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-
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

Supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). All authors report no conflicts of interest relevant to this article.

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


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

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

Please cite this article in press as: Giovanetti M, et al., Genetic evidence of Zika virus in mother’s breast milk and body fluids of a newborn with severe congenital defects, Clinical Microbiology and Infection (2018), https://doi.org/10.1016/j.cmi.2018.06.008