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Letter to the Editor

Genetic evidence of Zika virus in mother's breast milk and body fluids of a newborn with severe congenital defects

On the basis of the upsurge in the number of newborns with neurologic disorders in the northeast, in November 2015 the Brazilian Ministry of Health declared a public health emergency of national concern [1]. On the basis of evidence for a potential association between microcephaly and other neurologic disorders and Zika virus (ZIKV) infection, the World Health Organization declared a public health emergency of international concern on 1 February 2016 [1]. Here we report genetic evidence of ZIKV RNA in the mother's breast milk and in the serum and urine of a newborn with severe congenital defects.

The institutional review boards at the Instituto Gonçalo Moniz (Fiocruz-Bahia) approved the present study, and the subject provided written and informed consent before her participation.

A 32-year-old pregnant woman from the municipality of Feira de Santana (Bahia, Brazil) reported diffuse pruritic cutaneous rash and joint pain during the ninth gestational week. Serologic tests for toxoplasmosis, herpesvirus 1 and 2, dengue and chikungunya viruses were negative. Immunoglobulin G ELISA was positive and immunoglobulin M ELISA was negative for rubella and cytomegalovirus.

During her 22nd gestational week, morphologic ultrasound revealed alterations in the fetus' left hand, confirmed at week 23 via ultrasound and supported after birth by X-ray (Fig. 1(A) and (B)).

At week 30, foetal biometry appeared to be consistent with gestational age, with the exception of a reduced cephalic circumference, indicating microcephaly. Delivery was performed by C-

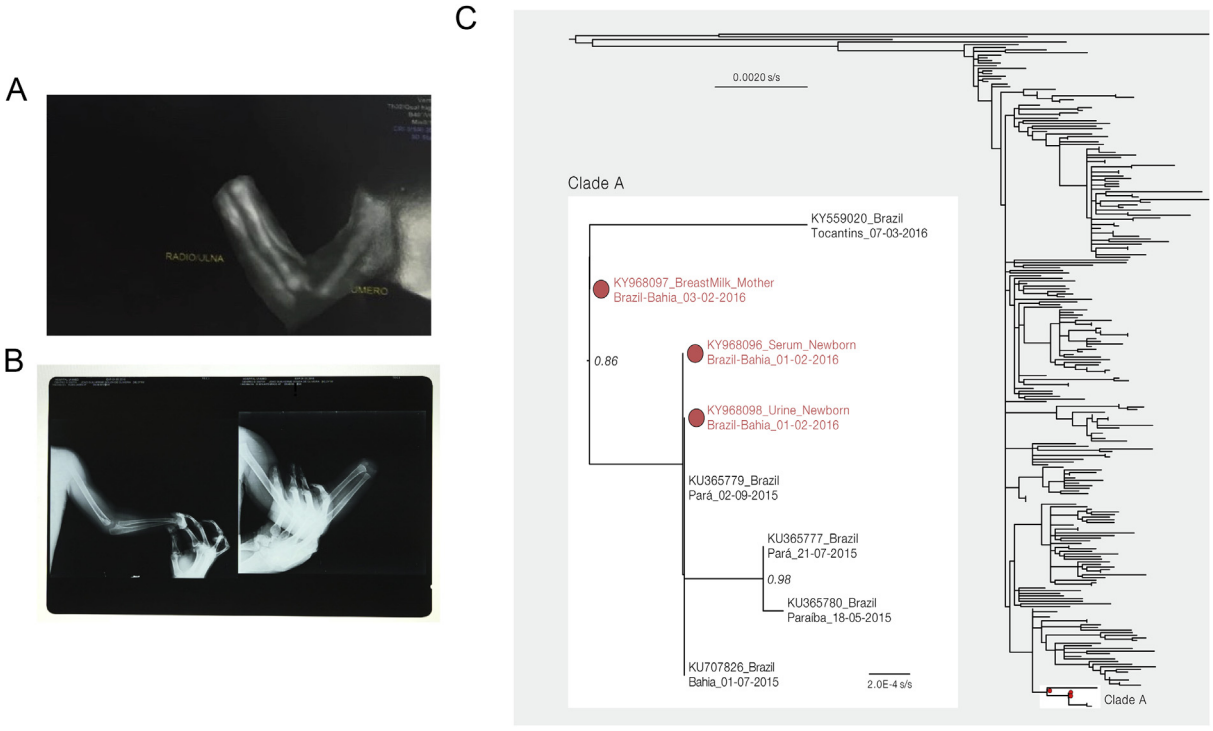

Fig. 1. (A, B) Clinical evaluation of congenital abnormalities. (A) Foetal ultrasound performed during 23rd gestational week revealed morphologic alteration in left hand of fetus. (B) X-ray evaluation performed after second year of birth confirmed defect of left upper limb. (C) Phylogenetic analysis of a newborn with congenital defects. Maximum likelihood phylogeny of NS5 sequences. Phylogeny was estimated using PhyML. Data set used contained new sequences recovered from mother's breast milk and from serum and urine of newborn, in addition to 254 publicly available complete genome sequences of Zika virus Asian genotype sampled in South America, Asia, Europe, Caribbean, North America and Pacific Islands. Sequences obtained from present study subjects are highlighted in red. Scale bar is in units of nucleotide substitutions per site. Inset shows close-up view of clade A containing newly isolated strains from Feira de Santana, Bahia (February 2016).

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section in the 38th gestational week, at which time the mother's blood and a fragment of placenta were collected. A male neonate was born with a weight of 2502 g and a head circumference of 29 cm, classified as severe microcephaly by Intergrowth-21st standard (*Z* score −3.4) [2]. A craniofacial disproportion, abnormal skull morphology with a sloping forehead and a defect of the left upper limb with ring constriction and residual nubbins were observed, indicating the presence of a possible amniotic band syndrome lesion (Fig. 1(A) and (B)).

Samples of the newborn's blood and urine were collected in the first 24 hours of birth, and breast milk was collected 2 days after delivery. Virus RNA was extracted from clinical samples and submitted to real-time quantitative PCR as a reference [3]. Although the placenta sample was positive with a threshold cycle of 33.0, the low virus loads in other samples prevented virus detection by real-time quantitative PCR. A specific set of primers corresponding to the NS5 gene were then designed for conventional nested PCR (Supplementary Material), obtaining ZIKV-specific reverse transcriptase PCR amplification products from newborn serum and urine and the mother's breast milk, while the mother's serum yielded no detectable products. NS5 gene fragments (426 bp) were then obtained using Sanger sequencing. The ZIKV sequences generated from the mother's breast milk and newborn urine and serum were deposited in GenBank under accession numbers KY968096, KY968098 and KY968097, respectively. Although genetic data alone cannot provide definitive evidence of direct vertical transmission, phylogenetic analysis indicated that the sequences generated from the mother's and newborn's body fluids clustered together with strong support (bootstrap support = 0.86, Fig. 1(C)). As expected, maximum likelihood analysis of the present and the other reference ZIKV genome sequences revealed that all belong to the Asian genotype.

Other authors have already described ZIKV in the breast milk of mothers of neonates born without microcephaly [4,5]. Here we report the presence of ZIKV both in the mother's breast milk and in the serum and urine of a newborn with congenital defects.

Transparency declaration

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cmi.2018.06.008.

References

[1] de Oliveirav WK, Araújo de Françaet GV, Carmo EH, Duncan BB, de Souza Kuchenbecker R, Schmidt MI. Infection-related microcephaly after the 2015 and 2016 Zika virus outbreaks in Brazil: a surveillance-based analysis. The Lancet 2017;390:861–70.

[2] 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.

[3] 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. Emerg Infect Dis 2007;2008:1232–9.

[4] Sotelo JR, Sotelo AB, Sotelo FJB, Doi AM, Pinho JRR, Oliveira RC, et al. Persistence of Zika virus in breast milk after infection in late stage of pregnancy. Emerg Infect Dis 2017;23:856–7.

[5] Dupont-Rouzeyrol M, Biron A, O'Connor O, Huguon E, Descloux E. Infectious Zika viral particles in breastmilk. Lancet 2016;12:387–10023.

M. Giovanetti[†]
Laboratorio de Patologia Experimental Instituto Gonçalo Moniz Salvador, Salvador, Bahia, Brazil

Laboratório de Flavivírus, Instituto Oswaldo Cruz Fiocruz, Rio de Janeiro, Brazil

J. Goes de Jesus[†]
Laboratorio de Patologia Experimental Instituto Gonçalo Moniz Salvador, Salvador, Bahia, Brazil

M. Lima de Maia[†]
Universidade Estadual de Feira de Santana, Salvador, Bahia, Brazil

Secretaria de Saúde de Feira de Santana, Feira de Santana, Salvador, Bahia, Brazil

J.X. Junior[†], M.F. Castro Amarante, P. Viana, F. Khouri Barreto
Laboratorio de Patologia Experimental Instituto Gonçalo Moniz Salvador, Salvador, Bahia, Brazil

E.M. de Cerqueira
Universidade Estadual de Feira de Santana, Salvador, Bahia, Brazil

N. Pedreira Santos, M. Barreto Falcão, M.C. Machado Costa
Secretaria de Saúde de Feira de Santana, Feira de Santana, Salvador, Bahia, Brazil

A.V. Barros Faiçal
Universidade Federal da Bahia, Salvador, Bahia, Brazil

A.L. Andrade de Melo
Secretaria de Saúde de Feira de Santana, Feira de Santana, Salvador, Bahia, Brazil

L. Santos Amorim, I.C. de Siqueira
Laboratorio de Patologia Experimental Instituto Gonçalo Moniz Salvador, Salvador, Bahia, Brazil

D.L. Macarenhas
Secretaria de Saúde de Feira de Santana, Feira de Santana, Salvador, Bahia, Brazil

A.M. Bispo de Filippis
Laboratório de Flavivírus, Instituto Oswaldo Cruz Fiocruz, Rio de Janeiro, Brazil

N. Rodrigues Faria
Department of Zoology, University of Oxford, Oxford, UK

A. Xavier Acosta
Universidade Federal da Bahia, Salvador, Bahia, Brazil

L.C. Júnior Alcântara[*]
Laboratorio de Patologia Experimental Instituto Gonçalo Moniz Salvador, Salvador, Bahia, Brazil

Laboratório de Flavivírus, Instituto Oswaldo Cruz Fiocruz, Rio de Janeiro, Brazil

[*] Corresponding author. L.C. Júnior Alcântara, Fundação Oswaldo Cruz (FIOCRUZ), Brazil.
E-mail address: lalcan@bahia.fiocruz.br (L.C. Júnior Alcântara).

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[†] The first four authors contributed equally to this article, and all should be considered first author.