

# Delayed childhood neurodevelopment and neurosensory alterations in the second year of life in a prospective cohort of ZIKV-exposed children

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**We report neurodevelopmental outcomes in 216 infants followed since the time of PCR-confirmed maternal Zika virus (ZIKV) infection in pregnancy during the Rio de Janeiro epidemic of 2015–2016 (refs. 1,2). Neurodevelopment was assessed by Bayley Scales of Infant and Toddler Development, third edition (Bayley-III; cognitive, language and motor domains) in 146 children and through neurodevelopment questionnaires/neurological examinations in 70 remaining children. Complete eye exams ( $n=137$ ) and hearing assessments ( $n=114$ ) were also performed. Below-average neurodevelopment and/or abnormal eye or hearing assessments were noted in 31.5% of children between 7 and 32 months of age. Among children assessed by Bayley-III, 12% scored below  $-2$  s.d. (score  $<70$ ; a score of  $100 \pm 2$  s.d. is the range) in at least one domain; and 28% scored between  $-1$  and  $-2$  s.d. in any domain (scores  $<85-70$ ). Language function was most affected, with 35% of 146 children below average. Improved neurodevelopmental outcomes were noted in female children, term babies, children with normal eye exams and maternal infection later in pregnancy ( $P=0.01$ ). We noted resolution of microcephaly with normal neurodevelopment in two of eight children, development of secondary microcephaly in two other children and autism spectrum disorder in three previously healthy children in the second year of life.**

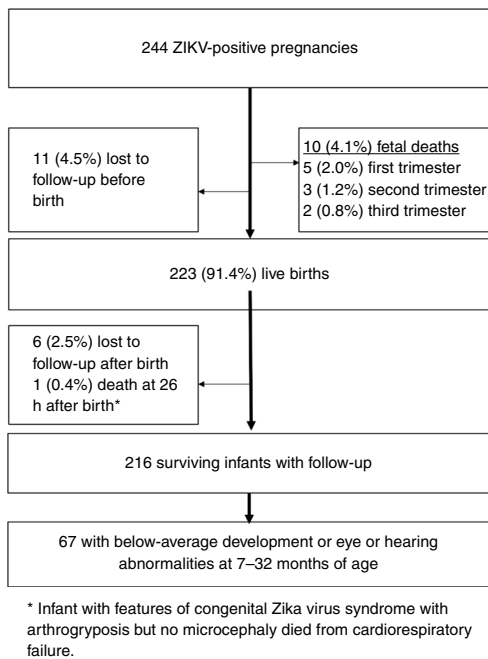
During the 2015–2016 Zika virus (ZIKV) epidemic in Rio de Janeiro, Brazil, we established a prospective cohort of symptomatic pregnant women with reverse-transcription PCR (RT-PCR)-confirmed ZIKV infection. We reported fetal ultrasound findings for the first 88 women enrolled during the epidemic (until February 2016)<sup>1</sup> and subsequently described gestational and infant outcomes for 125 pregnancies until July 2016 (ref. 2). All pregnancies had reached completion by December 2016. We report neurodevelopmental results obtained for children aged 7–32 months from our prospective cohort.

Between September 2015 and June 2016 we enrolled 244 pregnant symptomatic women who presented with a rash and tested positive for ZIKV by qualitative RT-PCR of serum or urine. Figure 1 depicts enrollment flow. Among 223 live births, the prematurity rate was 13% and ten infants (4.6%) were small for gestational age (Table 1). Microcephaly was identified in eight of 216 infants with follow-up (3.7%), including two with secondary microcephaly. Two of the eight cases of microcephaly resolved: one infant with proportionate microcephaly developed normal head circumference as he grew, while another developed normal head circumference following surgery for cranial synostosis. Both infants had normal neurodevelopmental outcomes, audiometric evaluations and eye exams in the second year of life.

The present report focuses on 216 infants who had prospective follow-up. All had clinical and neurologic evaluations; 146 infants (67.6%) had Bayley Scales of Infant and Toddler Development, third edition (Bayley-III) assessments. Sixty-seven of 70 remaining children (96%) had normal neurodevelopment questionnaires and neurological examinations (HINE). All children were offered Bayley-III assessments, but these were not performed if parents declined lengthier neurodevelopmental evaluations (that is, parents did not wish to spend additional time in the clinic or return another day for Bayley-III assessments). In that case, neurodevelopment was evaluated through HINE/neurodevelopmental questionnaires. The age range at the time of the final neurodevelopmental assessment was 7–32 months (median, 18 months). Only five children had Bayley-III assessments  $<12$  months of age. All HINE assessments were performed in the second year of life.

As seen in Table 1 and Fig. 2, 71.3% of children ( $n=154$ ) had normal neurodevelopmental assessments by Bayley-III or HINE evaluation. Fifty-nine of 146 children (40%) had Bayley-III results  $\leq -1$  s.d. in at least one of three functional domains. When children assessed through HINE and neurodevelopmental

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**Fig. 1** | Flow diagram of mother-infant pair enrollment and follow-up in the Rio de Janeiro Zika cohort.

questionnaires were included, 62 of 216 antenatally ZIKV-exposed children (28.7%) scored below average in at least one assessment. Among 146 children with Bayley-III results, 28.1% had below-average performance ( $-1$  to  $-2$  s.d.) and 12.3% performed well below average (below  $-2$  s.d.) in cognitive and/or motor and/or language domains. Language development was most affected, with 34.9% of children showing Bayley-III results below  $-1$  s.d.; 9.6% had delays in cognitive development and 16.4% in motor development. Bayley-III results had a skewed distribution towards lower scores; only 10% of children were above 1 s.d. in any domain, and none had scores  $>2$  s.d. In contrast, 30% of children had below-average scores.

Although 4.6% of infants were small for gestational age at birth, only 2 of 216 infants (1%) showed failure to thrive on follow-up ( $Z$ -score  $<-2$ ). Eye exams were abnormal in 9 of 137 infants (7%) and included chorioretinal atrophy, macular hypoplasia, optic nerve hypoplasia, optic nerve pallor, pigment mottling and increased optic cup. Hearing deficits were noted in 13 of 114 infants (12%). Sixty-eight of 216 children (31.5%) had neurodevelopment below average in at least one domain and/or eye and/or hearing abnormalities (Figs. 1 and 2 and Table 1).

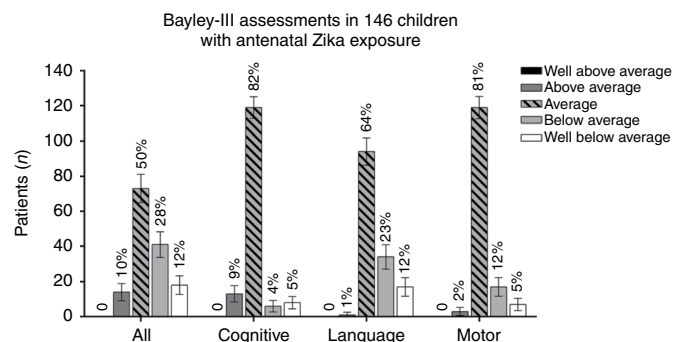
Among 18 children with well below-average performance (12%), six were microcephalic and three (2.1%) developed autism spectrum disorder (ASD). The two children with resolving microcephaly had average scores on Bayley-III assessments in the second year. Three children with ASD were normal initially and developed ASD in year 2.

Parameters associated with a Bayley-III score  $<-1$  s.d. in 146 children included prematurity (odds ratio, 5.41; 95% confidence interval, 1.66–20.56;  $P < 0.01$ ) and an abnormal funduscopic exam (odds ratio, 20.35; 95% confidence interval, 3.02–412.72). Female gender was a protective risk factor (odds ratio, 0.36; 95% confidence interval, 0.16–0.78) against a below-average Bayley-III score (Supplementary Tables 1 and 2). An abnormal funduscopic exam was associated with a Bayley-III score  $<-2$  s.d. (odds ratio, 7.92; 95% confidence interval, 1.24–48.89). Statistically significant associations with maternal age, education, mode of delivery, infant birth weight and hearing function were not seen.

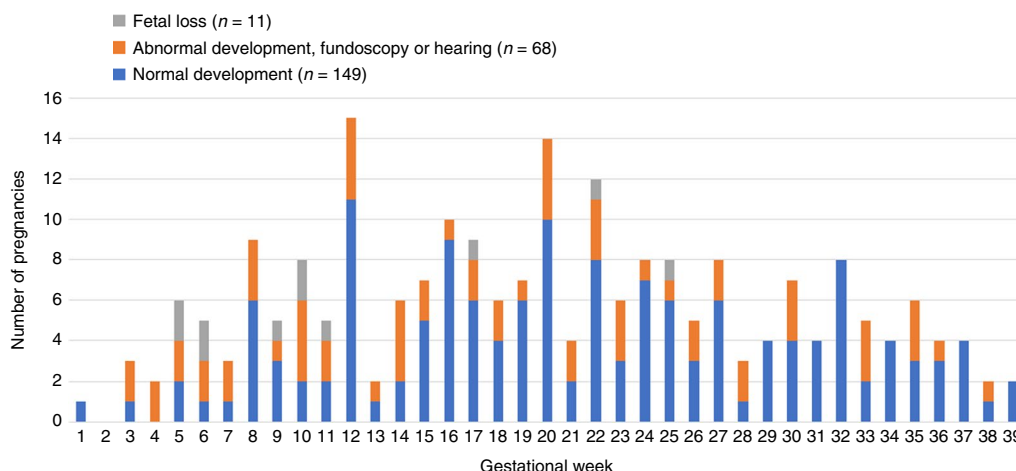
**Table 1** | Characteristics of ZIKV-exposed neonates and neurodevelopmental and neurosensory assessments

Demographics at birth	<i>n</i> (out of 216)	Percentage				
Mean maternal age at birth (years) and s.d.	30.3	±6.3				
Infant gender						
Female	106	49.1				
Male	110	50.9				
Preterm infants	28	13.0				
<37 to ≥35 weeks	18	8.3				
<35 weeks	10	4.6				
Small for gestational age	10	4.6				
Microcephaly	8	3.7				
Primary	4	1.9				
Secondary <sup>a</sup>	2	0.9				
Resolved <sup>b</sup>	2	0.9				
Interviews and Bayley-III neurodevelopmental assessments at 7-32 months of age ( <i>n</i> = 216) <sup>c</sup>	Normal	Abnormal				
	<i>n</i>	%	<i>n</i>	%		
	154	71.3	62	28.7		
Bayley-III assessments ( <i>n</i> = 146)			Between $-1$ and $-2$ s.d.	Below $-2$ s.d.		
			<i>n</i>	%	<i>n</i>	%
All ( <i>n</i> = 146)	87	59.6	41	28.1	18	12.3
Cognitive ( <i>n</i> = 146)	132	90.4	6	4.1	8	5.5
Language ( <i>n</i> = 146)	95	65.1	34	23.3	17	11.6
Motor ( <i>n</i> = 146)	122	83.6	17	11.6	7	4.8
Other neurosensory assessments						
Hearing ( <i>n</i> = 114)	101	89	13	12		
Funduscopic eye exam ( <i>n</i> = 137)	128	94	9	7		

<sup>a</sup> Two infants born with normal head circumference developed microcephaly in the first year of life. <sup>b</sup> One infant born with proportionate microcephaly developed normal head circumference over time and one infant born with cranial synostosis showed improved head circumference after surgery. <sup>c</sup> Neurodevelopmental interviews evaluated time of achievement of developmental stages by an adaptation of the HINE scheme, evaluating neurological exam, motor function and state of behavior in infants from 2 to 36 months. For Bayley-III, the 'All' category depicts the lowest score observed in one of the three functional domains. One s.d. below normal corresponds to a Bayley-III composite score  $<85$ ; 2 s.d. below normal corresponds to a Bayley-III composite score  $<70$ .



**Fig. 2** | Bayley-III assessments in 146 children between the ages of 7 and 32 months. Well above average:  $>2$  s.d., score  $>131$ ; above average: 1–2 s.d., score 116–130; average:  $-1$  to 1 s.d., score 85–115; below average:  $-1$  to  $-2$  s.d., score 84–70; well below average:  $<-2$  s.d., score  $<70$ . Populational percentages are shown at the top of each bar.



**Fig. 3 | Gestational age at time of maternal Zika virus infection in 244 pregnancies.** Adverse pregnancy and infant outcomes by week of maternal ZIKV infection during gestation. Adverse pregnancy outcome is fetal loss. Infant outcomes include below-average/abnormal neurodevelopment and/or abnormal eye and/or abnormal hearing assessments between 7 and 32 months of age.

An earlier gestational age at the time of ZIKV infection was a significant predictor of below-average neurodevelopment (Supplementary Table 2 and Fig. 3). Later gestational age at the time of ZIKV infection was less likely to be associated with below-average neurodevelopment, fetal loss and microcephaly (Supplementary Table 2). For each trimester by which ZIKV infection was delayed, risk for below-average development decreased by 46% (odds ratio, 0.54, adjusted; 95% confidence interval, 0.34–0.86). Eye and hearing abnormalities were not associated with gestational age of ZIKV infection. Fetal loss was noted in women infected as late as 25 weeks' gestation (Fig. 3).

Infants were followed from the time of maternal antenatal infection until the second and third years of life, allowing investigation of longer-term neurodevelopmental outcomes than previously recorded. We noted eight cases of microcephaly (3.7%): two developed postnatally and two resolved over time, one spontaneously and the other following corrective surgery for craniosynostosis. Congenital ZIKV has been associated with early fusion of sutures<sup>3</sup>.

A concern following in utero ZIKV exposure was whether infants born without obvious structural brain abnormalities would have normal development<sup>2,4–14</sup>. It appears that this is not a safe assumption. Nearly one-third of infants had findings impacting future development regardless of head size. A Bayley-III score  $< -2$  s.d. ( $< 70$ ) in any functional domain is the accepted cut-off value for severe developmental delay<sup>15</sup>. A Bayley-III score between  $-1$  and  $-2$  s.d. (70–84) is the cut-off value associated with risk of developmental delay<sup>16</sup>. In the general population, a normal distribution of neurodevelopmental scores would be expected when using a standardized tool such as Bayley-III. We did not see a bell-shaped curve when we plotted results for ZIKV in utero-exposed children. Instead, there was a skewed distribution to the right: no children had scores  $> 2$  s.d. from the norm, whereas 12% had scores  $< -2$  s.d. A higher rate of below-average performance on the Bayley-III language scale as compared to the cognitive scale was noted; this is consistent with studies performed in preterm and very low-birth weight infants, where the Bayley-III scale seriously underestimated developmental delay by age 2 years in one study<sup>17</sup> and underdiagnosed developmental delay when compared to Bayley-II (ref. 18). If this is the case in our population, neurodevelopmental results could actually be worse than reported.

In a previous report comparing neuroimaging findings to neurodevelopmental performance in 94 children, we noted a significant association between normal results on brain imaging and higher Bayley-III scores<sup>19</sup>. Nevertheless, neuroimaging failed to predict

severe developmental delay in 2% of children and normal development in 16% (ref. 19). We surmise that non-structural or non-specific findings identified in brain imaging in the first month of life following in utero ZIKV exposure are not necessarily predictive of subsequent suboptimal development, nor are poor growth measures or non-specific early clinical findings. In our original report of 125 pregnancies, 42% of children had abnormal clinical and or imaging findings around the time of birth<sup>2</sup>. In the present analysis of 216 children evaluated as late as 32 months of age, 31.5% had below-average performance in developmental assessments and/or abnormal neurosensory function.

Interestingly, 24 of 49 children (49%) with abnormal findings in early infancy in our previous report<sup>2</sup> had normal assessments in their second or third years of life. This includes children with early functional abnormalities such as seizures and hypotonia, as well as non-specific neuroimaging findings in the neonatal period. Conversely, a normal exam at birth did not guarantee future normal development; 17 of 68 children (25%) with normal assessments in infancy in our earlier report<sup>2</sup> had below-average neurodevelopmental assessments and/or abnormal hearing or ophthalmologic assessments on follow-up. This includes three children who developed ASD in the second year of life. Autism has been reported to have higher prevalence in congenital infections; ASD was as high as 5% in children with congenital rubella syndrome<sup>20</sup> and is more prevalent in children with congenital cytomegalovirus<sup>21</sup>. It has been postulated that neuroimmune modulation may play a role in the genesis of autism in infants exposed to congenital ZIKV infection<sup>22</sup>.

In our analyses attempting to identify potential predictors of abnormal development, we found significant associations with abnormal eye exams, prematurity, male gender and gestational age at infection. Female infants are known to have improved outcomes following serious perinatal infections as compared to males<sup>23</sup>. Women infected in later periods of pregnancy were less likely to have fetal loss, or to have infants with structural brain defects or below-normal development. In a previous study we did not find associations between abnormal infant findings following in utero ZIKV exposure and maternal disease severity during pregnancy, but it is worthy of mention that all infants in our cohort were born to women with symptomatic ZIKV infection<sup>24</sup>. Since there is no evidence to suggest that children of women with asymptomatic ZIKV infection have better outcomes than those of symptomatic women, close follow-up of all antenatally ZIKV-exposed children is warranted.

The longitudinal nature of our unique infant cohort with RT-PCR-confirmed maternal ZIKV infection during pregnancy allows

us to estimate the frequency of specific clinical features in ZIKV-exposed infants. The frequency of eye manifestations was 7% and hearing deficits 12%. Previous studies, including our own<sup>25–30</sup>, reported higher rates of ophthalmic findings in infants with congenital ZIKV infection. It is important to remember that some studies were not prospective in design and were subject to referral bias.

One of our study limitations is that parents of children who appear healthy are often reluctant to consent to further evaluations. Zika virus can be a highly stigmatizing diagnosis. Thirty-two percent of the children in this study had neurologic/developmental functions assessed through an adapted version of the HINE scheme, which included a detailed neurodevelopmental questionnaire in lieu of Bayley-III. Another study limitation is that we did not have a control group of children who underwent neurodevelopmental testing in parallel. Because ZIKV was highly epidemic in Rio de Janeiro, and because of the diagnostic challenges in detection of antenatal ZIKV exposure (asymptomatic/unidentified maternal infection), we could not establish a control group in which we could confidently demonstrate absence of antenatal ZIKV exposure. Nevertheless, since the ZIKV epidemic has subsided, there are not many cohort studies of children with PCR-confirmed antenatal ZIKV exposure with extended longer-term follow-up. Our data demonstrate that there are subtle, but nevertheless important, neuro-repercussions of antenatal ZIKV exposure arising over time beyond the severe structural brain defects and microcephaly noted at birth. These children need to be monitored far beyond early infancy. In our opinion, all children born to mothers with ZIKV infection during pregnancy should be followed longitudinally in the first 3 years of life by a multidisciplinary team, with biannual neurodevelopmental, ophthalmologic and auditory evaluations even if early evaluations were normal. As Zika is a recently recognized congenital infection, it remains to be seen whether future repercussions such as learning disabilities, further hearing loss or other problems can affect exposed children during school-age years, so yearly assessments until age 7 years should be performed.

Our neurodevelopmental findings in children have been corroborated by animal studies, including a recent study by Zhao et al. (personal communication) demonstrating abnormal neurologic functions in mice following ZIKV infection, including features of ASD. These mice had perturbations in social interaction and showed signs of depression, impaired learning and memory, in addition to severe motor defects. Similar to the children in our study, following infection with an Asian strain of ZIKV, mice demonstrated significant impairment of visual cortical function, circuit organization and experience-dependent plasticity. Long-term outcomes may be influenced by more subtle inflammation-immune responses mediated by ZIKV, raising the possibility that early interventions could improve the neurodevelopmental trajectory of children exposed to ZIKV in utero, a possibility that requires further investigation.

### Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at <https://doi.org/10.1038/s41591-019-0496-1>.

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### Author contributions

K.N.-S., P.B., C.R.G., J.P.P. and M.E.M. conceived and designed the study. K.N.-S., P.B., T.K., Z.V., L.D., M.P., L.M.A.d.C., S.M.P., A.A.Z., I.T., T.R.S.S., D.C.d.C., R.P.C., J.M., A.B.R., R.H.H., C.Y.P.A., F.F.G., J.P.P., S.L.G. and M.E.M. were responsible for data collection and accuracy checking of data. K.N.-S., P.B., T.K. and Z.V. were responsible for

data analysis. K.N.-S., P.B., T.K., Z.V., K.A., C.R.G., L.D., A.A.Z., I.T., C.E., P.B.M., J.D.C., Z.X., G.C. and M.E.M. were responsible for interpreting the data. K.N.-S., P.B., Z.V., T.K. and M.E.M. drafted the manuscript. All authors critically revised the manuscript and gave final approval of the version to be published.

### Competing interest

The authors declare no competing interests.

### Additional information

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## Methods

**Study population.** In this cohort study, pregnant women at any week of gestation who presented to the acute febrile illness clinic at the Fundação Oswaldo Cruz with a rash as previously described were enrolled<sup>2</sup>. Infants from PCR-confirmed<sup>31</sup> ZIKV-positive mothers were followed prospectively.

**Study oversight.** The study protocol was approved by the institutional review boards at Fundação Oswaldo Cruz (Fiocruz) and the University of California, Los Angeles. Participants provided written informed consent. The authors vouch for the accuracy and completeness of the data and the analyses and for the fidelity of the study to the protocol.

**Infant clinical assessments.** Anthropometric measures at birth were obtained for all live births and subsequent visits as previously described<sup>2</sup>. Microcephaly at birth (primary) was defined as head circumference Z-score <−2 (moderate) and <−3 (severe). Secondary microcephaly developed postnatally<sup>32</sup>. Detailed eye exams were performed by pediatric ophthalmologists as previously described<sup>25–27</sup>. Hearing assessments were performed through brainstem evoked response audiometry<sup>2,19</sup>.

**Neurodevelopmental assessments.** All infants had detailed neurologic assessments with accompanying neurodevelopmental interviews performed concurrently. Bayley-III<sup>33</sup> was performed by trained personnel for three domains: cognitive, language (receptive and expressive) and motor (fine and gross). Bayley-III was chosen as the developmental tool, validated cross-culturally in Brazil<sup>34</sup>. Reported results are for the latest Bayley-III assessment performed for each child, ranging from 7 to 32 months of age. A Bayley-III score within 1 s.d. above or below the norm of 100 was considered normal for that specific domain. A score of 1–2 s.d. above the norm (116–130) was considered above average and 1–2 s.d. below the norm (<85 to 70) moderately below average. A score >2 s.d. from the norm (>130) was considered well above average and 2 s.d. below the norm well below average (<70). The scoring system is consistent with previously published developmental studies<sup>15,21,35–37</sup>. In the absence of Bayley-III, time of achievement of developmental stages was investigated by an adaptation of the HINE scheme to evaluate neurological exam, motor function and state of behavior in infants from 2 to 36 months<sup>38–40</sup>. Key psychomotor milestones were included in our interview. Gross motor skills included emergence of head control, onset of sitting free, standing and walking freely. Time of appearance of babbling, single words (for single words, at least 5–10 words with a semantic role), responsive smiling and index finger pointing towards an object were obtained from parental interview and patient observation.

**Statistical analysis.** A multivariate logistic regression was used to examine the associations between development ( $n = 146$ ) and medical predictors (prematurity, sex, size for gestational age and cesarean delivery), clinical outcomes (ZIKV hearing and eye abnormalities) and demographic predictors (mother's age at delivery and education level). Associations between gestational age at infection and clinical outcomes, including hearing ( $n = 114$ ), neurodevelopment ( $n = 216$ ), fetal loss ( $n = 233$ ), microcephaly ( $n = 216$ ) and fundoscopic evaluation ( $n = 137$ ), were explored by multivariate logistic regression. Covariates (including maternal age at delivery, prematurity, birth weight, cesarean delivery, mother's education level, child's gender, hearing abnormality and eye abnormality) were examined

for collinearity, using chi-square for nominal covariates and correlation analysis for two continuous variables. Potential confounding variables were individually examined for the association of both the outcome and predictive factor, then evaluated by stepwise regression analysis. Covariates that demonstrated an association with both the outcome and predictive variable, and changed the crude beta regression by 10% from the unadjusted analysis in at least one analysis, were included in all models. These included maternal age at birth, child's gender, prematurity, size for gestational age, cesarean section and mother's educational level. When examining developmental delay as an outcome, analyses were further adjusted for hearing and eye abnormalities. Odds ratios, 95% confidence intervals and two-sided  $P$  values were reported. Analyses were conducted using the statistical package R (v.3.0.1, The R Foundation for Statistical Computing, [www.r-project.org](http://www.r-project.org)).

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

## Data availability

The data sets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

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## Reporting Summary

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*Our web collection on [statistics for biologists](#) contains articles on many of the points above.*

### Software and code

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Data collection

Data was collected from chart abstraction of patient medical records and recorded into Microsoft Access. Data was coded using R, version 3.5.1 from the R Foundation for Statistical Computing.

Data analysis

R, version 3.5.1 from the R Foundation for Statistical Computing was used for data analysis. Code was derived from the following R libraries: Base, epibasix, car, xlsx, pastecs, psych, ggplot2, and lme4.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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# Behavioural & social sciences study design

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Study description	This study is a cohort of infants born to ZIKV positive women who were followed over time to examine quantitative data including hearing, visual abnormalities, and neurodevelopment.
Research sample	As described previously in Brasil et al, 2017: "In this cohort study, pregnant women at any week of gestation who presented to the acute febrile illness clinic at the Oswaldo Cruz Foundation with a rash that had developed within the previous 5 days were offered enrollment and were included in the study after they had provided written informed consent. After the women were enrolled, detailed demographic, medical, and prenatal history information, as well as clinical findings, were entered into case-report forms. Laboratory data on rubella, cytomegalovirus, and Venereal Disease Research Laboratory (VDRL) serologic testing were abstracted from prenatal medical records and entered in case-report forms. Serum and urine specimens were obtained at study entry. Weekly follow-up was conducted by telephone, and a second visit was scheduled within 30 days after enrollment for clinical and laboratory follow-up. Women were referred for fetal ultrasonography before 20 weeks of gestation, between 20 and 30 weeks of gestation, and after 30 weeks of gestation. No women had had a diagnosis of fetal malformations in the current pregnancy before enrollment. The study population was generally healthy; women reported no coexisting conditions or medication use. Infants born to ZIKV-positive mothers are being followed prospectively."
Sampling strategy	This was a prospective cohort study of live infants born to mothers with a rash who had PCR-confirmed Zika virus infection during the Rio de Janeiro epidemic of 2015-2016. In that cohort 244 women were identified during the Rio epidemic; these were all pregnant women who came to the Fiocruz Febrile Illness Clinic with a rash that had developed in the last 5 days; all had a positive Zika virus PCR result in blood or urine the day they came to clinic. There were 10 fetal deaths and 11 women who were lost to follow-up before giving birth. As such there were 223 live births. Among those live births, 6 infants were lost to follow-up shortly after birth and 1 died in the second day of life. The sample size reflects the remaining 216 surviving infants who were followed over time. Enrolment into the pregnancy cohort stopped once the Zika epidemic in Rio de Janeiro ended; that is, when no further women with a rash due to ZIKV were identified. This was a sample of convenience as these were the patients identified prospectively during the ZIKV epidemic in Rio de Janeiro.
Data collection	The data was collected through standardized case report forms in real time and included patient interviews, medical history, physical examination findings and laboratory/ imaging/ and neurodevelopmental results. The data was collected by hand with pen and paper; our study coordinators collected the data during the time of each patient visit. As this was during and in the aftermath of the ZIKV epidemic, they were not blinded to the fact that infants were ZIKV exposed. Patients were first enrolled at the time of the ZIKV epidemic and followed subsequently over time.
Timing	The start date for enrollment of infants was December 2015 to December 2016. There was no gap during the 13 month enrollment period.
Data exclusions	Incomplete data from 11 fetal deaths and 17 children lost to follow-up were not included in the final analysis because they lacked the outcome variable (neurodevelopmental assessment).
Non-participation	17 mothers enrolled with their infant and were lost to follow-up (11 before birth and 6 after birth). Because the mother was not able to be located for further follow-up, potential due to relocation, no reason was given for non-participation.
Randomization	Because there was no intervention, there was no need for randomization in this prospective cohort of infants. Covariates were measured and controlled for in analysis. Covariates were examined for collinearity and as potential confounders.

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## Human research participants

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Population characteristics	See above
Recruitment	<p>All children enrolled in this prospective observational cohort had in utero exposure to ZIKV. Their mothers were recruited for participation into a longitudinal ZIKV cohort during the Rio de Janeiro Zika virus epidemic of 2015-2016 (Brasil et al, NEJM Dec 2016). Pregnant women at any week of gestation who presented to the acute febrile illness clinic at the Oswaldo Cruz Foundation with a rash that had developed within the last 5 days were offered enrollment into the study. Those women with a positive PCR result for ZIKV in blood or urine were enrolled into the prospective Zika cohort. Live born infants to mothers enrolled in the prospective ZIKV cohort comprise the infants followed in the present study. They all had PCR-confirmed ZIKV in utero exposure. Recruitment site was the Fiocruz Foundation medical facilities in Rio de Janeiro for mothers and their infants. Because of the nature of maternal inclusion criteria we only enrolled infants who were born to mothers with symptomatic ZIKV infection during pregnancy (all women had a rash, and this is why they came to medical attention). Infants were not recruited because mothers had abnormal ultrasounds or there were abnormalities at birth. All children included in the present study had to be enrolled antenatally to a mother who had a rash during pregnancy in the last 5 days and was found to have a positive PCR for ZIKV in blood or urine.</p>
Ethics oversight	<p>The study protocol was approved by the institutional review boards at Fundação Oswaldo Cruz (Fiocruz) and the University of California, Los Angeles. Participants provided written informed consent. The authors vouch for the accuracy and completeness of the data and the analyses and for the fidelity of the study to the protocol.</p>

Note that full information on the approval of the study protocol must also be provided in the manuscript.