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Detection and sequencing of Zika virus in normocephalic newborns with congenital Zika infection

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ABSTRACT

Fourteen asymptomatic normocephalic newborns with confirmed congenital Zika infection were investigated. All newborns presented Zika virus (ZIKV) positivity on reverse transcriptase polymerase chain reaction. Following ZIKV-specific *NS5* gene fragment sequencing in one child, phylogenetic analysis revealed that this isolate belonged to the Asian genotype, and clustered closely with other sequences previously isolated in north-east and northern regions of Brazil.

Introduction

In early 2015, an outbreak of Zika virus (ZIKV) infections emerged in north-east Brazil (Campos et al., 2015). In late 2015, an unexpected outbreak of cases of microcephalic newborns was observed in several north-eastern capital cities. Subsequently, maternal–fetal transmission of ZIKV, which leads to congenital infection, was confirmed (Calvet et al., 2016; Mlakar et al., 2016).

Congenital ZIKV infection (CZI) is characterized by neurological malformation, including subcortical calcifications, corpus callosum abnormalities, ventriculomegaly and cerebellar hypoplasia (de Fatima Vasco Aragao et al., 2016). In addition, CZI has occasionally been linked to chorioretinal abnormalities, dysphagia and arthrogryposis (van der Linden et al., 2016).

The molecular detection of ZIKV in newborns with CZI presents challenges due to the prolonged period between maternal infection and the time of analysis in neonatal samples. This article reports a series of normocephalic infants with CZI confirmed by reverse transcriptase polymerase chain reaction (RT-PCR), in addition to genomic sequencing and viral phylogenetic analysis.

Materials and methods

Study design

This case series included normocephalic newborns with confirmed molecular detection of ZIKV, born between February and August 2016. All study participants were enrolled at José Maria de Magalhães Netto Maternity Hospital in Salvador, Brazil during a surveillance protocol in 2016 (Oliveira et al., 2020).

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Study proceedings

Clinical and epidemiological data on all newborns were obtained through review of medical records and interviews with mothers. Data management was performed using REDCap 6.18.1 (Vanderbilt University, Nashville, TN, USA). Biological samples, including placental tissue, umbilical cord blood and newborn urine, were collected by staff nurses on the maternity ward as part of institutional protocols. Following delivery, umbilical cord blood was obtained from the umbilical vein by venepuncture. Fetal-side placental tissue samples were collected near the umbilical cord insertion. Newborn urine was collected within the first 24 h of life. All samples were kept frozen at -80°C until RNA extraction.

Newborns were considered normocephalic in accordance with head circumference (HC) measurements within two standard deviations (SD) from the mean, considering the newborn's gender, gestational age and HC at birth, as established by the INTERGROWTH-21st criteria (Villar et al., 2014).

In order to exclude the presence of other congenital infections, including toxoplasmosis, syphilis, cytomegalovirus (CMV) and human immunodeficiency virus (HIV), serological test results from parturients and newborns were obtained.

ZIKV diagnosis was performed by extracting viral RNA from clinical samples, followed by RT-PCR amplification as described previously (Lanciotti et al., 2008). Next, a specific set of primers corresponding to the *NS5* gene was designed, and the ZIKV-specific amplification products obtained were submitted to a nested RT-PCR protocol (Giovanetti et al., 2018). Finally, Sanger sequencing and phylogenetic analysis were performed as described previously (Appendices 1 and 2, see online supplementary material).

Results

In total, 14 newborns (Table S1, see online supplementary material), eight (57.1%) of whom were female, presented a mean gestational age at birth of 38.57 (SD 1.40) weeks; two were born prematurely.

All 14 mothers reported symptoms suggestive of ZIKV infection at differing times during their gestation: six (42.9%) occurred in the first trimester of pregnancy, two (14.3%) in the second trimester and two (14.3%) in the third trimester, while four (28.5%) mothers did not recall in which period they experienced symptoms.

The mean HC at birth of the newborns was 33.71 (SD 1.66) cm; birth weight was considered appropriate for gestational age (AGA) in 11 (78.6%) newborns and small (SGA) in three (21.4%) neborns. Only one child presented a low Apgar score (score of 5) in the first minute of life, but all values were normal by the fifth minute. Maternal serological results for toxoplasmosis IgM, CMV IgM, HIV and syphilis were negative.

Neonatal cranial ultrasonography was performed in 13 (92.9%) newborns, with no alterations observed in any cases. No newborns required neonatal ICU hospitalization, and none of them died. All patients were discharged together with their mothers from the maternity hospital after a mean of 5.50 (SD 2.10) days, and were subsequently referred to paediatricians for primary care.

Mothers were asked to bring their infants to a reference paediatric clinic for multi-disciplinary follow-up; however, only five (36%) complied, and these infants were subsequently followed during the first 2 years of life. Nevertheless, all presented normal neurological evaluations, with average HC measurements in accordance with age and gender. Ophthalmologic and auditory evaluations were also normal.

With regard to the molecular diagnosis of ZIKV in newborns, eight (57.1%) presented positivity in urine samples, five (35.7%) in umbilical cord plasma, two (14.3%) in placental tissue, and one (7.1%) in serum. In two cases, positivity was observed in two samples of different origin (plasma and urine; plasma and placenta).

ZIKV-specific RT-PCR amplification products were obtained successfully by Sanger sequencing from the plasma of a single newborn (NB 9). Phylogenetic analysis (Figure 1) indicated that the identified strain (MK216746) belonged to the Asian genotype, which clustered closely with other sequences isolated in north-east and northern regions of Brazil, as evidenced by strong bootstrap support (>90%).

Discussion

In babies whose mothers had a confirmed diagnosis of ZIKV during pregnancy, the prevalence of neurological abnormalities, including microcephaly, ranges from 5% (Shapiro-Mendoza et al., 2017) to 42% (Brasil et al., 2016). However, the proportion of asymptomatic or mild symptoms among infants with confirmed CZI has yet to be described.

Most findings related to CZI have been reported in studies involving infants with microcephaly. However, van der Linden et al. (2016) described children born with normal HC who presented abnormalities such as dysphagia and arthrogryposis, as well as abnormalities on brain imaging studies. In the present study, none of the 14 infants presented abnormal findings upon initial physical examination or on cranial ultrasound, and no abnormalities were detected in those followed-up for a period of 2 years.

Most mothers in this case series reported symptoms suggestive of ZIKV infection within the first trimester of pregnancy. While some studies have suggested a stronger association between microcephaly and maternal infection in the first trimester, the risk of adverse outcomes consistent with CZI is estimated to range between 4% and 17% in mothers infected during this period (Ades et al., 2021).

In the present study, CZI was confirmed by molecular diagnosis (RT-PCR). Identifying ZIKV in neonates is usually difficult due to the extended period between the time of infection *in utero* and diagnostic testing in newborns. However, the identification of ZIKV in neonatal samples confirms the prolonged persistence of the virus in newborns months after maternal infection (Oliveira et al., 2016). While RT-PCR has detected some instances of ZIKV infection in mothers of asymptomatic newborns (Cristante et al., 2017), the current literature contains no cases of ZIKV RT-PCR positivity documented in samples from asymptomatic newborns.

A previous study demonstrated superior ZIKV detection in urine samples compared with serum in infected adults (Gourinat et al., 2015). Most of the newborns evaluated in the present study presented ZIKV positivity in urine samples on RT-PCR, highlighting the applicability of this biological fluid as a reliable means of virus detection.

Unfortunately, in previous studies, few samples of infants with CZI were submitted for ZIKV genomic sequencing. Nonetheless, a ZIKV genome that grouped closely with other ZIKV sequences isolated in north-east and northern regions of Brazil was identified, demonstrating similarity with the Asian lineage responsible for the 2015 epidemic in north-east Brazil and French Polynesia (Faria et al., 2017). In addition, a previous study identified mutations that may be related to virulence factors associated with viral neuropathogenesis in 11 partial ZIKV genomes belonging to the Asian lineage (Melo et al., 2016). Nevertheless, the analysis of more viral sequences is necessary to make predictive inferences regarding the viral mutations detected.

The present study aimed to contribute to the body of knowledge surrounding CZI, the prevalence of which has surged recently in Brazil and which presents a highly variable spectrum. The confirmed diagnosis of CZI in asymptomatic newborns corroborates

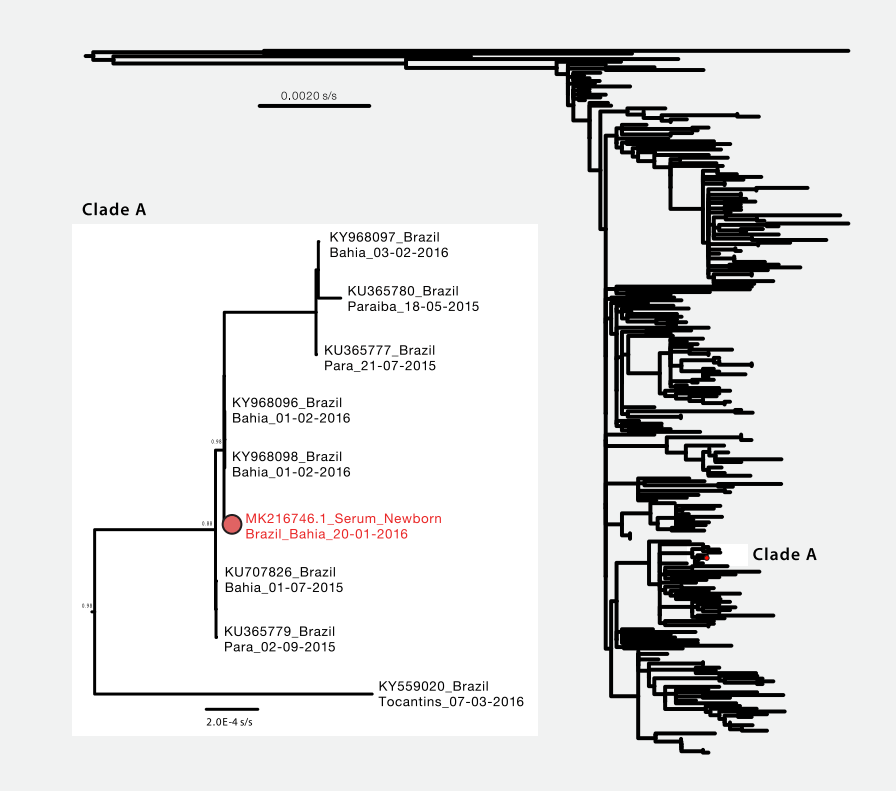

Figure 1. Phylogenetic analysis of the *NS5* sequences of Zika virus, including the newly identified sequence from an asymptomatic newborn (without microcephaly) in Salvador, Bahia, Brazil.

previous recommendations for neonatal screening in endemic areas.

A relevant limitation is that only five of 14 infants were followed-up in this study. However, all five remained asymptomatic through the second year of life. Despite this, it is notable that asymptomatic newborns with CZI may evolve with neurological complications, including late-onset microcephaly (van der Linden et al., 2016). Accordingly, active monitoring is recommended to enable early detection of neurological or other clinical complications of CZI in order to conduct timely interventions.

Declaration of Competing Interest

None declared.

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Ethical approval

This study was approved by the Institutional Review Board of Gonçalo Muniz Institute, Oswaldo Cruz Foundation (Protocol No. 1.935.854/2016). The legal guardians of all newborns provided written informed consent.

Author contributions

ICS and LCJA contributed to the study design. BLA, MG and ICS contributed to data analysis and writing of the manuscript. JVO, TCXC, EMF, RP, JIC, JMGDO, AVF, IAA, CS, EKEAL, RL and AXA contributed to participant enrolment, review of medical records, and collection of samples and data. MG, MW, LCJA and ICS contributed to laboratory analysis.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2021.10.051.

References

Ades AE, Soriano-Arandes A, Alarcon A, Bonfante F, Thorne C, Peckham CS, et al. Vertical transmission of Zika virus and its outcomes: a Bayesian synthesis of prospective studies. Lancet Infect Dis 2021;21:537–45.

Brasil P, Pereira JP, Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, et al. Zika virus infection in pregnant women in Rio de Janeiro. N Engl J Med 2016;375:2321–34.

Calvet G, Aguiar RS, Melo ASO, Sampaio SA, de Filippis I, Fabri A, et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. Lancet Infect Dis 2016;16:653–60.

Campos GS, Bandeira AC, Sardi SI. Zika virus outbreak. Bahia, Brazil. Emerg Infect Dis 2015;21:1885–6.

Cristante C, Blask A, Mulkey SB, DeBiasi RL, du Plessis AJ, Khademian Z, et al. Neuroimaging findings in normocephalic newborns with intrauterine Zika virus exposure. Pediatr Neurol 2017;78:75–8.

de Fatima Vasco Aragao M, van der Linden V, Brainer-Lima AM, Coeli RR, Rocha MA, Sobral da Silva P, et al. Clinical features and neuroimaging (CT and MRI) findings in presumed Zika virus related congenital infection and microcephaly: retrospective case series study. BMJ 2016;353:i1901.
Faria NR, Quick J, Claro IM, Thézé J, de Jesus JG, Giovanetti M, et al. Establishment and cryptic transmission of Zika virus in Brazil and the Americas. Nature 2017;546:406–10.
Giovanetti M, Goes de Jesus J, Lima de Maia M, Junior JX, Castro Amarante MF, Viana P, et al. Genetic evidence of Zika virus in mother's breast milk and body fluids of a newborn with severe congenital defects. Clin Microbiol Infect 2018;24:1111–12.
Gourinat A-C, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. Emerg Infect Dis 2015;21:84–6.
Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis 2008;14:1232–9.
Melo AS de O, Aguiar RS, Amorim MMR, Arruda MB, Melo F de O, Ribeiro STC, et al. Congenital Zika virus infection. JAMA Neurol 2016;73:1407.
Mlakar J, Korva M, Tul N, Popović M, Poljšak-Prijatelj M, Mraz J, et al. Zika virus associated with microcephaly. N Engl J Med 2016;374:951–8.
Oliveira DBL, Almeida FJ, Durigon EL, Mendes ÉA, Braconi CT, Marchetti I, et al. Prolonged shedding of Zika virus associated with congenital infection. N Engl J Med 2016;375:1202–4.
Oliveira JV, Carvalho TCX, Giovanetti M, de Jesus JG, Santos CS, Pessoa LB, et al. Neonatal surveillance for congenital Zika infection during the 2016 microcephaly outbreak in Salvador, Brazil: Zika virus detection in asymptomatic newborns. Int J Gynecol Obstet 2020;148:9–14.
Shapiro-Mendoza CK, Rice ME, Galang RR, Fulton AC, VanMaldeghem K, Prado MV, et al. Pregnancy outcomes after maternal Zika virus infection during pregnancy –U.S. Territories, January 1, 2016–April 25, 2017. MMWR Morb Mortal Wkly Rep 2017;66:615–21.
van der Linden V, Pessoa A, Dobyns W, Barkovich AJ, Júnior H, van der L, Filho ELR, et al. Description of 13 infants born during October 2015–January 2016 with congenital Zika virus infection without microcephaly at birth – Brazil. MMWR Morb Mortal Wkly Rep 2016;65:1343–8.
Villar J, Cheikh Ismail L, Victora CG, Ohuma EO, Bertino E, Altman DG, et al. International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. Lancet 2014;384:857–68.