Journal of Infection and Public Health 17 (2024) 1050–1056

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: www.elsevier.com/locate/jiph

Possible vertical transmission of Chikungunya virus infection detected in the cord blood samples from a birth cohort in Vietnam

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ARTICLE INFO

Article history:
Received 30 October 2023
Received in revised form 6 April 2024
Accepted 15 April 2024

Keywords:
Chikungunya virus infection
Vertical transmission
Birth Cohort
Vietnam

ABSTRACT

Background: Chikungunya virus (CHIKV) is an alphavirus (genus *Alphavirus*, family *Togaviridae*) that is primarily transmitted to humans by *Aedes* mosquitoes, and can be transmitted from mother to child. Little is known about CHIKV transmission in Vietnam, where dengue is endemic and Aedes mosquitoes are abundant. This study aimed to determine the prevalence and characteristics of vertical CHIKV infection in a birth cohort, and seroprevalence of anti-CHIKV antibodies with or without confirmation by neutralization tests among women bearing children in Vietnam.

Methods: We collected umbilical cord blood plasma samples from each newly delivered baby in Nha Trang, Central Vietnam, between July 2017 and September 2018. Samples were subjected to molecular assay (quantitative real-time RT-PCR) and serological tests (anti-CHIKV IgM capture and IgG indirect enzyme-linked immunosorbent assay, and neutralization tests).

Results: Of the 2012 tested cord blood samples from newly delivered babies, the CHIKV viral genome was detected in 6 (0.3%) samples by RT-PCR, whereas, 15 samples (0.7%) were anti-CHIKV-IgM positive. Overall, 18 (0.9%, 95% CI: 0.6–1.5) samples, including three positives for both CHIKV IgM and viral genome on RT-PCR, were regarded as vertical transmission of CHIKV infection. Of the 2012 cord blood samples, 10 (0.5%, 95% CI: 0.2–0.9) were positive for both anti-CHIKV IgM and IgG. Twenty-nine (1.4%, 95% CI: 1.0–2.1) were seropositive for anti-CHIKV IgG while 26 (1.3%, 95% CI: 0.8–1.9) of them were also positive for neutralizing antibodies, and regarded as seropositive with neutralization against CHIKV infection.

Conclusion: This is the first report of a possible CHIKV maternal-neonatal infection in a birth cohort in Vietnam. The findings indicate that follow-up and a differential diagnosis of CHIKV infection in pregnant women are needed to clarify the potential for CHIKV vertical transmission and its impact in the newborn.

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https://doi.org/10.1016/j.jiph.2024.04.012

Introduction

Chikungunya virus (CHIKV) is an *alphavirus* in the *Togaviridae* family [1]. The virus is classified as an arthropod-borne virus (arbovirus) transmitted primarily by *Aedes aegypti* and *Aedes albopictus* mosquitoes, which are endemic in tropical and subtropical regions [2]. CHIKV was first reported as a human pathogen in 1952 during an outbreak in Tanzania that led to an epidemic of debilitating arthritic disease [3]. The virus re-emerged during an outbreak in Kenya in 2004, leading to its expansion to areas beyond this geographical region [4]. During the outbreak, the virus spread to several islands in the Southwest Indian Ocean, India, Southeast Asia, and temperate regions in Europe and America, however it did not reach the level of Public Health Emergency of International Concern (PEIC) [4]. Within the same period, a major outbreak was reported on the Island of La Réunion, which is in the Western Indian Ocean [5]. Approximately 38.2% of the inhabitants of the island were infected by CHIKV with an attack rate of 34% symptomatic CHIKV clinical infections [6,7]. It was during the 2005–2006 Reunion CHIKV outbreak that the case-fatality rate of this outbreak was, lower than 1% caused by severe atypical CHIKV infection [8]. The first case of mother-to-child transmission was reported during the outbreak on the La Réunion island, and a vertical transmission rate of up to 48.7% was reported [9]. Infected infants were observed to have a broader range of cutaneous manifestations like pigmentation, bullous rash, and blistering [10]. The usual symptoms of chikungunya are fever, rash, and joint pain. Children can have features distinct from adults, such as more frequent dermatological and hemorrhagic manifestations and less frequent rheumatologic manifestations [11]. Neurological manifestations including seizures, encephalopathies, encephalitis, and hemorrhagic manifestations associated with thrombocytopenia and lymphopenia are commonly reported among children with severe CHIKV infection [9,12,13]. Furthermore, a study conducted on the neurodevelopmental outcomes of perinatally CHIKV-infected infants revealed that 51% of the infants had global neurodevelopmental delay [14]. Laboratory diagnosis during the acute stage of CHIKV infection is achieved by either reverse transcription polymerase chain reaction (RT-PCR) or serological assays. The anti-CHIKV IgM antibodies are detectable 1–12 days after onset, while IgG is detected in the samples several weeks later and can persist for years [15]. Most of the vertical transmission of CHIKV were reported from India, Reunion Island [16], Latin America (Brazil, Colombia, Jamaica) [17], and case reports from China, Sri Lanka [18].

Limited information is available on CHIKV transmission in Vietnam. Dengue virus (DENV) is endemic in the region and shares similar vectors (*Aedes* mosquitoes) with CHIKV [19]. CHIKV infection is frequently misdiagnosed as DENV infection because their primary symptoms are indistinguishable [20]. The first report of CHIKV among febrile patients in Southern Vietnam was in 1967 [21]. A retrospective study of febrile patients' samples collected in 2006 in Northern Vietnam revealed a 25% positivity for CHIKV neutralization antibodies [22]. Furthermore, a cross-sectional study conducted on residual serum samples collected in 2015 from inpatients or outpatients confirmed evidence of past CHIKV transmission in Central and Southern Vietnam, with a CHIKV IgG positivity of 13.4% [23].

To date, no vertical transmission of CHIKV has been reported in Vietnam. In Asia, several cases involving vertical transmission of CHIKV have been reported in regions in which the virus circulates [24,25]. Therefore, this study aimed to determine (1) the seropositive proportion and characteristics of vertical transmission of CHIKV at birth among newborn babies in a birth cohort in Vietnam and (2) the seropositive and seropositive with neutralization confirmed of CHIKV among women bearing children in Vietnam using blood samples in a birth cohort.

Material and Methods

Samples and study population

This study retrospectively analysed samples that had been used in a previous birth cohort study [26]. From July 2017 to September 2018, we enrolled newborn babies born to asymptomatic mothers who delivered at Khanh Hoa General Hospital (KHGH), Nha Trang, Vietnam from morning to 4 pm from Monday to Friday. The mothers were residing in the catchment area (16 communes) of Nha Trang city, and were 18 years or older at the time of the delivery of their babies. Among 3223 deliveries at KHGH from women residing in the catchment area during the study period, 2015 met the enrollment criteria and were enrolled in a birth cohort. The others were not enrolled mainly because the delivery occurred at night or weekends when the manpower was not sufficient for the safe collection of samples and information. Excluding three cases with missing samples, a total of 2012 umbilical cord plasma samples were available and analyzed retrospectively for the presence of CHIKV infection by specific serological tests or RT-qPCR in this study.

Women who had spontaneous/induced abortions, stillbirths, multiple pregnancies, or serious complications during their pregnancy were excluded from the study. Blood samples were collected from the umbilical cord immediately after delivery [26]. To take the umbilical cord blood, the umbilical cord was double clamped and cut to separate the baby after delivery. Approximately 10 mL of the cord blood sample was withdrawn from the umbilical vein of the placenta side of the umbilical cord by using a 21-gauge needle and syringe. The samples were collected in EDTA tubes. A total of 2012 umbilical cord plasma samples were frozen and transferred to the Institute of Tropical Medicine, Nagasaki University, to be tested for Zika virus, Rubella virus, and Cytomegalovirus [26,27]. The remaining plasma samples were kept at − 80 ℃ and used for serological and molecular testing for this study.

Viruses and cell lines

CHIKV strain S-27 (the African prototype) belonging to the East-Central-South African (ECSA) divergent clade [28] was used for CHIKV IgG indirect and IgM capture enzyme-linked immunosorbent assay (ELISA) and neutralization testing. The virus was propagated in mosquito cells. Vero cells were used for virus titration and neutralization tests.

Detection of anti-CHIKV IgG antibodies

To screen for anti-CHIKV IgG in the plasma samples, in-house indirect IgG ELISA was performed using purified CHIKV as the assay antigen [29]. Detection of IgG antibodies was performed following the procedure described in previous studies [22,30]. Briefly, 125 ng/100 µL of viral antigen diluted in coating buffer was coated onto 96-well microplates (Nalge Nunc International, Denmark), except for the blank wells. After overnight incubation at 4 °C, the test samples and positive and negative controls were distributed into duplicate wells. Subsequently, horseradish peroxidase (HRP)-conjugated anti-human IgG (American Qualex, USA) was added. Colour development was achieved by adding *o*-phenylenediamine dihydrochloride solution (OPD; Sigma Chemical Co, USA) to each well. After 30 min of incubation at room temperature, the reaction was stopped with 1 N sulfuric acid (H_2SO_4), and the optical density was read at 492 nm using a Synergy H1™ Hybrid Multi-Mode Microplate Reader (BioTek Instruments Inc., USA). The IgG titres of patients' serum samples were determined from the positive standard curve. A sample titre of ≥ 3000 was considered IgG-positive. [30].

Detection of anti-CHIKV IgM antibodies

An in-house IgM capture ELISA system was used to detect the presence of anti-CHIKV IgM, as described previously [22,30]. All wells, except the blank wells, were coated with anti-human IgM goat IgG (Cappel ICN Pharmaceuticals, USA). After overnight incubation at 4 °C, the test samples and positive and negative controls were distributed into duplicate wells. Then, 128 ELISA units of CHIKV assay antigen (prototype strain; S-27) were added to each well and incubated for 1 h at 37 °C. HRP-conjugated anti-CHIKV mouse-derived recombinant E1 monoclonal antibody was added and incubated for 1 h at 37 °C. Colour development and optical density reading were performed as described above. A positive control (or sample) OD_{492}/negative control OD_{492} (P/N) ratio ≥ 2.0 was considered positive. The sensitivity of the in-house anti-CHIKV IgM capture ELISA was 98.3% (95% CI: 90.9–100%), specificity was 88.0% (95% CI: 71.8–96.6%), and accuracy was 94.6%. It was documented in the previous study [30].

Neutralization assay

The IgG- or IgM-positive samples were confirmed with a 50% focus reduction neutralization test ($FRNT_{50}$), as described in previous studies [22,30]. The heat-treated plasma samples were mixed with equal volumes of 40 focus-forming units and incubated at 37 °C with 5% CO_2 for 36 h. After fixation, the cells were blocked and permeabilised as described previously [22]. To detect viral foci, immunostaining was performed as described in a previous study [30]. The endpoint serum dilution that produced a ≥ 50% reduction over the mean number of the control well was considered the FRNT50 titer and IgG- or IgM-positive samples with a neutralizing titer of ≥ 10 were classified as CHIKV infected [31].

Chikungunya virus case classification

Considering the guidelines from WHO (World Health Organization / PAHO (Panamerican Health Organization) [32], the results of a published study [9], and our previous study [22], it was defined as "vertical transmission of CHIKV" if laboratory tests demonstrated at least one of the following: (a) positive real-time reverse transcription PCR (RT-qPCR) for CHIKV, or (b) positivity for CHIKV IgM. If the CHIKV IgG was positive, it was considered seropositive. If a seropositive sample had a neutralization titer of ≥ 10 against CHIKV, it was considered seropositive with neutralization.

Real-time reverse transcription PCR

To detect the viral genome in the samples, RNA was extracted from plasma using a viral RNA Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. Two-step RT-qPCR was performed following the procedure described in the previous study [31]. First, reverse transcription was performed following the manufacturer's instructions using the PrimeScript™ RT reagent kit (Takara, Japan). Then, real-time PCR was performed using SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (Takara, Japan) following the manufacturer's instructions. The cycle threshold value < 40 was considered as CHIKV positive for RT-qPCR. A standard curve for quantification of viral genome levels was produced with complementary DNA at 10-fold serial dilutions from 10^8 to 10^2 genomic copies. Samples with CHIKV IgG titre ≥ 3000 and CHIKV IgM P/N ratio ≥ 2.0 were evaluated with RT-qPCR.

Data analysis

Data analysis and graphical presentation of the figures were performed with GraphPad Prism 9.0.1 (GraphPad Software).

Results

Demographic characteristics

Among 2015 pairs of mothers and babies recruited to the birth cohort study, a total of 2012 umbilical cord plasma samples were available and analysed retrospectively for the presence of CHIKV infection by specific serological tests or RT-qPCR in this study. All the 2012 babies were born in the mother's third trimester of pregnancy (median gestational age of 39.3 weeks, interquartile range 38.6–40.0), except for 11 born at unknown gestational age with birth weight ranging from 2700 to 4700 g. The median age of the mothers at delivery was 28 years, (interquartile range 25–32). Of the 2012 mothers, 101 (5%) reported fever and one reported arthralgia during pregnancy. No cases of rash, lymphadenopathy, or conjunctivitis were reported.

Prevalence of vertical transmission of CHIKV infection

Of the 2012 cord blood samples, 34 (1.7%) were positive for CHIKV viral genome or/and anti-CHIKV IgM and/or IgG. Out of 2012 samples, 15 (0.7%) were anti-CHIKV-IgM positive. The viral genome was detected via RT-qPCR in 6 /34 samples which were positive for anti-CHIKV IgM and/or IgG, with an average of 29,733 copies (Table 1). Overall, 18 of the 2012 newborn babies 0.9% (95% confidence interval (CI): 0.6–1.5) were positive for CHIKV IgM and/or RT-qPCR, regarded as vertical transmission of CHIKV infection. Out of 2012 cord blood samples, 10 (0.5%, 95% CI: 0.2–0.9) were positive for both anti-CHIKV IgM and IgG. No mothers of babies with vertical transmission of CHIKV reported fever, rash, arthralgia, or lymphadenopathy/conjunctivitis during their pregnancy. All of the 18 babies were born without apparent symptoms except for one girl reported to have vomited repeatedly and hospitalised in the paediatric department and recovered well in a couple of days. None of them had congenital Zika, Rubella, or Cytomegalovirus infection together [26,27]. The babies with vertical transmission of CHIKV were born between August and December 2017 and between May and September 2018 in our study population (Fig. 1). The higher rate of vertical transmission was occurred in 2017 than in 2018.

Seropositive with or without neutralization against CHIKV infection

Among the 2012 cord blood samples, 34 (1.7%) samples were positive for anti-CHIKV IgM and/or IgG. Anti-CHIKV IgG was positive in 29 out of the 2012 cord blood samples, the seroprevalence was 1.4%; 0.0%, 0.8%, 1.0%, 1.8%, 3.1% and 5.7% in age group (years) less than 20, 20–24, 25–29, 30–34, 35–39, and 40 or more, respectively (Fig. 2). Among the 2012 cord blood samples, 29 (1.4%, 95% CI: 1.0–2.1) were anti-CHIKV IgG positive and 26 (1.3%, 95% CI: 0.8–1.9) had anti-CHIKV neutralizing antibodies.

Anti-CHIKV neutralizing antibodies were detected in 30 of the 34 samples that were positive for anti-CHIKV IgM and/or IgG (Table 1). The prevalence of mothers positive for anti-CHIKV IgM and/or IgG with its neutralizing antibody titre ≥ 10, regarded as seropositive with neutralization against CHIKV infection which was 1.5% (30/2012). However, four (0.1%) of 2012 cord blood samples were positive for anti-CHIKV IgM and/or IgG with its neutralizing antibody titre < 10, regarded as seropositive without neutralization against CHIKV infection.

Discussion

This is the first report of a possible vertical transmission of CHIKV in a birth cohort in Vietnam. Of the 2012 study participants, 1.7% were positive for anti-CHIKV IgM and/or IgG antibodies while 1.5% of the subjects had anti-CHIKV neutralizing antibodies. The incidence

Table 1
Detection of anti-CHIKV antibodies and viral genome in the cord blood plasma samples.

Sample ID	CHIKV						Gestational age at birth	Birth weight (gram)	Head circumference (cm)	Mother's fever/rash/arthralgia/ lymphadenopathy/conjunctivitis during this pregnancy	Baby's symptom (s)
	IgM (P/N ratio)	IgG Titer	Neutralization Titer	real-time RT-PCR (copies/mL)	Vertical Transmission	(Seropositive, Neutralization)					
BC-1	**2.0**	**13,444**	160	Negative	yes	(+,+)	38w2d	3000	34	No	No
BC-2	**2.2**	**16,547**	320	Negative	yes	(+,+)	40w0d	3700	35.7	No	No
BC-3	**3.1**	490	20	Negative	yes	(+,+)	39w4d	3500	34.5	No	No
BC-4	**2.0**	**25,094**	1280	Negative	yes	(+,+)	38w3d	3000	34	No	No
BC-5	**5.4**	1151	80	Negative	yes	(+,+)	40w6d	3600	35	No	No
BC-6	**5.2**	595	40	Negative	yes	(+,+)	39w5d	2900	33	No	No
BC-7	**2.8**	**17,323**	640	Negative	yes	(+,+)	40w4d	4300	35	No	No
BC-8	**2.1**	**11,782**	160	Negative	yes	(+,+)	40w3d	3500	33	No	No
BC-9	**3.7**	**6712**	80	Negative	yes	(+,+)	40w4d	4000	33.5	No	No
BC-10	**2.3**	**5649**	80	27,300	yes	(+,+)	39w4d	3100	32.4	No	No
BC-11	**2.1**	272	< 10	27,100	yes	(+,-)	39w2d	3800	35.5	No	No
BC-12	0.8	**93,962**	5120	20,500	yes	(+,+)	41w0d	4200	36	No	No
BC-13	1.2	**62,971**	2560	46,900	yes	(+,+)	39w2d	3300	32	No	No
BC-14	1.2	**19,673**	320	18,800	yes	(+,+)	40w3d	3700	33.6	No	Vomiting repeatedly and hospitalized in the pediatric department
BC-15	**2.6**	660	40	37,800	yes	(+,+)	39w5d	3400	33	No	No
BC-16	**2.2**	**3882**	< 10	Negative	yes	(+,-)	38w6d	3200	33	No	No
BC-17	**2.9**	**97,188**	2560	Negative	yes	(+,+)	39w5d	3100	32.4	No	No
BC-18	**3.0**	**28,686**	1280	Negative	yes	(+,+)	39w4d	2700	32	No	No
BC-19	0.9	**14,754**	320	Negative	no	(+,+)	39w6d	2900	32	No	No
BC-20	0.6	**17,023**	320	Negative	no	(+,+)	39w3d	3000	33	No	No
BC-21	0.6	**10,524**	160	Negative	no	(+,+)	39w2d	2800	31.4	No	No
BC-22	1.2	**14,037**	320	Negative	no	(+,+)	39w6d	3200	32.7	Fever at 4 months of pregnancy	No
BC-23	1.0	**36,884**	640	Negative	no	(+,+)	38w2d	4300	35	No	No
BC-24	0.8	**61,954**	1280	Negative	no	(+,+)	40w3d	3500	33	No	No
BC-25	0.9	**3828**	40	Negative	no	(+,+)	38w4d	3000	32	No	No
BC-26	0.9	**3855**	40	Negative	no	(+,+)	40w2d	3500	32.7	No	No
BC-27	1.1	**12,241**	320	Negative	no	(+,+)	38w0d	3400	33.5	No	No
BC-28	0.9	**59,248**	1280	Negative	no	(+,+)	37w5d	3000	31.5	No	No
BC-29	0.8	**7809**	160	Negative	no	(+,+)	39w3d	3900	34	No	No
BC-30	1.2	**63,290**	1280	Negative	no	(+,+)	40w6d	3700	34	No	No
BC-31	1.1	**45,815**	640	Negative	no	(+,+)	39w0d	3500	33.5	No	No
BC-32	1.0	**11,865**	80	Negative	no	(+,+)	40w2d	3500	34	No	No
BC-33	1.3	**3882**	< 10	Negative	no	(+,-)	39w2d	3300	32	Fever at 7 months of pregnancy	No
BC-34	1.0	**3132**	< 10	Negative	no	(+,-)	39w3d	3500	33	No	No

CHIKV: Chikungunya virus.
Vertical transmission: Anti-CHIKV IgM positive or CHIKV real-time PCR positive.
Neutralization Confirmed: Seropositive (Anti-CHIKV IgG positive) with neutralization titer ≥ 10.
*Seropositive, Neutralization (+, +): Anti-CHIKV IgM and/or IgG positive with neutralization titer ≥ 10.
*Seropositive, Neutralization (+, -): Anti-CHIKV IgM and/or IgG positive with neutralization titer < 10.
Bold font indicates IgM positive (P/N ratio≥ 2), IgG positive (≥ 3000 titer) and neutralization value (≥ 10) in Table.

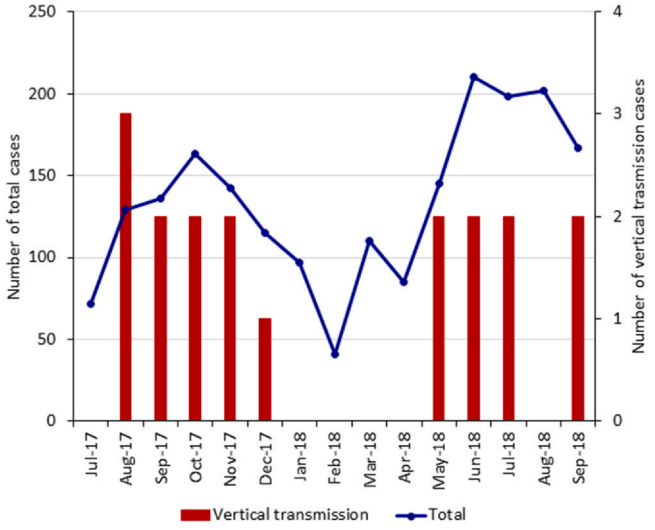

Fig. 1. Monthly number of births of babies born with vertical transmission of CHIKV infection. Line represents total number of cases (births of babies born) and bars represent the number of vertical transmission of CHIKV cases during July 2017 to Sep. 2018.

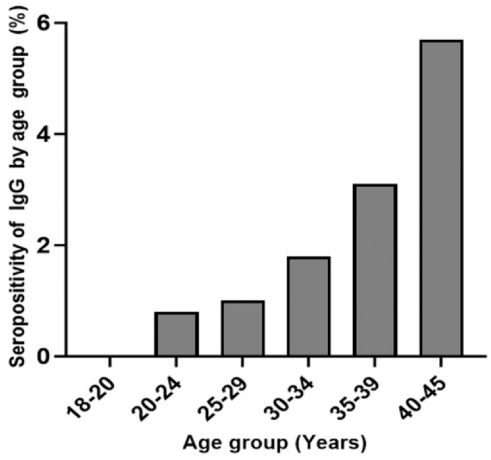

Fig. 2. Seropositivity rate of anti-CHIKV IgG by age group in pregnant women. Each bar graph represents the number of anti-CHIKV IgG positive by total cases in each age group.

of vertical transmission of CHIKV among the general new-born population in this study was determined by the presence of anti-CHIKV IgM or viral genome and it was 0.9%. All babies with mother-to-child transmission were apparently healthy at birth. Theoretically, viremia levels are typically detected by 3–7 days (range 1–12 days) and we assumed that the virus was passed from the mother to the fetus via the placenta. We detected CHIKV viremia in umbilical cord blood after delivery. IgM and IgG Ab against CHIKV are typically detectable 3 - 4 days and 6 - 7 days following onset of symptoms, respectively. Because the fetus makes IgM on their own before birth in utero, this may explain why we detected CHIKV IgM positive in umbilical cord blood. IgM against CHIKV typically remains detectable for 3 to 4 months after infection, whereas IgG against CHIKV is detectable for years. Of the 2012 cord blood samples, 1.4% were seropositive for CHIKV-specific IgG and most of those seropositive were also positive for neutralizing antibodies against CHIKV. CHIKV IgG positive with or without neutralizing antibodies in cord blood sample is sign of an exposure of the mother to the infection and an exposure of the fetus to the transfer of maternal transplacental antibodies.

The prevalence of neonates with the laboratory-confirmed vertical transmission of CHIKV was 0.25% (19 cases) among 7629 babies born during the outbreak on La Reunion island [9]. In an outbreak in the French Caribbean Islands of Martinique and Guadeloupe, prevalence of mother-to-child transmission of CHIKV was 0.16% among 9200 births during 2013–2015 [33]. The prevalence of vertical transmission of CHIKV in this study was 0.9%, which was higher than that reported in previous outbreaks in La Reunion and the French Caribbean Islands. To the best of our knowledge, no studies have reported the rate of vertical transmission among the general population in settings with no apparent outbreaks. The high proportion of CHIKV infection was mostly observed as the maternal ages increased, this supports the endemicity of CHIKV in Vietnam.

The main clinical features at presentation were fever, poor feeding, and pain, which were observed in all infants with vertical transmission (n = 19, 100%) on La Reunion Island. Severe neonatal disease was observed in 10 cases (52.6%) consisting of encephalopathy (n = 9) and hemorrhagic fever (n = 1). All neonates were asymptomatic at birth, and the median age at symptom onset was 4 days of age [9]. In the French Caribbean islands, an active hospital-based surveillance system detected 15 neonates with symptomatic CHIKV infections consecutive to mother-to-child transmission, seven of which were severe, with a mean age of 6 days at symptom onset [33]. In our study, all babies with mother-to-child transmission were apparently healthy at birth and had at least a couple of days of hospitalization. According to the Pediatric Dengue cohort Study in Nicaragua and the systematic review of asymptomatic rates by Carrillo et al. there is a strong inverse correlation between the force of infection (or the attack rate) and the asymptomatic rate regardless of the circulating genotype [34]. There is also a lineage-specific virulence, the Asian genotype being the less virulent, the East Central South African (ECSA) genotype being intermediate, and the ECSA-diverged Indian ocean lineage (IOL) being the most virulent [35]. Though we have no information on the infective genotype in our study, we believe that CHIKV is endemic in Vietnam and the attack rate at the time of our study was very low, as the low positivity of PCR and IgM antibodies in pregnant women and the national seroepidemiological survey by Nguyen et al. suggests [36]. There are three possible explanations for such apparent discrepancies between the previous reports and the present study. First, our study might have detected just transient viremia which did not establish infection. Second, we might have not detected symptoms appearing later because babies stayed at the hospital only for a couple of days after birth. Third, the presumably Asian or IOL circulating CHIKV strains circulating in Vietnam might have been less virulent in the neonate than feared given the low circulation of CHIKV in endemic Vietnam [36].

The CHIKV IgG seropositivity rate among the mothers in this study was 1.4% which is very low and adds credence to the third abovementioned hypothesis on the high asymptomatic rate. Comparatively, a recent study conducted in Nigeria to determine CHIKV infection during pregnancy of mothers in a non-outbreak setting revealed anti-CHIKV IgM seroprevalence of 11.8% (119/1006) and pregnant women who had acute CHIKV infection gave birth to babies with abnormal outcomes with possible antepartum transmission of CHIKV [37]. Previous studies on patients with fever, 25.0% were seropositive and confirmed with neutralization tests in 2006 [22] and 13.4% were seropositive for IgG in 2015 [23] in Vietnam. The low prevalence of CHIKV IgG among mothers in our study might be related to the difference in the target populations: febrile patients in both genders and all age groups in previous studies [22,23] and the cohort of pregnant women in our study. Also, the IgG levels might be different between umbilical cord samples and blood samples from the mothers [38,39]. The CHIKV IgG-positive cases were increased in older age among women in this study, which was consistent with the previous studies which reported that older age in women was associated with a higher chance of exposure [23,40,41].

Previous studies reported that mother-to-child transmission of CHIKV usually occurs secondary to prepartum or intrapartum maternal viremia that reflects a recent CHIKV infection [42,43]. In our

study, a viral genome was detected in cord blood suggestive of congenital infection (symptomatic or asymptomatic). However, to confirm a baby's infection rather than a transient viremia, we need further studies. The limitations of our study are the absence of (1) maternal blood at/before delivery to compare with the baby's blood serologically and virologically, (2) baby's blood in the early neonatal period and beyond 6 months of age for confirming the baby's viremia and persistence of high titre of IgG, (3) the baby's clinical symptoms in the early neonatal period beyond hospitalization, and (4) mid- or long-term follow-up of the babies to see late-onset clinical features. In addition, we reanalysed previous cohort samples in which women who had spontaneous/induced abortions, stillbirths, multiple pregnancies, or serious complications during their pregnancy were excluded in this study. Thus, all these exclusions had an effect on the results of the prevalence of vertical transmission.

Babies suspected of vertical CHIKV infection in our study were born between August and December 2017 and between May and September 2018 coinciding with the dry season (January through September) in Nha Trang when increased circulation of the *A. aegypti* mosquitoes is observed [44]. Although we detected the CHIKV viral genome in our study population, we could not determine the genotype of CHIKV because viral copy numbers were too low in our cord blood samples. It is important to determine which genotype of CHIKV is circulating in Vietnam and whether the strain circulating in Vietnam is less pathogenic than the strain on La Réunion Island [45]. It is also important to characterise the immune status of the general population in Vietnam in the future study.

We have shown the presence of vertical CHIKV infection in Vietnam, an arbovirus endemic area, with an incidence of 0.9% and seroprevalence of neutralizing antibodies against CHIKV with 1.3% in our birth cohort populations. Our study shows that CHIKV infection occurs in a non-negligible number of pregnant women in Vietnam, which might affect babies; however, the burden of CHIKV is still unknown. A major limitation of the study was that we had no detailed clinical and laboratory data on the birth cohort. In addition, we did not follow up on the neonates after discharge from the hospital. Consequently, we can neither conclude that those neonates had 'asymptomatic infection' nor rule out the possibility of 'delayed-onset disease'. Therefore, a systematic clinical and microbiological study should be conducted to determine the prevalence and impact of CHIKV infection in pregnant women and newborn babies in Vietnam.

This is the first report of possible maternal-neonatal CHIKV infection in a birth cohort in Vietnam; therefore, the information will be of great importance in the public health sector. The findings in this study highlight the need for surveillance for CHIKV infection in pregnant women and newborn babies, and clinical follow-up in endemic regions. Furthermore, systemic clinical and microbiological studies should be included in the differential diagnosis for symptomatic neonates in endemic regions.

Funding statement

This research work was supported by AMED (Japan Agency of Medical Research and Development) under Grant Number: JP223fa627004 and JP23fk0108656, and Japanese Society of Promotion and Science (JSPS) KAKENHI grant number 24K10246.

Ethical statement

Ethical approvals for this study were obtained from the Ethics Committees of the Institute of Tropical Medicine, Japan (160908158), and the National Institute of Hygiene and Epidemiology, Vietnam (IRB-VN01057–30/2015). Before sample collection, written informed consent was obtained from pregnant women participating in the first group and mothers of the neonates in the second group.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to thank the pregnant women who participated in this study and the staff in Vietnam who supported the study. The authors are grateful to Corazon C. Buerano for the valuable scientific suggestions and for proofreading the article.

References

[1] Powers AM, Logue CH. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. J Gen Virol 2007;88:2363–77.
[2] Burt FJ, Rolph MS, Rulli NE, Mahalingam S, Heise MT. Chikungunya: a re-emerging virus. Lancet 2012;379:662–71.
[3] Robinson MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. I. Clinical features. Trans R Soc Trop Med Hyg 1955;49:28–32.
[4] Silva LA, Dermody TS. Chikungunya virus: epidemiology, replication, disease mechanisms, and prospective intervention strategies. J Clin Invest 2017;127:737–49.
[5] Bessaud M, Peyrefitte CN, Pastorino BA, Tock F, Merle O, et al. Chikungunya virus strains, Reunion Island outbreak. Emerg Infect Dis 2006;12:1604–6.
[6] Gérardin P, Guernier V, Perrau J, Fianu A, Le Roux K, et al. Estimating Chikungunya prevalence in La Réunion Island outbreak by serosurveys: Two methods for two critical times of the epidemic. BMC Infect Dis 2008;8:99.
[7] Renault P, Solet JL, Sissoko D, Balleydier E, Larrieu S, et al. A major epidemic of chikungunya virus infection on Reunion Island, France, 2005-2006. Am J Trop Med Hyg 2007;77:727–31.
[8] Economopoulou A, Dominguez M, Helynck B, Sissoko D, Wichmann O, et al. Atypical Chikungunya virus infections: clinical manifestations, mortality and risk factors for severe disease during the 2005–2006 outbreak on Réunion. Epidemiol Infect 2009;137:534–41.
[9] Gérardin P, Barau G, Michault A, Bintner M, Randrianaivo H, et al. Multidisciplinary prospective study of mother-to-child chikungunya virus infections on the island of La Réunion. PLoS Med 2008;5:e60.
[10] van Keulen V, Huibers M, Manshande M, van Hensbroek MB, van Rooij L. Chikungunya virus infections among infants-who classification not applicable. Pedia Infect Dis J 2018;37:e83–6.
[11] Ritz N, Hufnagel M, Gérardin P. Chikungunya in Children. Pedia Infect Dis J 2015;34:789–91.
[12] Nyamwaya DK, Thumbi SM, Bejon P, Warimwe GM, Mokaya J. The global burden of Chikungunya fever among children: A systematic literature review and meta-analysis. PLOS Glob Public Health 2022;2:e0000914.
[13] Mehta R, Gerardin P, de Brito CAA, et al. The neurological complications of chikungunya virus: A systematic review. Rev Med Virol 2018;28:e1978.
[14] Gérardin P, Sampériz S, Ramful D, Boumahni B, Bintner M, et al. Neurocognitive outcome of children exposed to perinatal mother-to-child Chikungunya virus infection: the CHIMERE cohort study on Reunion Island. PLoS Negl Trop Dis 2014;8:e2996.
[15] Gopakumar H, Ramachandran S. Congenital chikungunya. J Clin Neonatol 2012;1:155–6.
[16] Ferreira F, da Silva ASV, Recht J, Guaraldo L, Moreira MEL, et al. Vertical transmission of chikungunya virus: A systematic review. PLoS One 2021;16:e0249166.
[17] Torres JR, Falleiros-Arlant LH, Dueñas L, Pleitez-Navarrete J, Salgado DM, et al. Congenital and perinatal complications of chikungunya fever: a Latin American experience. Int J Infect Dis 2016;51:85–8.
[18] Contopoulos-Ioannidis D, Newman-Lindsay S, Chow C, LaBeaud AD. Mother-to-child transmission of Chikungunya virus: A systematic review and meta-analysis. PLoS Negl Trop Dis 2018;12:e0006510.
[19] Moi ML, Nguyen TTT, Nguyen CT, Vu TBH, Tun MMN, et al. Zika virus infection and microcephaly in Vietnam. Lancet Infect Dis 2017;17:805–6.
[20] Chang SF, Su CL, Shu PY, Yang CF, Liao TL, et al. Concurrent isolation of chikungunya virus and dengue virus from a patient with coinfection resulting from a trip to Singapore. J Clin Microbiol 2010;48:4586–9.
[21] Deller J.J., Jr., Russell P.K. An analysis of fevers of unknown origin in American soldiers in Vietnam. Ann Intern Med 1967;66:1129–1143.
[22] Ngwe Tun MM, Inoue S, Thant KZ, Talemaitoga N, Aryati A, et al. Retrospective seroepidemiological study of chikungunya infection in South Asia, Southeast Asia and the Pacific region. Epidemiol Infect 2016;144:2268–75.
[23] Quan TM, Phuong HT, Vy NHT, Thanh NTL, Lien NTN, et al. Evidence of previous but not current transmission of chikungunya virus in southern and central Vietnam: Results from a systematic review and a seroprevalence study in four locations. PLoS Negl Trop Dis 2018;12:e0006246.
[24] Watanaveeradej V, Endy TP, Simasathien S, Kerdpanich A, Polprasert N, et al. The study transplacental chikungunya virus antibody kinetics, Thailand. Emerg Infect Dis 2006;12:1770–2.
[25] Yin X, Hu TS, Zhang H, Liu Y, Zhou Z, et al. Emergent chikungunya fever and vertical transmission in Yunnan Province, China, 2019. Arch Virol 2021;166:1455–62.

[26] Ngwe Tun MM, Moriuchi M, Toizumi M, Luvai E, Raini S, et al. Congenital Zika Virus Infection in a Birth Cohort in Vietnam, 2017-2018. Am J Trop Med Hyg 2020;103:2059–64.
[27] Toizumi M, Tanaka S, Moriuchi M, Nguyen HT, Takegata M, et al. Rubella seroprevalence among mothers and incidence of congenital rubella three years after rubella vaccine introduction in Vietnam. Hum Vaccin Immunother 2021;17:3156–61.
[28] Khan AH, Morita K, Parquet MDC, Hasebe F, Mathenge EGM, et al. Complete nucleotide sequence of chikungunya virus and evidence for an internal polyadenylation site. J Gen Virol 2002;83:3075–84.
[29] Inoue S, Morita K, Matias RR, Tuplano JV, Resuello RR, et al. Distribution of three arbovirus antibodies among monkeys (Macaca fascicularis) in the Philippines. J Med Prima 2003;32:89–94.
[30] Luvai EAC, Kyaw AK, Sabin NS, Yu F, Hmone SW, et al. Evidence of Chikungunya virus seroprevalence in Myanmar among dengue-suspected patients and healthy volunteers in 2013, 2015, and 2018. PLoS Negl Trop Dis 2021;15:e0009961.
[31] Ngwe Tun MM, Kyaw AK, Nabeshima T, Dumre SP, Soe AM, et al. Coinfection and circulation of chikungunya virus and dengue virus in pediatric patients in Myanmar, 2019. Microbes Infect 2023;25:105129.
[32] Chikungunya: case definitions for acute, atypical and chronic cases. Conclusions of an expert consultation, Managua, Nicaragua, 20-21 May 2015 Wkly Epidemiol Rec 90 2015 410 414.
[33] Dorléans F, Hoen B, Najioullah F, Herrmann-Storck C, Schepers KM, et al. Outbreak of Chikungunya in the French Caribbean Islands of Martinique and Guadeloupe: Findings from a Hospital-Based Surveillance System (2013-2015). Am J Trop Med Hyg 2018;98:1819–25.
[34] Bustos Carrillo F, Gordon A, Harris E. Reply to Gérardin et al. Clin Infect Dis 2019;68:172–4.
[35] Bustos Carrillo F, Collado D, Sanchez N, Ojeda S, Lopez Mercado B, et al. Epidemiological evidence for lineage-specific differences in the risk of inapparent chikungunya virus infection. J Virol 2019;93.
[36] Nguyen TV, Ngwe Tun MM, Cao MT, Dao HM, Luong CQ, et al. Serological and Molecular Epidemiology of Chikungunya Virus Infection in Vietnam, 2017–2019. Viruses 2023;15(10):2065.
[37] Sagay AS, Hsieh SC, Dai YC, Chang CA, Ogwuche J, et al. Chikungunya virus antepartum transmission and abnormal infant outcomes in a cohort of pregnant women in Nigeria. Int J Infect Dis 2024;139:92–100.
[38] Albrecht M, Arck PC. Vertically Transferred Immunity in Neonates: Mothers, Mechanisms and Mediators. Front Immunol 2020;11:555.
[39] Malek A, Sager R, Kuhn P, Nicolaides KH, Schneider H. Evolution of maternofetal transport of immunoglobulins during human pregnancy. Am J Reprod Immunol 1996;36:248–55.
[40] Laoprasopwattana K, Suntharasaj T, Petmanee P, Suddeaugrai O, Geater A. Chikungunya and dengue virus infections during pregnancy: seroprevalence, seroincidence and maternal-fetal transmission, southern Thailand, 2009-2010. Epidemiol Infect 2016;144:381–8.
[41] Patil HP, Gosavi M, Mishra AC, Arankalle VA. Age-dependent evaluation of immunoglobulin G response after chikungunya virus infection. Am J Trop Med Hyg 2021;104:1438–43.
[42] Foeller ME, Nosrat C, Krystosik A, Noel T, Gérardin P, et al. Chikungunya infection in pregnancy - reassuring maternal and perinatal outcomes: a retrospective observational study. Bjog 2021;128:1077–86.
[43] Duarte AO, Oliveira JV, Carvalho TCX, Pessoa LB, Filho CM, et al. Maternal and congenital infections arising from Zika, dengue and Chikungunya arboviruses in Salvador, Brazil. Trans R Soc Trop Med Hyg 2020;114:222–5.
[44] Higa Y, Yen NT, Kawada H, Son TH, Hoa NT, et al. Geographic distribution of Aedes aegypti and Aedes albopictus collected from used tires in Vietnam. J Am Mosq Control Assoc 2010;26:1–9.
[45] D'Ortenzio E, Grandadam M, Balleydier E, Jaffar-Bandjee MC, Michault A, et al. A226V strains of Chikungunya virus, Réunion Island, 2010. Emerg Infect Dis 2011;17:309–11.