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Silent circulation of Chikungunya virus among pregnant women and newborns in the Western Brazilian Amazon before the first outbreak of chikungunya fever

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ABSTRACT

The prevalence of immunity to Chikungunya virus (CHIKV) in pregnant women and newborns in the Western Brazilian Amazon was assessed at a time when previous studies did not report chikungunya fever in the area. In 435 asymptomatic pregnant women and 642 healthy unrelated newborns, the presence of IgM and IgG antibodies to CHIKV were determined by a commercial ELISA. All participants were negative to IgM anti-CHIKV. Anti-CHIKV IgG was identified in 41 (9.4%) pregnant women and 66 (10.3%) newborns. The presence of anti-CHIKV IgG was positively associated with the lowest socioeconomic status in pregnant women (OR 2.54, 95% CI 1.15-5.62, p=0.021) and in the newborns' mothers (OR 5.10, 95% CI 2.15-12.09, p< 0.001). Anti-CHIKV IgG was also associated with maternal age in both, the pregnant women (OR 1.06, 95% CI 1.00-1.11, p=0.037) and the newborns'mothers (OR 1.08, 95% CI 1.03-1.12, p=0.001). Pregnancy outcomes in which the mother or the newborn was anti-CHIKV IgG positive proceeded normally. Negative CHIKV serology was associated with being positive for DENV antibodies and having had malaria during pregnancy. These findings showed that there was already a silent circulation of CHIKV in this Amazon region before the first outbreak of chikungunya fever. Furthermore, seropositivity for CHIKV was surprisingly frequent (10%) in both, pregnant women and newborns, affecting mainly low-income women.

KEYWORDS: CHIKV. Seroprevalence. Asymptomatic infection. IgG antibodies. Pregnancy. Neonates. Amazon region.

INTRODUCTION

Chikungunya fever is a mosquito-borne illness transmitted by the vectors *Aedes aegypti* and *Aedes albopictus*. The disease is caused by chikungunya virus (CHIKV), an RNA virus belonging to the togaviridae family and the genus alphavirus. After an incubation period of 2-10 days, the acute phase of the disease lasts 7-14 days with the sudden onset of fever in association with arthralgia and arthritis. The subacute phase lasts up to three months and the chronic phase can persist for more than three additional months[1]. However, many infected individuals are asymptomatic. Anti-CHIKV IgM antibodies are detectable from day 3 after the onset of symptoms, reaching 80% positivity after the first week. IgG anti-CHIKV antibodies are also detected from the first week of infection, usually one or two days after the onset of IgM antibodies. Elevated IgG antibody levels are detectable between 3-5 weeks and can persist for years[2].

In September 2014, the first autochthonous case of chikungunya fever was reported in Northern Brazil (Amapa State) and one week later, new cases appeared in the Northeastern region (Bahia State). Since then, the disease became endemic in every State of Brazil. In 2019, the country recorded 132,205 probable cases, corresponding to an incidence rate of 62.9 cases/100,000 inhabitants. The Southeast region had the highest number of cases (69.9%), followed by the Northeast (25.6%), North (3.2%), Midwest (0.8%) and 0.3% in the South region[3].

Considering other arboviruses, the first cases of Dengue virus (DENV) fever in Acre State were reported in 1995 in the city of Rio Branco, the State capital, but they were all imported. In 2000, 2,110 cases of DENV were reported in Acre State, according to the Brazilian Ministry of Health[4], with a subsequent series of epidemics in the years 2004, 2005, 2009 and 2014. According to the Brazilian Ministry of Health, the number of probable cases of DENV in Acre State were 5,253 in 2015 and 2,258 in 2016, with an incidence rate of 653.8 and 281 per 100,000 inhabitants, respectively[5].

Based on data from the Brazilian Ministry of Health, the first case of chikungunya fever was reported in Acre State in 2015, and the number of probable cases was only 30 in 2015 and 345 in 2016, with incidence rates of 3.7 and 42.9 per 100,000 inhabitants, respectively[5]. These 2015 and 2016 chikungunya cases were reported mostly in the State capital and neighboring cities, all located at the Eastern end of the State.

In the Alto Jurua region, located in the Northwest of Acre State where the municipality of Cruzeiro do Sul is located, no cases of Chikungunya fever were reported until 2014. In contrast, the city has been reporting autochthonous cases of dengue. In the year 2014, Cruzeiro do Sul was among the cities with the highest number of dengue cases in the country (23,130 cases/100,000). The Brazilian Ministry of Health in 2015/2016 reported the presence of *Aedes aegypti* in the city of Cruzeiro do Sul based on the LI/LIRAa stands for household larval survey (LI) and the Rapid Assessment of Infestation by (LIRAa), and the result was 3.1, meaning that there was a dengue (arbovirus) outbreak in the period[5,6].

In the present study, we investigated if CHIKV was circulating in an area where arboviruses vectors were abundant[6-8], but at a time (2015 and 2016) in which arbovirus outbreaks were not occurring. To this end, seropositivity to CHIKV was assessed in pregnant women and unrelated newborns prior to the first report of CHIKV fever by local and governmental public health authorities.

MATERIALS AND METHODS

Study site and study population

This study is part of a prospective population-based birth cohort investigation, the Maternal and Child Health and Nutrition in Acre, Brazil (MINA-Brazil). It is the first cohort study of mothers and newborns in the Brazilian Amazon, initiated in the city of Cruzeiro do Sul in July 2015, until June 2016. Other findings of the MINA-Brazil study have already been reported[7].

Cruzeiro do Sul is the second most populated city in Acre, it is located at the West end of the State (07º37' S and 72º40'W), bordering the Amazonas State and the country of Peru. It is more than 600 km far from the State capital Rio Branco, located at the Eastern end of the State. The weather in Cruzeiro do Sul is equatorial, warm and humid, with an average annual temperature around 25 °C, and a rainfall index exceeding 2,000 mm/year, thus presenting ideal environmental conditions for the reproduction of mosquitoes. Cruzeiro do Sul has approximately 82,000 inhabitants, 70.5% of whom live in the urban area[7,8]. In 2010, the Human Development Index of Cruzeiro do Sul was 0.663 (medium), and only 12.7% of the households had access to proper sanitation facilities[8]. More than 95% of the total births are delivered at the only Maternity Hospital of Cruzeiro do Sul, the place in which this study took place.

Cruzeiro do Sul is a hotspot of malaria in the Western Brazilian Amazon, still reporting a high rate of malaria transmission, and harboring the largest focus of *Plasmodium falciparum* malaria in the country[9,10]. According to the Malaria Epidemiological Surveillance and Information System (SIVEP) database from the Brazilian Ministry of Health, the Acre State reported 11,454 cases of malaria in 2014; 10,875 cases in 2015 and 10,735 cases in 2016[11]. These years comprise the period in which the present study was performed. Pregnant women up to 20 weeks of gestation, living in the urban area of Cruzeiro do Sul, were recruited from July 2015 to June 2016 while booking an appointment for the antenatal care at one of the 13 primary health care units, covering the entire urban area of Cruzeiro do Sul. They were eligible for the study if they intended to give birth at the maternity hospital in Cruzeiro do Sul. Upon acceptance, a home visit was scheduled to obtain informed written consent and to collect socioeconomic and health information. Medical assessments were scheduled in the second and third trimesters of pregnancy to collect clinical data and blood samples[7]. In addition to clinical data information, data on previous episodes of malaria and malaria during pregnancy were retrospectively obtained

from the Epidemiological Surveillance System (SIVEP)[11]. In the present study, the number of samples tested was 1,077.

Ethical issues

Written informed consent for participation was obtained from mothers before the enrollment. For adolescent mothers, consent was given by the participant and their caretakers. This study was approved by the ethical review board of the School of Public Health, University of Sao Paulo, Brazil (protocol Nº 872.613, November 13th, 2014).

Serum Samples

Blood samples from 435 pregnant women were collected during medical assessments in the second and third trimesters of pregnancy. Then, samples underwent processing to obtain serum samples. We initially analyzed maternal serum samples from the third trimester, and tested the corresponding second trimester samples only if the initial serological results were inconclusive. Regarding the newborns, 642 blood samples were collected from the placenta (umbilical cord) immediately after delivery and processed to obtain serum samples, as previously described[7]

Detection of anti-CHIKV IgG and IgM

The presence of anti-CHIKV IgG and IgM antibodies were assessed in serum samples by ELISA (Euroimmun, Lübeck, Germany), according to the manufacturer's instructions. Results were expressed as index values calculated by dividing the optical density (OD) of the samples by the OD of a kit-supplied calibrator serum included in the same run. Serum samples were considered IgG or IgM positive if the ratio was ≥1.1, borderline (inconclusive) if the ratio was ≥ 0.8 and <1.1, and negative if the ratio was <0.8[12].

Detection of anti-DENV IgG

The presence of anti-DENV IgG was assessed by ELISA, using as antigen the recombinant NS1 (rNS1) and E (rE) proteins of dengue 2 (HQ026763, DENV-2 strain/BR0690/RJ/2008), as previously described[13]. Cutoff values were calculated as the mean OD of negative controls plus three standard deviations (SDs). Serum samples from pregnant women with an average OD above the cutoff value were considered positive.

Statistical analysis

The wealth index was used as a proxy for the socioeconomic status of the participant's family and calculated through the principal component analysis of assets owned by the family[14]. We used the first component (which explained 18.9% of the variation between the households) to generate this index and then we categorized it into tertiles.

The chi-square or Fisher's exact test were used for comparisons of maternal and neonatal characteristics according to the results of anti-CHIKV IgG antibodies. Multiple logistic regression models were performed considering anti-CHIKV IgG antibodies as the dependent variable. Independent variables were initially selected when they were associated with the dependent variable at $P \leq 0.10$. Afterwards, covariates associated with the outcome at $P < 0.05$ remained in the final multiple regression model. Missing data were included in the multiple regression models by creating missing-value categories. All statistical analyses were performed with the SPSS v.25 software (IBM, New York, USA).

RESULTS

Characteristics of the pregnant women in relation to CHIKV IgG antibody status are described in Table 1. None of the 435 serum samples from women in the third trimester were IgM-positive, while 41 (9.4%) were positive for anti-CHIKV IgG. IgG-positivity was associated with age ≥ 35 years old (p = 0.012), having the lowest wealth index (p = 0.020), being a beneficiary of Bolsa Familia (p = 0.013), a government aid for the neediest families, and being positive for anti-DENV IgG (0.029). In the multiple adjusted model, maternal age remained associated with being positive for anti-CHIKV IgG antibodies (OR 1.06, 95% CI: 1.00-1.11, $p = 0.037$), as did belonging to the lowest tertile of the wealth index (OR 2.54, 95% CI: 1.15 – 5.62, $p = 0.021$).

Characteristics of the newborns' mothers in relation to the newborns' CHIKV IgG antibody status are described in Table 2. There were no IgM-positive anti-CHIKV serum samples, while 66 (10.3%) were anti-CHIKV IgG positive. IgG positivity was associated with maternal age ≥ 35 (p = 0.001), being non-white (p = 0.001), having had > 1 pregnancy (p = 0.002), being in the lowest economic group (p < 0.001) and receiving the governmental aid (p = 0.002). In terms of pregnancy outcome, a higher percentage of IgG-positive women had a vaginal delivery (p = 0.026) and a newborn with a higher birth weight (p = 0.031). In addition, malaria during pregnancy was

Table 1 - Sociodemographic characteristics of pregnant women in relation to the presence or absence of anti-CHIKV IgG antibodies.

Sociodemographic characteristics		Anti - CHIKV IgG		
		Positive N= 41 (%)	Negative N= 394 (%)	*p value*
Age (years)	≤ 19	6 (14.6)	74 (18.8)	**0.012**
	20 to 34	26 (63.4)	289 (73.3)	
	≥ 35	9 (22.0)	31 (7.9)	
Self-declared ethnicity	White	5 (12.2)	57 (14.5)	0.692
	Non-white	36 (87.8)	337 (85.5)	
1st pregnancy	yes	15 (36.6)	177 (45.0)	0.330
	no	26 (63.4)	217 (55.1)	
Number of antenatal care visits	<6	6 (15.0)	52 (13.2)	0.808
	≥ 6	34 (83.0)	335 (85.0)	
Wealth index (tertiles)	1 (poorest)	17 (41.5)	87 (22.1)	**0.020**
	2	10 (24.4)	144 (36.5)	
	3	14 (34.1)	163 (41.4)	
	no	35 (85.4)	335 (85.0)	
Job	paid	19 (46.3)	132 (33.5)	0.072
	unpaid	22 (53.7)	262 (66.5)	
Beneficiary of Bolsa Familia	yes	23 (56.1)	143 (36.3)	**0.013**
	no	18 (43.9)	251 (63.7)	
Dengue Serology (IgG)	Positive	6	20	**0.029**
	Negative	34	357	

Totals differ due to missing values for some independent variables.

reported in 74.2% of the women with anti-CHIKV IgG, as opposed to 55.0% of the antibody negative women ($p < 0.001$). In the multiple adjusted model, maternal age (OR 1.08, 95% CI: 1.03-1.12, $p = 0.001$) and wealth index (OR 5.10, 95% CI: 2.15 – 12.09, $p = < 0.001$) remained associated with the presence of anti-CHIKV IgG antibodies. Regarding the IgG-positive newborns, 55% were male, only four were delivered preterm and 98.5% were appropriate for the gestational age according to the Intergrowth 21st birth weight charts[15]. All newborns, including those born preterm, had Apgar scores ≥ 7 in the 5th minute of life. Information on the maternal DENV serology was not available for these newborns.

DISCUSSION

The analysis of serum samples from 435 pregnant women and 642 healthy unrelated newborns revealed that none of the pregnancies was associated with an acute CHIKV infection (all were negative to CHIKV IgM). However, 9.4% and 10%were positive for CHIKV IgG, respectively, indicating a prior exposure to the virus. The association of being IgG positive in both groups of subjects with low economic status and with being non-white suggests that living in a crowded environment with poor nutrition and/or sanitation likely contributes to an increased susceptibility to this infection[5,16]. The association of CHIKV IgG with maternal age suggests that the older the woman, the more she will be exposed to the virus. The associations between not having IgG antibodies to CHIKV, being seropositive for antibodies to DENV and having reported gestational malaria were not expected, as the same low socioeconomic conditions should similarly increase the susceptibility to these three infections[17]. However, there are some studies in the literature on a possible "cross-protection" provided by an arbovirus infection in relation to the following one[18-20], but so far, no pathophysiological mechanisms has been proposed.

The association of seropositivity to CHIKV with low socioeconomic status are similar to findings from previous reported studies[10,21,22]. It is noteworthy that the frequency of CHIKV infections detected in this study by our serological analysis is much higher than that reported by the government surveillance system. They reported only 30 suspected cases of CHIKV fever in 2015 and 345 cases in 2016 in Acre State, with low incidence rates per 100,000 inhabitants, of 3.7 and

Table 2 - Sociodemographic characteristics of the 642 newborns and their mothers according to the presence or absence of anti-CHIKV IgG antibodies in the newborns.

Characteristics		Anti – CHIKV IgG		
		Positive N= 66 (%)	Negative N= 576 (%)	*p* value
Maternal age (years)	≤ 19	14 (21.3)	129 (22.4)	**0.001**
	20 to 34	37 (56.0)	398 (69.1)	
	≥ 35	15 (22.7)	49 (8.5)	
Self-declared ethnicity	White	2 (3.0)	66 (11.5)	**0.001**
	Non-white	53 (80.3)	435 (75.5)	
1st pregnancy	yes	13 (19.7)	233 (40.5)	**0.002**
	no	44 (66.7)	311 (54.0)	
Number of antenatal care visits	< 6	23 (35.0)	141 (24.5)	0.060
	≥ 6	41 (62.0)	422 (73.3)	
Wealth index (tertiles)	1 (poorest)	35 (53.0)	168 (29.2)	**< 0.001**
	2	15 (22.7)	182 (31.6)	
	3	7 (10.6)	194 (33.7)	
	no	49 (74.2)	463 (80.4)	
Job	Paid	12 (18.1)	150 (26.0)	0.291
	Not-paid	45 (68.2)	394 (68.4)	
Beneficiary of Bolsa Familia	yes	35 (53.0)	217 (37.7)	**0.002**
	no	22 (33.3)	327 (56.8)	
Type of Delivery	Vaginal	52 (78.8)	375 (65.1)	**0.026**
	Cesarean	14 (21.2)	201 (34.9)	
Newborn sex	Female	30 (45)	287 (49.8)	0.468
	Male	36 (55)	285 (49.5)	
Birth weight (grams)	≤ 2,499	1 (1.5)	31 (5.4)	**0.031**
	2,500 – 3,750	65 (98.5)	503 (87.3)	
	≥ 3,751	0 (0)	39 (6.8)	
Gestational malaria	yes	49 (74.2)	317 (55.0)	**< 0.001**
	no	8 (12.1)	227 (39.4)	

42.9, respectively[23]. The true incidence rates were much higher as noted by the 10% seropositivity to CHIKV that we found in pregnant women and newborns. Furthermore, the cases of CHIKV fever reported by the government surveillance system in 2015 and 2016 were from the State capital and surroundings that are located at the Eastern end of the State, at least 600 km far from Cruzeiro do Sul, a municipality located at the Western end of the State, where the present study was carried out.

According to the literature, the percentage of asymptomatic CHIKV infection varies from one epidemic to another, according to the circulating isolate (viral strain lineage), age groups, and possibly to the research design and laboratory methods used to assess specific antibodies anti-CHIKV[24,25]. Other recent studies have also reported on the percentage of asymptomatic CHIKV infections. A study in the Phillipines performed between 2012 and 2013[24] included individuals of different age groups (> 6 months up to > 50 years), and participants were followed-up for 12 months. Among 853 individuals who completed the 12 months follow-up, 19 symptomatic infections and 87 asymptomatic infections were detected (2.19 and 10.03 per 100 person-years, respectively), a proportion of asymptomatic CHIKV infections of 82%. According to the authors, the nonspecific nature of symptoms and the high rate of subclinical and mild infections may account for the lack of reported cases. A prospective study in Managua, Nicaragua, in 2015[26] evaluated the percentage of asymptomatic CHIKV infections during a CHIKV epidemic. In this study, 60 CHIKV-infected symptomatic children and 236 household contacts were followed-up. All index cases had classic symptoms of the disease, while

among the household contacts 29 (12.3%) had classic signs and symptoms of CHIKV, three (1.3%) had only fever, while 31 (13.1%) were asymptomatic. The observed symptomatic-asymptomatic ratio was 1:0.97, or 49% of asymptomatic infections. Based on this study and other published studies, asymptomatic CHIKV infections can vary from 3 to 82%[26-29].

In humans, a silent circulation of some viruses has been reported in the absence of clinical disease. Aubry *et al.*[30] conducted a serological survey on 593 blood donors in order to verify if the Ross River Virus (RRV) had already been introduced and was circulating in the French Polynesia (FP). The serum samples were collected from July 2011 to October 2013. The 204 (34.4%) serum samples were positive for RRV-IgG by ELISA; 14 (6.86%) of them were also positive by a commercial ELISA, confirming the circulation of RRV in the FP. According to the authors, RRV may have circulated in the FP without being detected for several reasons, mainly due to the asymptomatic nature of most infections (55% to 75%), and many symptomatic patients have mild symptoms that may not require medical attention. These results supported the existence of autochthonous RRV transmission and suggested that this pathogen has silently circulated in the French Polynesia.

Sanderson *et al.*[31] evaluated the prevalence of IgG and IgM antibodies against the Rift Valley fever virus (RVFV) in human serum samples (n = 1,276) collected from government health facilities within the Chobe District in 2013 and 2014. The authors did not find any record of previous, suspected or confirmed RVFV infections in the Botswama's health surveillance systems. Notwithstanding, 5% of tested serum samples were positive for IgG antibodies and 11% of these cases were also positive for IgM, confirming that RVFV was actively circulating in humans without clinical disease in this region.

Our study has limitations that must be acknowledged. Paired serum samples from the newborns of mothers who were tested in the present study were not available for analysis due to their utilization in prior investigations. This was the reason for the replacement by serum samples collected from the umbilical cord of unrelated newborns. However, since the presence of anti-CHIKV IgG antibodies in newborns' serum samples reflects their passive transfer from the mother through the placenta[32-36] to the fetus/newborn, our findings of a comparable percentage of anti-CHIKV IgG positivity in serum samples of both, pregnant women and unrelated newborns validates the analysis and corroborate the passive transfer of IgG antibodies through the placenta. Another limitation was our inability to assess DENV antibodies in cord blood samples. In addition, it would have been optimal to test our samples for anti-Mayaro virus IgG antibodies. This virus belongs to the same viral family as CHIKV, it is present in the same geographical area, and antibodies to Mayaro virus may cross-react with CHIKV[37]. However, no cases of Mayaro virus infections were reported to the local Department of Health or to the governmental health system at the time of our study. Furthermore, Mayaro virus is transmitted by mosquitoes of the genus haemagogus which live in the woods, usually at the top of the trees, being rarely found at the ground level or in urban areas, making the transmission of Mayaro virus to humans less likely in urban areas[37], such as the one in which our study was carried out.

CONCLUSION

Asymptomatic CHIKV infections likely outnumber symptomatic infections in women of low socioeconomic status in this region of the Amazon and studies that only focus on the latter will greatly underestimate the prevalence of this infection. Furthermore, the presence of asymptomatic infections at a time when outbreaks of symptomatic CHIKV fever have not yet been reported emphasizes the value of serological surveys during quiescent periods in susceptible populations living in areas of occurrence of CHIKV, aiming at implementing earlier and effective preventive actions, before the reporting of the first outbreak of symptomatic chikungunya fever.

AUTHORS' CONTRIBUTIONS

KAK performed the serological CHIKV assays, formal data analysis, and wrote the first draft; MCR performed the serological CHIKV and data analysis; MBM curated the data on pregnant women and neonates; RMS planned the study design and curated the data of pregnant women and neonates; MCC worked on the planning of the cohort and data management, and edited the manuscript; SBB and HFSS performed dengue serology; MAC worked in the planning of the cohort, supervised the cohort data management, formal data analysis, and edited the manuscript; SSW analyzed the experimental data and edited the manuscript for content and English language usage; TSO conceptualized the study, coordinated and wrote the manuscript. All authors have reviewed the manuscript and have approved the final version submitted for publication.

CONFLICT OF INTERESTS

The authors have no conflict of interests relevant to this article to disclose.

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