmission of chikungunya virus

Of the 26 CHIKV infections among symptomatic pregnant women (13 IgM+IgG– and 13 IgM+IgG+) with babies followed, one was detected in the first trimester, eight second trimester and 17 third trimester (Table 1). We further examined the babies' samples to assess the possibility of antepartum transmission; of the 19 available samples from babies followed, three were CHIKV IgM reactive (all in the third trimester with two normal and one preterm outcomes), suggesting antepartum transmission of CHIKV (Table 1). The time course of three pregnant women with possible antepartum CHIKV infection and IgM/IgG reactivity of their babies were summarized in Figures 3d-f. Case OL-ZP-0028 gave birth to a preterm baby at 36 weeks; the baby at 6 weeks (the first follow-up) showed CHIKV IgM+/IgG+ (Figure 3d). Another past flavivirus infection confirmed by NT was noted in case OL-ZP-0020, who was enrolled at 37 weeks with CHIKV IgM+/IgG+ and high NT_{50} titer to CHIKV; due to ZIKV and DENV ELISAs IgG+, NT to ZIKV and

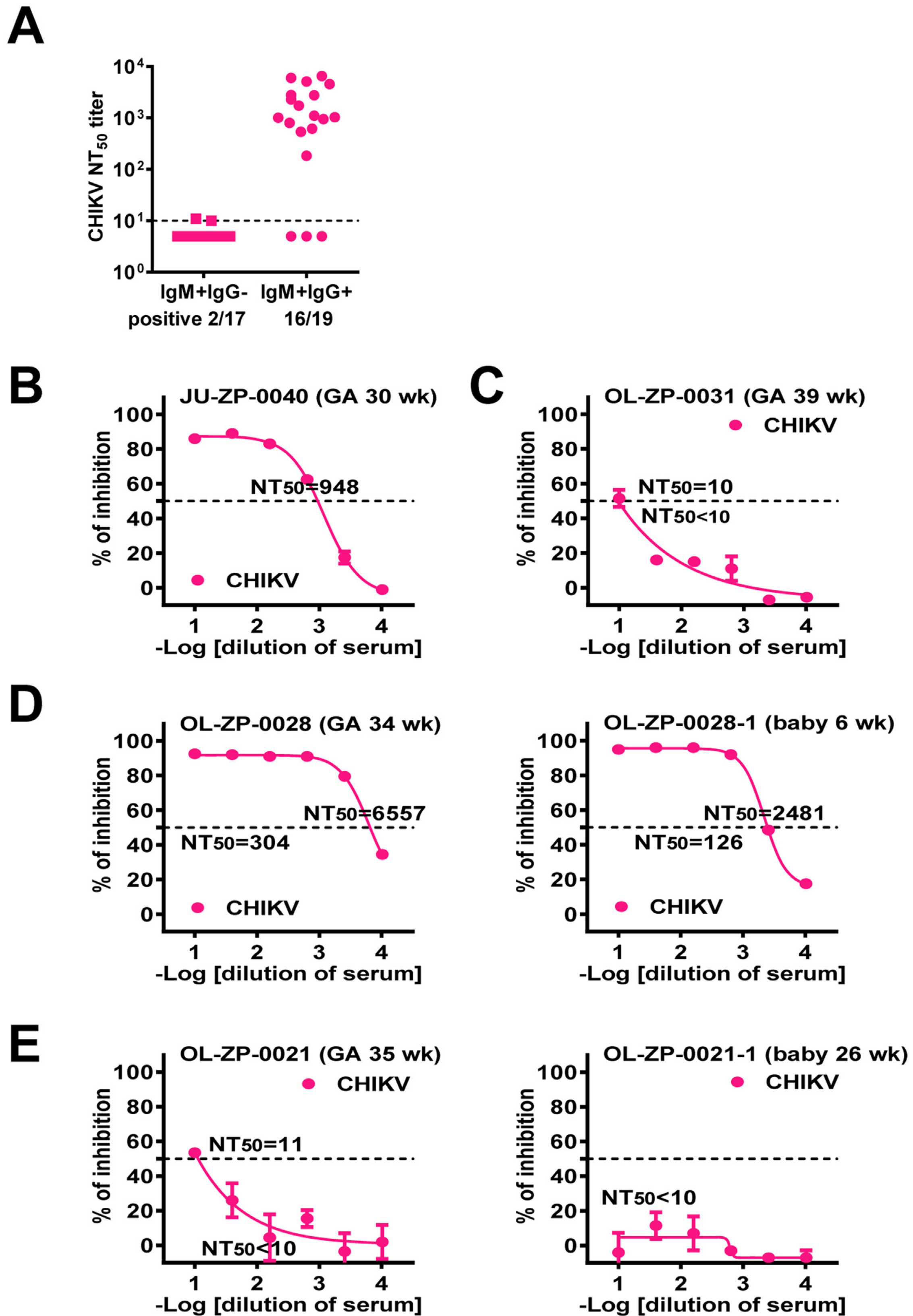

Figure 2. CHIKV pseudoviruses NT in Nigeria pregnant women. (a) CHIKV NT_{50} titers in different subgroups based on CHIKV IgM/IgG results using Chembio DPP® ZCD IgM/IgG rapid test. (b-e) Neutralization curves and NT_{50} titers to CHIKV pseudoviruses of four pregnant women with acute CHIKV infection and abnormal infant outcomes. Data are means and standard deviations of duplicates from one experiment.
CHIKV, chikungunya virus; Ig, immunoglobulin; NT, neutralization test.

Table 1
Serological evidence of CHIKV infection during pregnancy by trimester[a] and infant outcomes.

CHIKV serological tests[b]	Pregnant women	Symptomatic pregnant women	Infants followed up	Infant outcomes	
				Normal	Abnormal
N =	36	30	26	19	7
IgM+IgG−	17	15	13		
NT+	2	2	**2**	0	2
1st trimester				*0*	*0*
2nd trimester				*0*	*1 (c)*
3rd trimester				*0*	*1 (d)*
NT-	15	13	11	8	3
1st trimester				*1*	*0*
2nd trimester				*4*	*0*
3rd trimester				*3*	*3 (e,f,g)*
IgM+IgG+	19	15	13		
NT+	16	12	**10**	8	2
1st trimester				*0*	*0*
2nd trimester				*1*	*0*
3rd trimester				*7*	*2 (h,i)*
NT−	3	3	3	3	0
1st trimester				*0*	*0*
2nd trimester				*2*	*0*
3rd trimester				*1*	*0*

CHIKV, chikungunya virus; Ig, immunoglobulin; NT, neutralization test.
[a] 1st, 2nd, and 3rd trimesters: gestational age ≤12 weeks, >12 weeks to <28 weeks, and ≥28 weeks, respectively. No women in this subset were sampled during intrapartum period: within 2 days prior to or after delivery.
[b] Chembio DPP® ZCD IgM/IgG rapid test was performed to detect CHIKV IgM and IgG, followed by CHIKV pseudovirus NT.Seven Infants with abnormal outcomes are shown in parenthesis:
[c] Polydactyly/sepsis/jaundice (OL-ZP-0021, Figure 3c).
[d] Multiple congenital anomalies (cleft lip/palate/microcephaly) (OL-ZP-0031, Figure 3a).
[e] Stillbirth and preterm (JU-ZP-0016, Figure S1A).
[f] Stillbirth (OL-ZP-0010, Figure S1B).
[g] Multiple congenital anomalies (hypotonia/seizure/microcephaly) (OL-ZP-0042, Figure S1C).
[h] Stillbirth (ZU-ZP-0040, Figure 3b).
[i] Preterm (IgM+/IgG+ infant) (OL-ZP-0028, Figure 3d).The time course of three pregnant women with acute CHIKV infection not confirmed by NT and their infants with abnormal outcomes was summarized in Supplementary Figure S1.

DENV was performed and showed NT_{90} titers of 163 to ZIKV, 39 to DENV2 and <10 to DENV1, 3 and 4, suggesting past ZIKV and DENV infection. She gave birth to a normal baby at 40 weeks; the baby followed up at day 1 showed CHIKV IgM+/IgG+ (Figure 3e).

Discussion

In this study, we examined CHIKV infection during pregnancy in a non-outbreak setting in Nigeria and reported presumed antepartum transmission of CHIKV and association of antepartum CHIKV infection with abnormal infant outcomes.

Based on previous reports from the 2005 Reunion Island outbreak that MTCT of CHIKV occurred predominantly during the intrapartum period, subsequent studies of CHIKV infection during pregnancy primarily focused on the perinatal period and confirmed the contribution of intrapartum CHIKV infection to the MTCT of CHIKV with IOL and Asian genotypes in Asia and Latin America [11–14]. Our finding of presumed CHIKV transmission to three infants out of 19 (15.8%) pregnant women with antepartum CHIKV infection was unexpected compared with the Reunion Island study, in which three abortions out of 678 (0.4%) antepartum CHIKV infections were attributed to in utero CHIKV infection. The higher rates of antepartum CHIKV MTCT might be attributed to pathogenesis of endemic infection and/or CHIKV strain differences. Furthermore, we reported seven abnormal infant outcomes including three stillbirths, two multiple congenital anomalies, one polydactyly with sepsis and jaundice, and one preterm among 26 antepartum CHIKV infections (26.9%). The seven abnormal infant outcomes were a subset of 17 abnormal infant outcomes associated with arbovirus (ZIKV, DENV, and CHIKV) infection during pregnancy, in which birth registry data were compared [15]. Consistent with our findings, another study of the CHIKV outbreak in Colombo, Sri Lanka in 2006-2007 reported CHIKV IgM seropositivity among 6 infants out of 31 (19.4%) tested and born to pregnant women with antepartum CHIKV infection and 10 out of 40 (25.0%) abnormal outcomes including two abortions, one intrauterine death, one preterm, five congenital heart diseases (atrial septal defect, patent ductus arteriosus and persistent foramen ovalis) and one intrauterine growth retardation, after excluding intrapartum infections (postpartum and viremic at delivery) [24].

A recent review of 13 CHIKV outbreaks in Africa from 1999 to 2020 revealed that viral lineage was identified in eight outbreaks; all were associated with ECSA genotype (which the IOL originated from) except one outbreak of West African genotype in Kedougou, Senegal during 2009-2010, in which 14 out of 144 acute sera at five health care facilities were confirmed by CHIKV RT-PCR and six CHIKV IgM+ cases were identified from 1409 sera collected [1–3]. The timing of sample collection after symptom onset differed between studies and may account for the difference in detection by RT-PCR.

After CHIKV infection, individuals develop an IgM response starting from 3 to 8 days post-symptom onset, followed by an IgG response at 7 to 14 days [25]. A recent study reported five distinct antibody patterns (IgM−IgG−/NT−, IgM+IgG−/NT−, IgM+IgG−/NT+, IgM+IgG+/NT− and IgM+IgG+/NT+) during acute febrile phase and the presence of NT or IgG antibody was associated with protection against developing chronic arthritis in the future, which was supported by two studies of outbreaks in India (2010-3 and 2014-6) [23,26]. Although most individuals of the IgM−IgG− and IgM+ IgG− subgroups developed IgG and NT antibodies at the convalescent-phase, this may explain some IgM+IgG− and NT− samples observed in our study.

The proportion of inapparent CHIKV infection has been reported to be 3-25%, corresponding to a symptomatic to inapparent ratio of 1:0.03 to 1:0.33 [1]. Recent studies of the outbreak in Nicaragua reported a higher proportion of inapparent infection

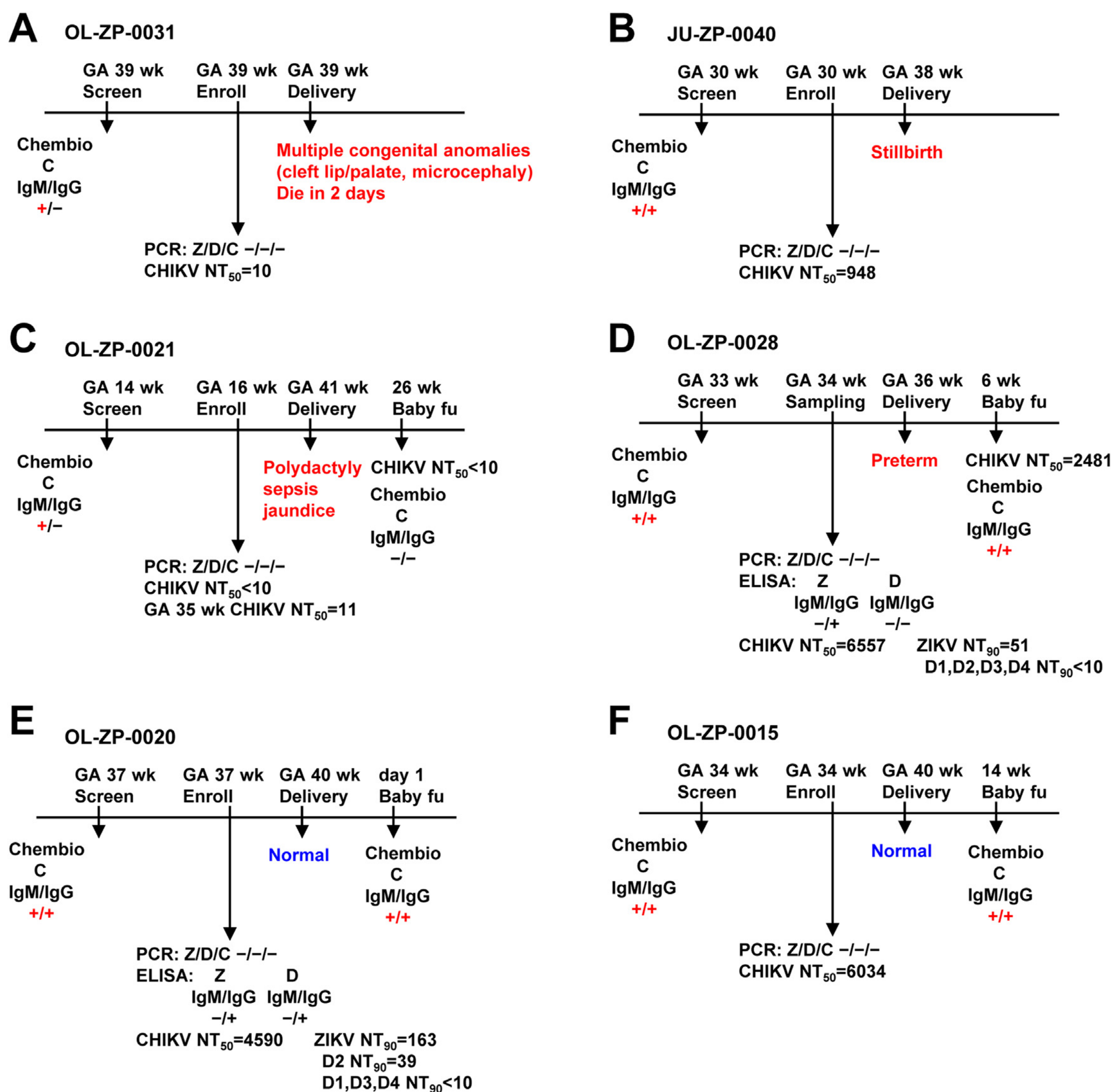

Figure 3. Time course of pregnant women who had acute CHIKV infection confirmed by NT and gave birth to babies with abnormal outcomes or antepartum transmission. (a-d) Time course of four pregnant women with acute CHIKV infection and abnormal infant outcomes. Multiple congenital anomalies with cleft lip/palate and microcephaly (a), stillbirths (b), polydactyly/sepsis/ jaundice (c), and preterm (d). (d, e-f) Time course of three pregnant women with acute CHIKV infection and possible antepartum transmission of CHIKV to fetus.
C, chikungunya virus; D, dengue virus; GA, gestational age; Ig, immunoglobulin; NT, neutralization test; wk, weeks; Z, Zika virus.
CHIKV IgM-reactive infants highlighted in red.

with a symptomatic to inapparent ratio of 1:0.65 to 1:1.20, which varies between lineages and epidemics [27,28]. Our findings of a symptomatic to inapparent ratio of 1:2 is unexpected, which may be related to viral lineage differences, the non-outbreak setting, underreporting of symptoms, and competing febrile illnesses.

Conventional plaque-reduction NT (PRNT) for CHIKV requires a biosafety level III laboratory and is labor intensive and time-consuming. Several NTs based on different CHIKV pseudoviruses, replicon particles or genetically modified infectious clones containing luciferase or green fluorescent protein as reporters have been developed [29,30]. Compared with live virus PRNT, our CHIKV pseudovirus NT can be performed in a biosafety level II laboratory and requires fewer plates (one 96-well plate for six samples in duplicates vs 18 six-well plates for PRNT), less time (72 hours vs >4 days for PRNT), and less sample volume (20 µl vs 100 µl for PRNT in duplicates, starting from serum at 1:10 final dilution). Compared with CHIKV pseudoviruses using luciferase reporter, our CHIKV pseudovirus employed miRFP reporter which can be quantified by one step of direct imaging without multiple laborious steps (5 minutes scan vs ∼60 min for a 96-well plate) (Figure 1). Moreover, the same plate can be quantified multiple times for kinetic study without generating numerous replicates. Compared with GFP reporter, miRFP has minimal autofluorescence and can be quantified by either direct imaging or flow cytometry to determine the percentage of positive cells. Together, these features suggest our CHIKV pseudovirus with miRFP reporter is a simple, practical, and cost-effective tool for neutralization.

There are several limitations to our study. First, this was an observational study with a small sample size of pregnant women with CHIKV IgM+ infection (n = 36); future studies involving larger sample sizes that enable statistical analyses are needed to validate these observations. Second, the higher arbovirus IgM seropositivity in asymptomatic women was unexpected and suggests that our overall seropositivity rates are underestimated given the biased sampling towards symptomatic women. Third, although the babies' samples after delivery or the first follow-up were assessed to identify antepartum transmission, the possibility of neonate CHIKV infection cannot be completely ruled out. For ex-

ample, the first available follow-up sample for the baby of case OL-ZP-0015 was at 14 weeks (Figure 3f); excluding this case resulted in a presumed antepartum transmission rate of 10.5% (2/19), which was still higher than that (0.4%) reported in the Reunion Island study. Peripartum transmission is also a possibility that cannot be ruled out. We were not able to confirm acute maternal CHIKV infection with PCR; given the persistence of CHIKV IgM 13 to 18 months after the acute phase, we chose to focus only on symptomatic CHIKV IgM+ pregnant women to reduce the possibility of pre-conception infection, but advise caution in assessing infant outcome associations. Finally, NT to rule out O'nyong-nyong virus (ONNV), a closely related alphavirus which caused intermittent yet explosive outbreaks in East Africa, was not performed [31]. Despite previous report of the unidirectional antigenic relationships between the two viruses, some sera still cannot be distinguished by NT [32]. Since the ONNV outbreak involving both the East and West Africa in 1959-1962, ONNV has been apparently silent for 35 years until 1996-1997 when another outbreak in East Africa (Uganda and Kenya and Tanzania) took place [31–33]. In Nigeria, only three confirmed-ONNV cases (by virus isolation) were reported in 1966 and 1969, suggesting the possibility of ONNV infection among the participants in Nigeria is remote [31].

CHIKV was first described in Africa, yet subsequent to the Reunion Island outbreak the epidemiology and pathogenesis in the continent has been infrequently studied. Our study provides new and convenient diagnostic tools for future study. In addition, we describe significant asymptomatic infection and abnormal pregnancy and infant outcomes potentially associated with antepartum CHIKV infection. Future studies are needed to further characterize the risk of antepartum CHIKV infection to pregnant women and their infants in Africa.

Declarations of competing interest

The authors have no competing interests to declare.

CRediT authorship contribution statement

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Ethical approval

The study of coded serum or plasma samples was approved by the Institutional Review Boards (IRB) of the Harvard Longwood Campus (IRB# 17-0654), University of Jos (IRB# 127/XIX/5940), and University of Hawaii at Manoa (IRB# 2021-00044 and CHS#17568).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.11.036.

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