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Seroprevalence of Zika virus in pregnant women in Brazil: a cohort study in the macroregion of Dourados - Mato Grosso do Sul

Seroprevalencia del vírus Zika en mujeres embarazadas em Brasil: um estudio de cohorte em la macrorregión de Dourados – Mato Grosso do Sul

Soroprevalência do Zika virus em gestante no Brasil: um estudo de coorte na macroregião de Dourados – Mato Grosso do Sul

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ABSTRACT

Objective: Estimate the seroprevalence of Zika virus in pregnant women in the macroregion of Dourados - MS. **Methods:** Pregnant women admitted to the Tertiary Hospital from January 2016 to December 2017 were selected, and a serological diagnosis was made using the antibody neutralization technique for the quantification of antibodies against ZIKV with Operetta high-performance image analysis system (PerkinElmer). The study was approved by the Research Ethics Committee. **Results:** 100 samples were analyzed, and the prevalence of identified ZIKV was 2%. Myalgia and ostealgia (90%) and high fever (75%) were the most common symptoms. Moreover, >50% of the cases reported a regular use of repellents. Maternal ZIKV infection was associated with premature childbirth and Inter-Atrial Communication but not with neurological alterations. **Conclusion:** These results demonstrated the importance and need for creating public policies to mitigate the possibility of damage caused by ZIKV, raising awareness among pregnant women regarding the use of repellents and other forms prevention, such as combating vectors, and investing the availability of new ZIKV vaccines.

Keywords: Newborn, Pregnant women, Seroprevalence, Zika.

RESUMO

Objetivo: Estimar a soroprevalência do vírus Zika em gestantes da macrorregião de Dourados-MS. **Métodos:** Foram selecionadas gestantes internadas no Hospital Terciário no período de janeiro de 2016 a dezembro de 2017 e realizado diagnóstico sorológico utilizando a técnica de neutralização de anticorpos para quantificação de anticorpos contra ZIKV com sistema de análise de imagens de alto desempenho Operetta (PerkinElmer). O estudo foi aprovado por Comitê de Ética em Pesquisa. **Resultados:** Foram analisadas 100 amostras e a prevalência do ZIKV identificado foi de 2%. Mialgia e ostealgia (90%) e febre alta (75%) foram os sintomas mais comuns. Além disso, >50% dos casos relataram uso regular de repelentes. A infecção materna pelo

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ZIKV foi associada ao parto prematuro e à comunicação interatrial, mas não a alterações neurológicas. **Conclusão:** Esses resultados demonstraram a importância e a necessidade da criação de políticas públicas para mitigar a possibilidade de danos causados pelo ZIKV, conscientizando as gestantes quanto ao uso de repelentes e outras formas de prevenção, como o combate aos vetores e investindo na disponibilidade de novas vacinas contra o ZIKV.

Palavras-chave: Recém-nascido, Gestantes, Soroprevalência, Zika.

RESUMEN

Objetivo: Estimar la seroprevalencia del virus Zika en mujeres embarazadas en la macrorregión Dourados-MS. **Métodos:** Se seleccionaron mujeres embarzadas ingresadas en el Hospital Terciario desde enero de 2016 a diciembre de 2017 y se realizó diagnóstico mediante la técnica de neutralización de anticuerpos para cuantificar anticuerpos contra ZIKV con el sistema de análisis de alto rendimiento Operetta (PerkinElmer). El estudio fue aprobado por el Comité de Ética en Investigación. **Resultados:** Se analizaron 100 muestras y la prevalencia de ZIKV identificada fue del 2%. Los síntomas más comunes fueron mialgia y osteoalgia (90%) y fiebre alta (75%). Además, >50% de los casos informaron el uso regular de repelentes. La infección materna por ZIKV se asoció con parto prematuro y comunicación interauricular, pero no con cambios neurológicos. **Conclusión:** Estos resultados demostraron la importancia y necesidad de crear políticas públicas para mitigar la posibilidad de daños causados por el ZIKV, concientizando a las mujeres embarazadas sobre el uso de repelentes y otras formas de prevención, como combatir vectores e invertir en la disponibilidaed de nuevos Vacunas contra el ZIKV.

Palabras clave: Recién nacido, Mujeres embarazadas, Seroprevalencia, Zika.

INTRODUCTION

Zika is an infection caused by a virus transmitted by arthropod vectors through the Aedes aegpyti mosquito, present in almost all of Brazil due to the tropical climate that is predominant, providing breeding grounds in homes and uninhabited places, as outbreaks can be in any container that accumulate clean water, being more common in unfavorable social conditions such as inadequate waste disposal, lack of sanitation and irregular occupations (PELLISSARI BP, et al., 2016).

In 1947, the Zika virus was isolated for the first time in a Rhesus monkey with fever in the Zika Forest of an African country called Uganda and later identified in the same forest in the Aedes africanus mosquito (Dick GW, et al., 1952), with the first three cases of human infection in Nigeria in 1954 (MACNAMARA FN, 1954). Even though it has been a long-recognized virus, in Brazil it was only identified in 2015 in the states of Bahia and São Paulo (BRASIL, 2017).

In 2016, the World Health Organization (WHO) declared Zika virus (ZIKV)-related microcephaly and other neurological disorders a Public Health Emergency of international interest. Since the beginning of 2015, the geographical distribution of ZIKV increased in Brazil with a potential for higher dissemination due to a lack of pre-existing exposure of the populations, an absence of effective preventive measures, and the widespread transmission of the mosquito vector *Aedes aegypti*. Until the epidemiological week 32 (1 March 2016 to 13 August 2016), 196,976 potential cases of fever caused by the Zika virus were registered in the country (incidence rate of 96.3 cases per 100,000 people).

Of these, 132,524 (67.3%) cases were reported among women, of which 96,494 (72.8%) were of childbearing age (10 to 49 years old). During the same period, 16,264 (16.9%) potential cases were reported in pregnant women in Brazil. The highest prevalence of cases was reported between February and March of the same year.

The Midwest and Northeast regions had the highest incidence rates of the disease in the population, with 270.1 and 172.1 cases per 100,000 inhabitants, respectively. In the analysis of the incidence rate of fever caused by ZIKV in women of childbearing age by the Federation Unity (UF), it was observed that the states of

Mato Grosso, Rio de Janeiro, and Bahia had the highest rates, with 1,022.6, 566.3, and 461.6 cases per 100,000 inhabitants, respectively (BRAZIL, 2016). The first reported case of ZIKV in a resident of the city of Dourados, Mato Grosso do Sul state, was on 25 February 2016.

According to the information obtained from the Epidemiological Surveillance Center (SEMS) from January 2016 to December 2017, 35 suspected cases of ZIKV infection were reported and investigated, with 19 laboratory confirmed cases, of which 13 were among women of childbearing age.

In the current epidemiological context, fever caused by ZIKV shows signs and symptoms like dengue and other flaviviruses, as well as other diseases, obscuring the clinical diagnosis of this disease. Therefore, confirmation of the causative agent of the disease by laboratory tests is crucial in determining the appropriate clinical management of patients (CDC 2010; BRAZIL, 2016).

ZIKV infection is diagnosed using molecular techniques, including qRT-PCR during viremia, which can continue for ≤7 days after the onset of symptoms. Serological tests can detect IgM/IgG antibodies against ZIKV 5-6 days after the onset of symptoms, with an increase in the titer over two weeks. Serological tests for the ZIKV may cross-react with antibodies to the dengue virus (DENV).

Studies by the World Health Organization (WHO) in 2015 reveal that serological reactions for the IgM and IgG antibodies should be interpreted with caution because they yield false-positive results for dengue or other Flaviviruses such as yellow fever (vaccine and disease).

Also, as several people with clinical symptoms do not seek health services for treatment, it is believed that the disease is underreported, and because diagnostic confirmation by laboratory tests is not always available, the actual prevalence of the infection may be different from registered records.

Therefore, the objective of the study was to estimate the seroprevalence of ZIKV in pregnant women in the macroregion of Dourados - MS from January 2016 to December 2017. This study also analyzed variables associated with ZIKV, such as the socioeconomic conditions, signs, and symptoms associated with seropositivity to ZIKV infections, as well as health complications of children born to pregnant women included in the study.

METHODS

Study setting

Mato Grosso do Sul (MS) is a state in mid-west Brazil that borders Paraguay and Bolivia and has a population of 2.5 million people. This study was conducted from January 2016 to December 2017, with parturient residents of Dourados-MS, admitted to the maternity ward of the Tertiary Hospital in MS, Brazil.

This is a public hospital with 237 beds, providing assistance of medium and high complexity in various specialties. This is the only reference hospital for high-risk pregnancy care in 34 municipalities (covering ~900,000 people), with an average of 1000 visits and 300 deliveries per month.

Parturient study population

The study, conducted between January 2016 and December 2017, included pregnant women hospitalized at the HU maternity hospital, who were aged ≥18 years, were mentally capable of understanding the study, and who had collected serological samples during childbirth care for a previous study on the seroprevalence of syphilis with 674 parturients women attended at HU-UFGD living in Dourados-MS and with telephone contact registered in the database, in which we managed to contact 100 pregnant women due to the difficulty of contact via telephone to apply the questionnaire for our Zika virus study.

Data collection

Data were collected upon admission for delivery. Each pregnant woman was interviewed using a standardized questionnaire covering the variables age, sex, marital status, education, drug use, sexual history, STI diagnosis and prenatal care. Participant race/color (white and non-white) was self-reported.

The interview and data collection were carried out by a team of previously trained health professionals, with an initial part involving direct contact and a second specific moment with a questionnaire aimed at Zika virus infection. The second moment happened two years later, these pregnant women were contacted to collect information about their babies and possible consequences of ZIKV.

Collection of biological samples

Sample collection was performed using a vacuum tube system at the Tertiary Hospital. Ten milliliters of venous blood were obtained from each participant and processed to obtain serum, which was stored at –20°C until the serological test was carried out by the Molecular Virology Laboratory at FIOCRUZ in Curitiba-PR. Blood collection was carried out by a team of previously trained health professionals.

Serological tests

The samples were tested with a previously standardized ZIKV and DENV fluorescent neutralization technique (KOISHI AC, et al., 2018). Briefly, serum samples were inactivated at 56ºC for 30 min to reduce the effects of the complement system, and then diluted 1/20 (followed by serial 1/3 dilutions). The ZIKV strain ZV BR 2015/15261 and DENV strain DENV1-FGA/89 were used in this study.

An equal volume of the virus suspension (MOI of 0.4–300 pfu) was mixed with the diluted samples and incubated at room temperature for 1 h for neutralization of the virus. Then, each mixture was inoculated onto plates containing Huh 7.5 cells (1.5×10^4 cells in 96-well plates) and incubated at 37ºC for 1 h.

The inoculum was replaced with fresh medium, and the plates were further incubated at 37ºC for 48 h. The cells were fixed with cold methanol/acetone and immunostained using the monoclonal antibody 4G2 and secondary antibody anti-mouse IgG Alexa Fluor 488. Cell nuclei were counterstained with DRAQ5.

Imaging was performed using the Operetta High-Content Imaging System (PerkinElmer). The percentage of infected cells was obtained and normalized per the positive and negative controls; antibody titer was determined as the serum dilution inhibiting 90% of the viral infection (NT90).

To reduce false-positive results, samples were considered positive for ZIKV when the NT90 was ≥20, whereas samples with an NT90 of <10 were considered negative. When the NT90 varied from ≥10 to <20, the results were recorded as inconclusive (KOISHI AC, et al., 2018).

Data analysis

The results of the interview and the serological tests were recorded in the Electronic Data Capture program (REDCap) and analyzed using the statistical software SAS version 9.2 (SAS Institute, Cary, NC, USA).

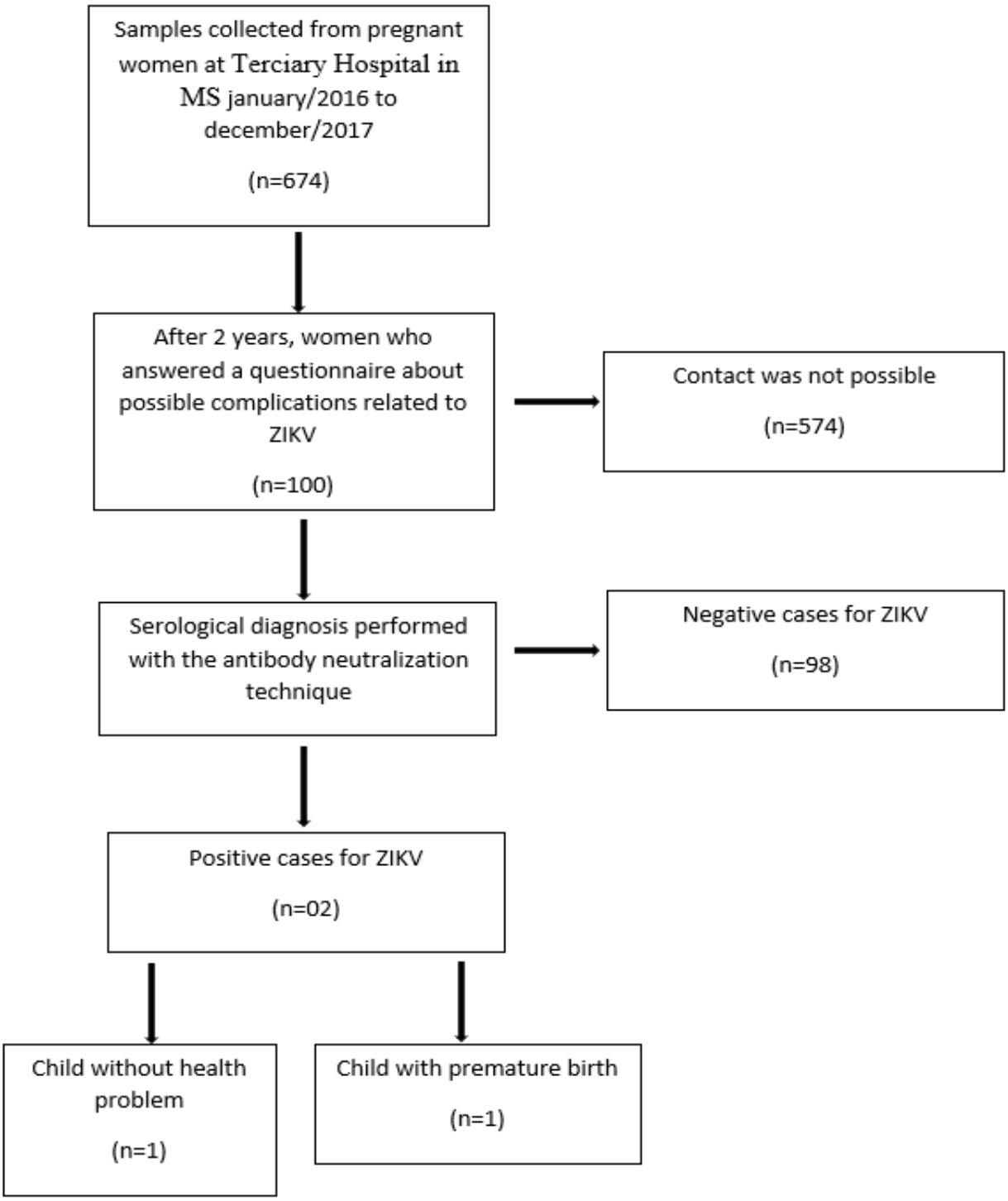
Ethical consideration

This investigation was approved by the research ethics committee of the Federal University of Grande Dourados (n° 1,402,529), CAAE n. 50965715.3.0000.5160. The informed consent of all eligible participants was obtained in writing before participation, as stipulated in Resolution 466 on 12 December 2012 by the National Health Council, thus protecting the identity of the women and using only the variables necessary for the study.

RESULTS

In this study, samples from 674 parturients were collected between January 2016 and December 2017, of which, 100 women were followed-up two years later for analysis of data on possible complications due to ZIKV in children after birth, with 2 cases positive for ZIKV, being 1 child without health problems and 1 with prematurity (**Figure 1**).

Figure 1 - Study profile carried out on parturient women out Dourados-MS, 2016-2017.



Source: Dan CS, et al., 2024.

Of the 100 pregnant women admitted for delivery, 61% (61/100) were >25 years old, 65% (65/100) studied at least till high school, 64% (64/100) declared themselves non-White, 78% (78/100) were married, 39% (39/100) were housewives, 98% (98/100) lived in the city of Dourados, and 93% (93/100) had an income below one minimum wage. Regarding the symptoms, 9% (9/100) had fever for <7 days during pregnancy, 7% (7/100) had a skin rash for ≥7 days during pregnancy, 2% (2/100) reported that the partner had a skin rash for <7 days during pregnancy, 7% (7/100) reported that some family member had a skin rash for <7 days during pregnancy, 17% (17/100) had arthralgia for <7 days during pregnancy (**Table 1**).

Table 1 - Variables associated with ZIKV seropositivity in pregnant women hospitalized for delivery at the Tertiary Hospital in Dourados-MS, 2016-2017.

Variables	N. Total	
	n = 100	%
Did you have a fever for less than 7 days during pregnancy?		
Yes	9/100	(9%)
No	91/100	(91%)
Did you have skin rash for less than 7 days during pregnancy?		
Yes	7/100	(7%)
No	93/100	(93%)
Did the partner/partner have skin rash for less than 7 days during pregnancy?		
Yes	2/100	(2%)
No	98/100	(98%)
Has any family member had skin rash for less than 7 days during pregnancy?		
Yes	7/100	(7%)
No	93/100	(93%)
Had arthralgia for less than 7 days during pregnancy?		
Yes	17/100	(17%)
No	83/100	(83%)

Source: Dan CS, et al., 2024.

Of those obtainde, 49% (49/100) exhibited symptoms of dengue in the past, of which 71% (35/49) were confirmed by serological test. Muscle and bone pain were the most reported symptoms, observed in 90% (44/49) of dengue patients, followed by high fever, reported in 75% (37/49) of the patients. Moreover, 4% (4/100) did not receive the yellow fever vaccine, and 47% (47/100) did not regularly use repellents during pregnancy (**Table 2**).

Table 2 - Variables associated with ZIKV seropositivity in pregnant women hospitalized for delivery at the Tertiary Hospital in Dourados-MS, 2016-2017.

Variables	N. Total	
	n = 100	%
Have you had dengue symptoms in the past?		
Yes	49/100	(49%)
No	51/100	(51%)
If yes, was it confirmed by serological examination?		
Yes	35/49	(71%)
No	14/49	(28%)
If yes which ones		
High fever	37/49	(75%
Headache	39/49	(79,6%)
Muscle and bone pain	44/49	(90%
Abdominal pain with vomiting and diarrhea	20/49	(41%)
Red spots on the skin	35/49	(71,5%)
Have you received the yellow fever vaccine?		
Yes	96/100	(96%)
No	4/100	(4%)
Did you regularly use repellent during pregnancy?		
Yes	53/100	(53%)
No	47/100	(47%)

Source: Dan CS, et al., 2024.

For gestational outcomes, 14% (14/100) of the children were born with some health problem, of which 35.5% (5/14) were born prematurely and 7% (1/14) with encephalopathy not related to the positive case for ZIKV (**Table 3**). The serological results revealed that 2% (2/100) tested positive for ZIKV, of which one child had health problems such as premature birth, early sepsis, respiratory distress, and Inter-Atrial Communication (IAC) of 2.8 mm.

Table 3 - Variables with gestational outcomes associated with ZIKV seropositivity iadmitted for delivery at the Tertiary Hospital in Dourados-MS, 2016-2017.

Variables	N. Total	
	n = 100	%
Was the child born with any health problems?		
Yes	14/100	(14%)
No	86/100	(86%)
If yes which one?		
Prematurity	5/14	(35,5%)
Encephalopathy	1/14	(7%)
Others	8/14	(57%)
Result ZIKV		
Positive	2/100	(2%)
Negative	97/100	(97%)
Inconclusive	1/100	(1%)
FRNT90=		
> 10,00	04/100	(4%)
< 10,00	96/100	(96%)

Source: Dan CS, et al., 2024.

DISCUSSION

In Brazil, the ZIKV epidemic occurred in 2015 and 2016, after which it spread to other countries in South America, Central America, and the Caribbean, affecting people of all ages and with a significant number of congenital ZIKV (BARRETO ML, et al., 2016). In May 2017, following the reduction in ZIKV cases, health agencies in the country ended the emergency for the disease. This study describes the seroprevalence of ZIKV in pregnant women, analyzing the variables associated with seropositivity for the infection, confirmed by testing with a fluorescent neutralization technique based on high-throughput images for ZIKV infection by serology.

The main findings of our study based on the analysis of 100 samples were the observed prevalence of ZIKV in 2% (2/100) of the subjects, with the most common symptoms being myalgia, ostealgia, and high fever. In addition, one child had health problems such as premature birth, early sepsis, respiratory distress, and Inter-Atrial Communication (IAC) of 2.8mm from maternal ZIKV infection. Also, >50% of the cases reported a regular use of repellents during pregnancy. The prevalence of ZIKV among pregnant women from Thailand was significantly lower than most other subgroups (DENSATHAPORN T, et al., 2020). The explanations for the varying incidence rates of ZIKV infection in pregnant women include the use of potential mitigating factors, such as the use of repellents or vector control (KROW-LUCAL ER, et al., 2018), which agreed with the use of repellents by 53% (53/100) of the pregnant women observed in this study.

The smaller number of ZIKV-positive cases in our study may be attributed to the greater specificity of the tests used for the analysis of the samples to detect cross-reactivity with DENV antibodies. A significant difference in the seroprevalence of ZIKV obtained by ELISA and PRNT tests was observed, with only 53.9% of the cases confirmed by the PRNT test compared with samples positive for the anti-ZIKV IgG by NS1-ELISA. This difference may be attributed to the lower specificity of the NS1-ELISA test to detect specific anti-ZIKV IgG in individuals from hyperendemic areas of DENV (SLAVOV SN, et al., 2019). Studies by the World Health Organization (WHO) show that the interpretation of serological reactions to test for IgM and IgG antibodies must be done with great care because they give false positive results for Dengue or other Flaviviruses such as yellow fever (vaccine and disease). Furthermore, a large number of people with clinical symptoms do not seek health services for treatment, so it is believed that reporting is underestimated, and as diagnostic

confirmation by laboratory tests is not always available, the prevalence of infection may probably be different from the registered one. Socioeconomic factors such as low income was prevalent in the surveyed population. The relationship between low socioeconomic conditions and increased incidence of ZIKA virus has already been described, which may be associated with discrepancies in self-care and restricted access to health services (FONTOURA FC and CARDOSO MVLML, 2014; ABREU TT, et al., 2016; ARAÚJO TVB, et al., 2018). The most reported symptoms in the surveyed pregnant women were pain in the muscles and bones and high fever. Studies reveal that the most common symptoms in ZIKV-positive pregnant women in maternity clinics were rash, myalgia, arthralgia, fever, and headache (CABRAL CM, et al., 2017; NITHIYANANTHAM SF and BADAWI A, 2019).

In our study, one child had health problems such as premature birth, early sepsis, respiratory distress, and Inter-Atrial Communication (IAC) of 2.8mm in cases of maternal ZIKV infection. Among the 34 cases reported by Schuler-Faccini L, et al. (2015), only 9% had preterm delivery, which was similar to the normal rate of preterm delivery outside the ZIKV outbreak (Oliveira RR, et al., 2015; Souza RT, et al., 2016). However, even if children exposed to ZIKV during maternal pregnancy were not born with microcephaly, their postnatal follow-up is recommended (SOUZA VW, et al., 2018). In addition, an observacional study with 103 children in the northeast of Brazil, with alleged Zika vírus infection during pregnancy, 13,5% had echocardiograms compatible with congenital heart disease, and 43% of these cases showing changes in the atrial septal defect (CAVALCANTI DD, et al., 2017).

Notably, among the cases of pregnant women positive for ZIKV in our study, there were no reports of microcephaly in the children, which may be attributed to the low sample size of only 100 pregnant women, which is the main limitation of our study. According to SAMARASEKERA U and TRIUNFOL M (2016), in Brazil, microcephaly was initially defined as a head circumference of <33 cm, which was later corrected to 32 cm. Therefore, some experts suggest that the number of reported cases in Brazil may be partly attributed to an overestimation due to overdiagnosis and due to an active search following the mandatory notification of the cases of microcephaly. Also, several diseases or factors can cause microcephaly; for example, the Latin American Collaborative Study of Congenital Malformations estimated that 38% of the cases of congenital microcephaly in its database were caused by chromosomal and single-gene errors (ECLAMC, 2015).

Our study revealed a low prevalence of ZIKV in pregnant women during the study period, which may partly reflect the time of data collection, which was performed towards the end of the epidemic in 2017. During that year, it was announced that the disease outbreak in the country had ended, while over 50% of the interviewed pregnant women reported an increased regular use of repellents. To our knowledge, there are no reports of neurological alterations in children born to ZIKV-positive mothers. Therefore, it is recommended to conduct further studies analyzing the relationship between ZIKV positivity in pregnant women and the emergence of congenital diseases in children due to vertical transmission, create policies in public health services to mitigate potential harm due to ZIKV infection, provide health education to pregnant women through creating awareness about the use of repellents and other forms of virus prevention such as combating the mosquito vector *Aedes aegypti*, and invest in the availability of new vaccines against ZIKV.

FINAL CONSIDERATIONS

Our study revealed a low prevalence of ZIKV in pregnant women during the study period, which may partly reflect the time of data collection, which was performed towards the end of the epidemic in 2017. During that year, it was announced that the disease outbreak in the country had ended, while over 50% of the interviewed pregnant women reported an increased regular use of repellents. To our knowledge, there are no reports of neurological alterations in children born to ZIKV-positive mothers. Therefore, it is recommended to conduct further studies analyzing the relationship between ZIKV positivity in pregnant women and the emergence of congenital diseases in children due to vertical transmission, create policies in public health services to mitigate potential harm due to ZIKV infection, provide health education to pregnant women through creating awareness about the use of repellents and other forms of virus prevention such as combating the mosquito vector *Aedes aegypti*, and invest in the availability of new vaccines against ZIKV.

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