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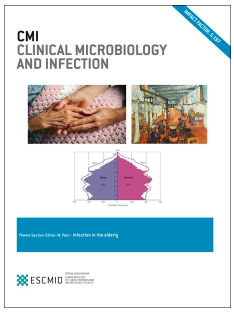

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Adverse birth outcomes associated with Zika virus exposure during pregnancy in São José do Rio Preto, Brazil

Running title: Zika virus exposure during pregnancy in São José do Rio Preto

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ABSTRACT

Objective: We aimed to report the first 54 cases of pregnant women infected by Zika vírus (ZIKV) and their virological and clinical outcomes, as well as the newborns' outcomes in 2016, after the emergence of ZIKV in dengue endemic areas of São Paulo, Brazil.

Methods: This is a descriptive study performed from February to October 2016 on 54 qPCR ZIKV-positive pregnant women identified by the Public Health Authority of São Jose do Rio Preto, São Paulo, Brazil. The women were followed and had clinical and epidemiological data collected before and after birth. Adverse outcomes in newborns were analyzed and reported. Urine or blood samples from newborns were collected to identify ZIKV infection by RT-PCR.

Results: 216 acute Zika-suspected pregnant women were identified, and 54 had the diagnosis confirmed by RT-PCR. None of the 54 women miscarried. Among the 54 newborns, 15 exhibited adverse outcomes at birth. The highest number of ZIKV infections occurred during the second and third trimesters. No cases of microcephaly were reported, though the broad clinical spectrum of outcomes, as lenticulostriate vasculopathy, subependymal cysts, auditive and ophtalmological disorders, were identified. ZIKV RNA was detected in 18 of 51 newborns tested and in eight of 15 newborns with adverse outcomes.

Conclusions: Although other studies have associated many newborn outcomes to ZIKV infection during pregnancy, these same adverse outcomes were rare or non-existent in this study. The clinical presentation in our newborns was mild compared to other reports, suggesting that there is significant heterogeneity of Congenital Zika Infection.

Abstract word count: 249 words

INTRODUCTION

Zika virus (ZIKV) infection has been associated with severe birth defects, such as newborn microcephaly(1, 2), meningoencephalitis(3) and Guillain-Barré syndrome(4, 5). Microcephaly represents a small part of a broad spectrum of teratogenic outcomes of intrauterine ZIKV infection referred to as congenital Zika syndrome (CZS)(6). Intrauterine growth restriction, ocular abnormalities, placental damage, fetal blood anomalies(7) and death are other findings that may be associated with ZIKV infection during pregnancy(1, 2, 8).

The city of São José do Rio Preto in São Paulo State, Brazil, is a region in which several arbovirus circulate(9-11). In 2016, a ZIKV outbreak was reported in the city(12), and a surveillance system was established to identify illnesses caused by ZIKV. Special attention has been given to pregnant women in an attempt to detect the impact of ZIKV infection on newborns. This study is a report of the first 54 confirmed cases of women infected by ZIKV during pregnancy and their virological and clinical outcomes, as well as the newborns' outcomes, identified through our surveillance system.

METHODS

Study Population

From February to October 2016, the Public Health Authority in the city identified 216 pregnant patients with Zika-like symptoms, among 1,674 pregnant women, in the elective and emergency services. The Brazilian Ministry of Health defines Zika-suspected cases based on macular or papular rash with two or more of the following signs/symptoms: fever, conjunctival hyperemia without secretion, pruritus, polyarthralgia, or joint edema(13). Fifty seven pregnant women with symptomatic acute Zika-suspected infection, between 5 and 38 weeks of pregnancy (gestational age defined as first trimester until 13rd weeks, second trimester from 14th to 26th weeks, and third trimester after 27th weeks)(14), attended in a health service in São José do Rio Preto, were notified as Zika-suspected patients and had blood sample collected during acute infection, with ZIKV RT-PCR

positive. These pregnant women were referred to Children's and Maternity Hospital (HCM) in São José do Rio Preto, São Paulo, Brazil, the reference hospital, and have been monitoring under a protocol approved by the São José do Rio Preto Medical School IRB. These blood samples were also tested for human immunodeficiency infection (HIV), toxoplasmosis, rubella, cytomegalovirus (CMV), hepatitis B and C, Herpes simplex virus (HSV), syphilis, and other infection (TORCHS), when the last one was relevant, using molecular and/or serological methods. The ZIKV-positive pregnant women were monitored by a multidisciplinary medical team through the use of clinical and radiological evaluations.

After the delivery, newborn's umbilical cord blood and/or urine were collected and tested for the presence of ZIKV using molecular and serological methods. The clinical exams of newborn and anthropometric measurements were performed according to the guideline of the Brazilian Ministry of Health(13), including the microcephaly definition, as newborns with 37 weeks of gestational age or less and cephalic perimeter lower than 2 standard deviations (sd) based on Intergrowth 21st to gestational age and sex(15) or newborns with 37 weeks or more and cephalic perimeter lower or equal to 31.5 cm for girls and 31.9 cm for boys, and equivalent to lower than 2sd based on WHO(16). It was considered as adverse outcomes findings: lenticulostriate vasculopathy, subependymal cysts, choroidal cyst, bilateral cranial bleed, chorioretinitis, premature birth, abnormal OAE (otoacoustic emission).

Ultrasounds (USs) were performed using a Philips HDI 5000 convex probe in order to generate fetal and post-natal images. Magnetic resonance imaging (MRI) was performed using a Philips Gyroscan Intera 1.5 T MRI scanner, and the images were analyzed by specialists in fetal medicine. Special attention was given to the fetus's or newborn's central nervous system. When available, otoacoustic emission tests (OAE) and fundus examinations were performed by specialists to identify any auditory or ophthalmologic disorders, respectively.

Virus and RNA Extraction

The viral strain used as positive control was ZIKV^{BR}. It was propagated in C6/36 *Aedes albopictus* cell cultures(17-19). Viral RNA was extracted from 140-mL blood and urine samples using the QIAamp Viral RNA Mini kit (Qiagen) according to the manufacturer's instructions.

qPCR for ZIKV

To detect the ZIKV genome in the mothers' blood or in the newborns' umbilical cord blood and/or urine samples, a one-step quantitative, real-time, fluorescent probed–based RT-PCR assay was performed using primers targeting the envelop (E) gene(20). All samples with Ct lower or equal to 38.5 were considered positive to ZIKV.

ZIKV ELISA

The umbilical cord blood samples found to be positive for ZIKV in qPCR were also tested for the Zika NS1 protein. The Zika Virus NS1 ELISA Kit (BioFront Technologies, Florida, USA) was used to capture anti-ZIKV NS1. All of the assays were performed according to the manufacturer's instructions. Each plate was read at 450 nm using a Spectramax Plus Microplate Reader (Molecular Devices, California, USA).

Complete Genome

Following RNA extraction, the cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Nineteen fragments were amplified by Nested PCR using Phusion high-fidelity DNA polymerase (Thermo Scientific). Fragment sizes ranged from 430 bp to 1461 bp. Primers are available upon request. Nested-PCR products were purified using the DNA Clean & Concentrator Kit (Zymo). Fragments were sequenced using the direct Sanger method with BigDye terminator v3.1 in an ABI 3130XL Genetic Analyzer (Applied Biosystems). Sequences were assembled and analyzed for coverage and quality using SeqMan software from the DNASTAR Lasergene package (Madison, WI).

Phylogenetic Reconstruction

The evolutionary history was inferred using the maximum likelihood method based on the general time reversible model(21) using a dataset compiled of 99 complete ORF (open reading frame) nucleotide sequences available in GenBank. The tree with the highest log likelihood (-35779.2777) is shown in Supplemental Figure 1. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach and then selecting the topology with superior log likelihood value. A discrete gamma distribution was used to model differences in evolutionary rates among sites (5 categories; +G, parameter = 0.2918). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 0.0010% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Codon positions included were $1^{st}+2^{nd}+3^{rd}$+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 10208 positions in the final dataset. Evolutionary analyses were conducted in MEGA7(22).

Statistical Analysis

All statistical analyses were carried out using the Epi-Info software for Windows (Centers for Disease Control and Prevention, Georgia, USA). We used chi-squared and Wilcoxon rank sum tests to compare the characteristics according to birth outcomes for categorical and continuous data, respectively.

RESULTS

Among 216 symptomatic acute ZIKV-suspected pregnant women in the Public Health System of São José do Rio Preto, São Paulo, Brazil between February 2016 and October 2016, this

descriptive study included 57 pregnant women (26%), which had ZIKV infection confirmed by RT-PCR in blood. Three pregnant women (5%) were lost during follow up, resulting in a final sample size of 54 women. ZIKV infection was detected in all trimesters of gestation. Fifteen pregnant women (28%) experienced adverse birth outcomes. The clinical and demographic characteristics of the 54 mothers and their respective newborns are shown in Table 1. The distribution of suspected and confirmed cases of ZIKV according to epidemiological week and gestational week of ZIKV exposure, and the associations between these data and adverse outcomes, are shown in Figures 1 and 2.

No pregnant woman in this study miscarried, and only eight (15%) of the fetuses were born at less than 37 weeks. The APGAR score median of the newborns was 9/10 and 10/10 at 1 and 5 minutes, respectively, two newborns had APGAR lower than 7 at 1 minute and none at 5 minutes, and no abnormalities were detected in the neurological exams. The additional serological screening to infectious diseases during pregnancy are shown in Tables 1 and Supplemental Table 4, while performed radiologic exams and their findings are shown in Table 1 and 2.

Almost a quarter of pregnant women (28%, 15/54) who received follow-up care presented adverse fetal/birth outcomes (Supplemental Table 1). In three cases (20%), there were histories of co-morbidities, and in seven cases, the mother reported exposure to alcohol, tobacco or illicit drugs (Table 1 and Supplemental Table 1). One newborn, which was born prematurely, encountered all of the anthropometric parameters below those expected for gestational age compatible with intrauterine growth restriction. In this same newborn, unilateral US, abnormal OAE, and ZIKV in cord blood (RT-PCR) were all identified, without other infectious agents. but with exposure to illicit drugs (marijuana) during gestation. The adverse outcomes observed in each case of ZIKV exposure in utero are described in Supplemental Table 1, 2 and 3.

Among the 39 newborns with no adverse birth outcomes, the profile of ZIKV exposure was similar to those with adverse outcomes. Clinical and laboratory data of these newborns are presented in Table 2. The serological and molecular tests to ZIKV are shown in Supplemental Table 6.

Evidence of ZIKV infection was detected in 18 out of 51 newborns (35%) that were evaluated by RT-PCR at birth using umbilical cord blood and/or urine samples (Table 3). Among the newborns who did not exhibit adverse outcomes, ZIKV RNA was detected in 10 out of 36 (28%) (Supplemental Table 4-7). The complete genome of the virus was amplified from 1 patient and 2 controls (Zika cases in male adults) and sequenced. The phylogenetic analyzes showed that the ZIKV identified in our mothers during outbreak in 2016 was cluster together with the same virus circulating in other areas of the country (Supplementary Figure 1).

DISCUSSION

Based on surveillance alerts, our health center has been conducting a prospective study on ZIKV in pregnancy and associated birth defects (with a focus on microcephaly) since January 2016. In ten months of surveillance, there were 216 cases of ZIKV-suspected pregnant women in our center, and here we reported 54 (26%) cases of pregnant women who were found to have ZIKV infection confirmed by RT-PCR based blood samples. Fifteen adverse fetal/birth outcomes and eighteen cases of congenital ZIKV infection in newborns were reported. Although ZIKV infection in the first trimester of gestation is associated with microcephaly (1, 2), no cases have been detected thus far among the newborns in our cohort. Most of the adverse neurological outcomes (14/15; 94%) occurred in the second and third trimester, and this may have been responsible for these mild outcomes.

This is not the first report to associate ZIKV infection after the first trimester with regular head circumference at birth but with adverse clinical outcomes, such as congenital brain injury acquired due to ZIKV(23). The outcomes associated with ZIKV infection during pregnancy may range from no effects to miscarriage to fetal infection resulting in CZS(24). An important study performed in Brazil in 2016 (1) reported several outcomes in fetuses and newborns exposed to ZIKV during pregnancy, as intrauterine growth restriction, cerebral calcifications, abnormal arterial

flow in the cerebral or umbilical arteries, global cerebral atrophy, microcephaly, macular hypoplasia and scarring, and placental insufficiency.

Congenital anomalies, including microcephaly, have a complex and multifactorial etiology and may be caused by other infections (such as TORCHS infections) during pregnancy, as well as chromosomal disorders, exposure to environmental toxins, and metabolic diseases(24, 25). Congenital toxoplasmosis(26), syphilis(27), HSV(28), and rubella(29, 30) may affect the central nervous system and cause neurological deficits. Out of all of the cases in this study in which sub-ependymal cysts were observed, only one pregnant woman had reagent toxoplasmosis IgM result and a newborn with ZIKV RT-PCR positive in the umbilical cord blood. Among those with vasculopathy, the only infection identified was by ZIKV. These factors lead us to believe that ZIKV can be the cause of neurological abnormalities. Knowing the cause of these issues is an important tool for prevention.

Since this is a descriptive study, a control group of women with no infection was not defined. A limitation presented by this study was the lack of data in some variables. The data were collected by the attending physician, based on a pre-established record, although it was not always filled completely. The clinical spectra observed in our newborns differed from those reported in other studies. Lenticulostriate vasculopathy, sub-ependymal cysts, auditory disorder, and chorioretinitis were the main outcomes observed, and there were no cases of macular hypoplasia, microcephaly, or abnormal neurological test results after birth. These findings showed that the symptoms of CZS might be broader than originally thought. The link to ZIKV may not be clearly established, neither excluded. In some cases, the only infectious agent detected was ZIKV. In cases where other infectious agents were identified by serological tests, the clinical findings were not usually related to this one.

In conclusion, our study highlights the importance of ZIKV infection in all trimesters of gestation. Brain abnormalities other than microcephaly, intra-cerebral calcifications, or severe outcomes detected by imaging exams during pregnancy may occur and reflect the significant heteroge-

neity of exposure to ZIKV during pregnancy. Adverse outcomes were mild or non-existent in our newborns, but their occurrence may affect neurological development, thus having an important negative impact on the patient specifically and on the population more generally. These impacts may only be measured some years after birth. This study provides additional evidence of the association between congenital ZIKV infection and certain fetal outcomes and contributes to a better understanding in the pathogenesis of birth defects caused by ZIKV.

Disclaimer: The opinions, assumptions, and conclusions or recommendations expressed in this material are the responsibility of the authors and do not necessarily reflect the views of the São Paulo Research Foundation (FAPESP).

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Author's Contribution:

Design of the study: MLN, FC, AHO, DCMVO, NV, AK

Collect/Analyze Patients Data: NRRNJ, CFE, GFG, LCFJ, AFNR, SAP, MWC, LEAAC, SHN, RCVM, INPS, SBAC, FC

Performed Tests/Analyses: NRRNJ, CFE, ACBT, NZ, RAS, GCDS, CB, BC, LG, ASS, CCBM, LCM

Contributed with Tools/Analysis: PR, PFVC, FAB, NV, AK

Wrote Manuscript: MLN, CFE, NRRNS, FC, NV, AK

The corresponding author had full access to all of the data in the study and assumed the final responsibility for the decision to submit this study for publication.

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TABLES

Table 1. Characteristics of women in the cohort and their pregnancies according to infants' birth outcomes

	Total (N=54)			Adverse Birth Outcomes# (N=15)			No Adverse Birth Outcomes (N=39)			
Characteristic	**No. of Responses**	**No. of Positives or Median**	**(% or IQR)**	**No. of Responses**	**No. of Positives or Median**	**(% or IQR)**	**No. of Responses**	**No. of Positives or Median**	**(% or IQR)**	***p-value***
Demographic										
Mother age (y)	54	27.5	(23 - 34)	15	23	(21 – 38)	39	28	(22 - 34)	0.68
Ethnicity										
White	45	31	(69)	14	9	(64)	31	22	(71)	0.83
Mestizo	45	10	(22)	14	4	(29)	31	6	(19)	-
Black	45	3	(7)	14	1	(7)	31	2	(6)	-
Other	45	1	(2)	14	0	(0)	31	1	(3)	-
Educational Level Completed										
College education	44	10	(23)	14	2	(14)	30	8	(27)	0.51
High school	44	26	(59)	14	10	(71)	30	16	(53)	-
Primary school	44	8	(18)	14	2	(14)	30	6	(20)	-
Prior Medical History										
Paras	37	1	(0 - 2)	12	1	(0.5 - 2)	25	1	(0 - 1)	0.60
Gravidas	37	2	(1.5 – 3.5)	12	2	(1.5 - 3)	25	2	(2.5 - 3)	0.80

Comorbidities*[1]	54	9	(17)	15	3	(20)	39	6	(15)	0.68
Prior Hx STD	45	4	(9)	14	2	(14)	31	2	(6)	0.39
Zika Infection during Pregnancy										
Trimester of ZIKV infection										
First trimester	54	4	(7)	15	1	(7)	39	3	(8)	0.20
Second trimester	54	26	(48)	15	4	(27)	39	22	(56)	-
Third trimester	54	24	(44)	15	10	(67)	39	14	(36)	-
Rash	53	51	(96)	15	14	(93)	39	37	(95)	0.49
Pruritis	54	34	(63)	15	10	(67)	39	24	(62)	0.73
Headache	54	23	(43)	15	5	(33)	39	18	(46)	0.39
Athralgias	54	21	(39)	15	7	(47)	39	14	(36)	0.47
Fever	54	18	(33)	15	3	(20)	39	15	(38)	0.20
Myalgias	54	15	(28)	15	4	(27)	39	11	(28)	0.91
Respiratory symptoms*[2]	54	8	(15)	15	0	(0)	39	8	(20)	0.06
Conjunctivitis	54	1	(2)	15	0	(0)	39	1	(3)	0.53
Serum ZIKV RT-PCR+	53	45	(85)	15	14	(93)	38	31	(82)	0.28
Urine ZIKV RT-PCR+	52	41	(79)	14	10	(71)	38	31	(82)	0.43
Pregnancy										
Current Alcohol drinker	44	2	(5)	14	1	(7)	30	1	(3)	0.57
Current Smoker	44	6	(14)	14	2	(14)	30	4	(13)	0.93
Medications*[3]	54	35	(65)	15	10	(67)	39	25	(64)	0.86
Complications*[4]	44	10	(23)	14	4	(29)	30	6	(20)	0.53
TORCH Serology										

Toxoplasmosis IgM+	47	2	(4)	13	1	(8)	34	1	(3)	0.47
CMV IgM+	47	0	(0)	13	0	(0)	34	0	(0)	NA
Rubella IgM+	47	4	(9)	13	0	(0)	34	4	(12)	0.20
VDRL+	49	1	(2)	13	1	(8)	36	0	(0)	0.09
UTI/MRI Exams										
No. of pre-natal US exams	51	3	(3 – 3)	14	3	(3 - 3)	37	3	(3 - 3)	0.80
Abnormal pre-natal US exam*[5]	51	2	(4)	14	2	(14)	37	0	(0)	0.02
Abnormal fetal MRI*[6]	25	6	(24)	8	1	(13)	17	5	(29)	0.36

Adverse outcomes: lenticulostriate vasculopathy, subependymal cysts, choroidal cyst, bilateral cranial bleed, chorioretinitis, premature birth, abnormal OAE
*[1] Comorbidities: With adverse outcomes: hypothyroidism (1), idiopathic thrombocytopenic purpura (1), chronic cardiopathy (1), hypertension (1); No adverse outcomes: hypothyroidism (2), hypertension (3).
*[2] Coryza, sore throat, or cough
*[3] Medications: with adverse outcomes: levothyroxine (1), prednisone (1), methyldopa (1), sulfadiazine plus pyrimethamine (1), acyclovir (1); No adverse outcomes: levothyroxine (1), methyldopa (2), methyldopa plus metformin (1), levothyrosin plus metformin (1), clindamycin (1), spiramycin (1)
*[4] Complications during pregnancy: With adverse outcomes: gestational diabetes (1), HSV infection (1), syphilis (1), acute toxoplasmosis (1); No adverse outcomes: gestational diabetes (2), rubella (4); acute toxoplasmosis (1)
*[5] US = ultrasound: with adverse outcomes: retro-ovulate hematoma (1), oligohydramnios (1)
*[6] MRI = magnetic resonance imaging (no significant findings): With adverse outcomes: eccentric placental insertion of umbilical cord (1); No adverse outcomes: placental thickening (1), asymmetrical thyroid lobes (1), increased subtentorial measures plus pericardial effusions (1), right renal cyst in fetus (1), swallowing failure and gastric distention (1)

Table 2. Characteristics of newborn infants according to birth outcome

	Total (N=54)			**Adverse Birth outcomes (N=15)**			**No Adverse Birth Outcomes (N=39)**			
	No. Of Responses	**No. of Positives or (%) or IQR Median**		**No. of Responses**	**No. of Positives or (%) or IQR Median**		**No. of Responses**	**No. of Positives or Median**	**(%) or IQR**	***p*-value**
Birth										
Gestational age at birth (wks)	54	38	(37.5 - 38)	15	38	(37 - 39)	39	38	(37 – 38.5)	0.83
Premature (<37 wks of gestation)	54	8	(15)	15	3	(20)	39	5	(13)	0.51
Male sex	54	30	(56)	15	6	(40)	39	24	(62)	0.15
Caesarean section delivery	35	29	(83)	12	11	(92)	23	18	(78)	0.32
Apgar score (median)										
At 1 min	33	9	(9 - 9)	11	9	(9 - 9)	22	9	(9 - 9)	0.30
At 5 min	33	10	(9 - 10)	11	10	(10 - 10)	22	10	(9.5 - 10)	0.09
Anthropometric Measurements										
Head circumference										
cm	53	35	(34 – 36)	15	35	(34 – 36)	39	35	(34 – 36)	0.71
percentile*[1]	53	89	(77 - 97)	15	92	(76 - 98)	39	89	(79 - 96)	0.78
Microcephaly*[2]	54	0	(0)	15	0	(0)	39	0	0	-
Weight										
grams	54	3097	(2901 - 3420)	15	2970	(2894 - 3486)	39	3098	(2929 - 3460)	0.62
Percentile*[2]	54	66	(39 - 82)	15	65	(44 - 85)	39	66	(39 - 84)	0.95
small for gestational age*[2]	54	0	(0)	15	0	(0)	39	0	(0)	-
Length										
cm	54	48	(46.8 – 49.5)	15	47	(46 - 48)	39	48	(47 – 49)	0.05

percentile	54	43	(27 - 71)	15	32	(14 - 56)	39	48	(34 - 73)	0.05
Clinical Evaluation										
Abnormal neurological evaluation	54	0	(0)	14	0	(0)	40	0	(0)	NA
Abnormal ophthalmological exam*[3]	22	2	(9)	10	2	(20)	12	0	(0)	0.10
Abnormal OAE/AABR*[4]	34	6	(18)	14	6*	(43)	20	0	(0)	0.00
Radiological Evaluations										
Abnormal exam	38	7	(18)	14	7	(50)	24	0	(0)	0.00
Cranial US*[5]	38	7	(18)	14	7*	(50)	24	0	(0)	0.00
Cranial MRI*[6]	3	0	(0)	1	0	(0)	2	0	(0)	NA
ZIKV Diagnostic Testing										
RT-PCR+	51	18	(35)	15	8	(53)	36	10	(28)	0.08
Serum	48	14	(29)	15	5	(33)	33	9	(27)	0.67
Serum Ct	14	36.5	(36 - 37)	5	36.3	(36.2 – 36.5)	9	36.8	(35.6 – 37.4)	0.31
Urine	46	4	(9)	15	3	(20)	31	1	(3)	0.06
Urine Ct	4	36.5	(31 – 36.6)	3	36.4	(31 – 36.6)	1	37.7	-	0.18
Infection in first trimester	18	2	(11)	8	1	(13)	10	1	(10)	0.20
Infection in second trimester	18	8	(44)	8	2	(25)	10	6	(60)	-
Infection in third trimester	18	8	(44)	8	5	(63)	10	3	(30)	-
MAC-ELISA+ ZIKV	16	0	(0)	7	0	(0)	9	0	(0)	NA
Hospitalization										
Days	37	2	(2 - 4)	14	2	(2 - 4)	23	2	(2.5 - 4.5)	0.64
NICU admission	54	5	(9)	15	0	(0)	39	5	(13)	0.15

*[1] (<-2 SD HC)
*[2] (<10th weight percentile)
*[3] Abnormal ophthalmological exam: unilateral chorioretinitis
*[4] OAE = otoacoustic exam: one case confirmed by automated auditory brainstem response (AABR)
*[5] US = ultrasound: With adverse outcomes: lenticulostriate vasculopathy (2), subependymal cysts (3), choroidal cyst (1), bilateral cranial bleed (1)
*[6] MRI = magnetic resonance imaging

Table 3. Outcomes among newborns from mothers exposed to ZIKV during pregnancy

Outcome	No. of Cases	Incidence (95% CI)*
Adverse Birth Outcomes	15/54	28 (17 - 41)
Exposure in first trimester	1/4	25 (0.63 - 81)
Exposure in second trimester	4/26	15 (5 - 33)
Exposure in third trimester	10/24	42 (23 - 62)
ZIKV detected at birth	8/18	44 (23 - 67)
ZIKV not detected at birth	7/15	47 (23 - 71)
ZIKV detection at birth	18/51	35 (22 - 48)
ZIKV exposure in first trimester	2/4	50 (9 - 91)
ZIKV exposure in second trimester	8/26	31 (15 - 50)
ZIKV exposure in third trimester	8/24	33 (14 - 52)
With adverse outcomes	8/15	53 (29 - 77)
No adverse outcomes	10/39	26 (14 - 41)

*Cumulative incidence shown as cases per 100 births

SUPPLEMENTAL MATERIALS

Supplemental Table 1. Clinical and laboratory findings of 14 infants with adverse birth outcomes

		During Pregnancy				At Birth				
Code	**Mother's Age (y)**	**Week of Gestation of ZIKV Infection**	**Symptoms during ZIKV Illness**	**Radiological Findings*[1]**	**Complications**	**Gestational Weeks/Sex**	**Z-score (Percentile) of Weight/Length/HC**	**Clinical Findings*[2]**	**US Findings*[1]**	**ZIKV RT-PCR**
5	38	35	exanthema pruritus	- (US/MRI)	-	38/F	-0.11(42)/-0.18(45)/1.53(93)	-	lenticulostriate vasculopathy	urine + / serum -
11	37	15	exanthema pruritus arthralgia	- (US/MRI)	-	39/M	-0.93(63)/0.10(54)/2.24(98)	abnormal OAE AD	-	serum +
14	37	32	exanthema pruritus fever myalgia arthralgia	- (US/MRI)	-	37/F	-2.28(46)/-1.47(7)/1.84(96)	abnormal OAE AD	lenticulostriate vaculopathy	urine + / serum -
16	36	28	exanthema	- (US/MRI)	gestational diabetes	36/F	-1.89(69)/0.09(53)/0.54(70)	abnormal OAE AD	-	serum -
18	17	25	exanthema fever myalgia arthralgia	- (US)	toxoplasmosis	38/F	0.06(88)/-0.62(60)/1.65(95)	-	subependymal cysts	serum +

20	22	12	exanthema head-ache arthralgia	- (US/MRI)	-	36/F	-2.32(1)/-2.09(22)/-2.13(1.65)	abnormal OAE AU	-	serum +
21	19	20	conjunctivitis	Eccentric insertion umbilical cord (MRI)	-	37/M	-2.18(54)/-0.46(32)/0.79(78)	abnormal OAE AD	-	serum -
24	23	35	exanthema pruritus	- (US)	VDRL+	39/F	-3.02(25)/-2.05(4)/-0.06(65)	-	choroidal cyst	serum -
31	17	16	exanthema pruritus conjunctivitis	- (US/MRI)	-	38/F	0.06(94)/0(50)/2.38(99)	-	bilateral cranial bleed*[3]	serum –
37	23	33	exanthema, pruri-tus headache myalgia arthralgia	- (US)	-	39/F	-0.67(25)/-3.05(25)/1.19(88)	abnormal OAE AU	-	serum -
39	21	25	exanthema	- (US/MRI)	-	39/M	-0.71(62)/-2(2.2)/0.83(80)	-	subependymal cysts	serum -
41	24	30	exanthema pruritus headache myalgia arthralgia conjunctivitis	- (US/MRI)	-	38/M	-0.49(90)/0.54(70)/1.8(96)	-	subependymal cysts	serum +
102	23	14	exanthema pruritus headache	Oligohydram-nios (US) in third trimester		28/M	-0.36(31)/-0.88(11)/0.71(62)	Premature birth	-	serum +
114	28	28	exanthema pruritus fever myalgia arthralgia conjunctivitis	Retro-ovulate hematoma (US) in first trimester	HSV infection	38/M	-2.62(30)/-0.41(34)/-0.45(32)	chorioretinitis OD	-	serum + / urine +

ZIKV = Zika virus; DENV = Dengue virus, CHIKV = Chikungunya virus, CMV = Cytomegalovirus; PCR = Polymerase Chain Reaction; RT-PCR = Reverse transcriptase - polymerase chain reaction; IgG = Immunoglobulin type G; NR: Non-reactive; NP = Not performed; CUS: Cranial ultrasound; MNR = Magnetic Nuclear Resonance; OAE = otoacoustic emissions; ITP = Idiopathic thrombocytopenic purpura; HC = head circumference; VDRL: Venereal disease research laboratory test

***[1]** The use of a dash (-) reflects no significant findings in the MRI, US or clinical evaluation

***[2]** AD, AS, OD, OS: right ear, left ear, right eye, left eye

***[3]** Degree 1 on right and degree 2 left hemisphere

Supplemental Table 2. Clinical and laboratory findings of 10 infants with ZIKV RNA detected at birth and who did not develop adverse outcomes

		During Pregnancy			At Birth				
Code	**Mother's Age (y)**	**Week of Gestation of ZIKV Infection**	**Symptoms during ZIKV Illness**	**Radiological Findings***	**Complication**	**Gestational Age/gender**	**Percentile of Weight/Length/HC**	**Radiological Findings***	**ZIKV RT-PCR**
7	28	21	Exanthema, pruritis	Increased subtentorial measures, pericardial effusions (MRI) / - (US)	-	38	-2.72(0.32)/0.06(52)/1.1(86)	-	serum +
9	29	30	Exanthema, pruritis, coriza	Right kidney cyst in fetal (MRI)/ - (US)	Acute toxoplas-mosis	38	0.06(52)/-063(27)/1.65(95)	-	serum +
22	33	13	exanthema, pruritis, headache, conjunctivitis	Swallowing failure and gastric distention (MRI) / - (US)	-	37	-2.57(38)/3-0.34(6)/1.24(89)	- (T-US)	urine +
23	21	31	exanthema pruritus myalgia arthralgia	- (US/MRI)	-	39/M	-0.93(51)/-1.12(13)/0.64(74)	-	serum +
26	30	25	pruritis headache con-junctivitis	- (US)	Rubella IgM+	39/M	-0.66(29)/-0.23(40)/0.88(81)	-	serum +
34	31	13	exanthema fever myalgia arthralgia	- (US/MRI)	gestational diabetes	32/M	0.2(6)5/0.2(58)/-0.13(44)	-	serum +
36	30	19	exanthema pruritis head-ache cough conjunctivitis	- (US)	-	37/M	-2.18(19)/-0.46(32)/-0.84(20)	-	serum +

38	25	31	exanthema pruritus headache	- (US)	-	38/F	-2.7(21)/-0.18(42)/0.7(76)	-	serum +
40	27	22	exanthema headache pruritis	- (US/MRI)	-	39/M	-0.88(23)/-0.46(32)/1.51(930	-	serum +
107	42	38	exanthema fever myalgia	- (US)	-	39/F	2.58(99)/1.99(99)/100REVER	-	urine +

ZIKV = Zika virus; DENV = Dengue virus, CHIKV = Chikungunya virus, CMV = Cytomegalovirus; PCR = Polymerase Chain Reaction; RT-PCR = Reverse transcriptase - polymerase chain reaction; IgG = Immunoglobulin type G; NR: Non-reactive; NP = Not performed; CUS: Cranial ultrasound; MNR = Magnetic Nuclear Resonance; OAE = otoacoustic emissions; ITP = Idiopathic thrombocytopenic purpura; HC = head circumference; VDRL: Venereal disease research laboratory test

* The use of a dash (-) reflects no significant findings in the MRI, US or clinical evaluation

Supplemental table 3. Clinical and laboratory findings of 23 infants with ZIKV RNA non detected at birth

		During Pregnancy			**At Birth**				
Code	**Mother's Age (y)**	**Trimester of Gestation of ZIKV Infection**	**Symptoms during ZIKV Illness**	**Radiological Findings***	**Complication**	**Gestational Age/gender**	**Percentile of Weight/Length/HC**	**Clinical or Radiological Findings***	**ZIKV RT-PCR**
1	29	2nd	Exanthema, pruritus, headache, arthralgia, conjunctivitis	- (US/MRI)	HSV IgM+	38/M	-0.25(40)/-0.41(34)/1.96(97)	- (C-US)	serum and urine -
2	33	2nd	Exanthema, pruritus, arthralgia, conjunctivitis	- (US)	-	38/F	-0.22(41)/0.33(63)/1.46(92)	- (C-US)	serum and urine -
4	35	3rd	Exanthema, pruritus	- (US/MRI)	Gestational Diabetes, Rubella IgM+	38/M	-0.18(42)/-1.48(7)/1.24(89)	- (C-US)	serum -
6	34	2nd	exanthema pruritus, headache	- (US/MRI)	-	37/F	-2.5(0.61)/-1.15(12)/0.88(81)	- (C-US)	serum -

8	22	1st	Fever, exanthema, pruritus, myalgia, headache, sore throat, cough, conjunctivitis	- (US/MRI)	Acute Toxoplasmosis	38/M	0.49(31)/-1.77(3)/0.18(57)	-	serum and urine -
10	32	2nd	Exanthema, pruritus, arthralgia	- (US)	-	39/M	-3.13(9.27)/-1.69(4)/0.64(74)	- (C-US)	serum and urine -
13	27	3rd	Fever, exanthema, pruritus headache, arthralgia, cough conjunctivitis	- (US/MRI)	Gestational Diabetes and Gestational Hypertension Disorder	36/M	-1.72(69)/0.56(71)/2.54(99)	-	serum and urine -
15	36	1st	Exanthema	- (US/MRI)	HSV IgM+	38/M	-0.18(38)/-1.99(1.7)/2.7(99)	- (C-US)	serum and urine -
16	22	3rd	Exanthema	- (US/MRI)	-	36/F	-1.89(70)/0.09(54)/0.54(71)	Abnormal OEA AD / - (C-US)	serum and urine -
17	26	2nd	Conjunctivitis	- (US)	-	38/M	-0.18(84)/-0.34(36)/2.7(99)	-	serum and urine -
19	26	2nd	Exanthema	- (US/MRI)	-	38/F	-0.28(90)/0.27(61)/-	-	serum and urine -
21	19	2nd	Conjunctivitis	- (US/MRI)	-	37/M	-2.18(53)/0.46(32)/0.79(78)	Abnormal OEA AS	serum and urine -
24	23	3rd	Exanthema, pruritus	- (US)	-	39/F	-3.02(25)/-2.05(4)/-0.06(65)	Abnormal OEA AD, choroidal cyst	serum and urine -
25	22	3rd	Fever, exanthema, pruritus	- (US)	-	37/F	0.31(96)/1.46(93)/1.8(96)	-	Serum -
27	30	2nd	Exanthema	- (US/MRI)	Gestational Hypertension Disorder	36/F	-1.96(56)/0.02(51)/-1.21(11)	- (C-US)	serum -

28	17	2nd	Exanthema, pruritus, headache, sore throat, arthralgia, conjunctivitis	- (US/MRI)	-	38/F	0.06(69)/0.62(73)/0.81(80)	-	Serum -
29	22	2nd	Exanthema, pruritus, headache, arthralgia,	- (US)	-	39/F	0.33(82)/1.47(93)/0.52(70)	-	serum and urine -
30	35	3rd	Exanthema, pruritus, headache, sore throat, arthralgia, conjunctivitis	- (US)	-	37/M	-2.41(28)/-1.28(10)/1.38(91)	- (C-US)	serum and urine -
31	17	2nd	Exanthema, pruritus, conjunctivitis	- (US/MRI)	HSV IgM+	38/F	0.06(93)/0 (49)/2.38(99)	Bilateral intracranial bleeding	serum and urine -
33	21	2nd	Fever, exanthema	- (US/MRI)	-	38/M	0.54(70)/0.49(31)/0.18(570	- (C-US)	Urine -
35	25	2nd	Exanthema, pruritus, myalgia	- (US/MRI)	-	38/M	-0.18(95)/-0.18(42)/2(97)	-	serum and urine -
37	23	3rd	Exanthema, pruritus, myalgia, headache, arthralgia	- (US)	-	39/F	-3.05(25)/-0.67(25)/1.19(88)	Abnormal OEA AS	serum and urine -
39	37	3rd	Exanthema	- (US/MRI)	-	39/M	-0.71(23)/-2(2.25)/0.83(80)	subependymal cysts (C-US)	serum and urine -
42	32	2nd	Exanthema	- (US/MRI)	-	34/F	-3.02(34)/-0.86(19)/0.7(76)	Abnormal OEA AS	Urine -
101	15	2nd	Exanthema, pruritus	- (US)	-	37/M	-0.06(47)/-0.22(41)/0.54(70)	-	serum and urine -
103	27	2nd	Fever, exanthema, pruri-tus, headache	- (US)	-	38/F	0.06(52)/1.23(89)/2.38(99)	Bilateral abnormal OEA	serum and urine -

105	33	3rd	Exanthema, headache	- (US)	-	38/M	-0.18(49)/-0.34(36)/1.24(89)	-	serum and urine -
109	37	3rd	Fever, exanthema,	-	-	-/M	-	-	Urine -
110	34	3rd	Fever, exanthema, pruritus, myalgia, headache, arthralgia, conjunctivitis	- (US)	-	40/F	-0.77(21)/0.39(65)/1.87(97)	-	Serum -
111	19	3rd	Exanthema, pruritus	-	-	-	-	-	serum and urine -
112	19	2nd	Fever, exanthema, myalgia	-	-	37/M	0.25(64)/-1.02(12)/0.79(75)	-	serum and urine -
115	27	3rd	Fever, exanthema, pruritus	-	-	39/M	-0.77(45)/-0.35)/0.78(78)	-	serum and urine -

*AD, AS: right ear, left ear
CUS: cranial ultrasound

Supplemental Table 4. Characteristics of 54 women in the cohort, according to detection of ZIKV RNA in their infant at time of birth

	ZIKV RNA Detection (N=18)			No ZIKV RNA detection (N=36)			
Characteristic	**No. of Responses**	**No. of Positives or Median**	**(% or IQR)**	**No. of Responses**	**No. of Positive or Median**	**(% or IQR)**	***p*-value**
Demographic							
Age (y)	18	28.5	(24 - 35)	36	27	(21 - 34)	0.22
Ethnicity							
White	18	11	(61)	27	20	(74)	0.14
Mestizo	18	6	(33)	27	4	(15)	-
Black	18	0	(0)	27	3	(11)	-
Other	18	1	(6)	27	0	(0)	-
Educational Level Completed							
College education	17	4	(24)	27	6	(22)	0.04
High school	17	13	(76)	27	13	(48)	-
Primary school	17	0	(0)	27	8	(30)	-
Prior Medical History							
Paras	14	1	(1 - 2)	23	1	(0.5 – 1.5)	0.18
Gravidas	14	2	(2.5 - 3)	23	2	(1 - 3)	0.48
Comorbidities*[1]	18	6	(33)	36	3	(8)	
Prior Hx STD	18	3	(17)	27	1	(4)	0.13
Zika Infection during Pregnancy							

Timing of illness							
First trimester	18	2	(11)	36	2	(6)	0.74
Second trimester	18	8	(44)	36	18	(50)	-
Third trimester	18	8	(44)	36	16	(44)	-
Rash	18	17	(94)	35	34	(97)	0.63
Pruritis	18	14	(78)	36	20	(56)	0.11
Headache	18	9	(50)	36	14	(39)	0.44
Athralgias	18	8	(44)	36	13	(36)	0.55
Fever	18	6	(33)	36	12	(33)	1.0
Myalgias	18	6	(33)	36	9	(25)	0.52
Respiratory symptoms*[2]	18	2	(11)	36	6	(17)	0.59
Conjunctivitis	18	1	(6)	36	0	(0)	0.15
Serum RT-PCR+	17	16	(94)	36	29	(81)	0.20
Urine RT-PCR+	18	14	(78)	34	27	(79)	0.89
Pregnancy							
Alcohol use	17	1	(6)	27	1	(4)	0.74
Smoking	17	3	(18)	27	3	(11)	0.54
Medications*[3]	18	8	(44)	36	4	(11)	
Complications*[4]	18	6	(33)	26	4	(15)	0.16
TORCH serology							
Toxoplasmosis IgM+	16	1	(6)	31	1	(3)	0.63
CMV IgM+	16	0	(0)	31	0	(0)	NA
Rubella IgM+	15	2	(13)	32	2	(6)	0.42
VDRL+	17	0	(0)	32	1	(3)	0.46

UTI/MRI exams							
No. pre-natal US exams	18	3	(3 - 3)	36	3	(2 – 2.5)	0.21
Abnormal pre-natal US exam*[5]	18	2	(11)	36	0	(0)	0.04
Abnormal fetal MRI*[6]	10	3	(30)	15	3	(20)	0.23

*[1] Comorbidities: With ZIKV-RNA detection: hypothyroidism (2), idiopatic thrombocytopenic purpura (1), chronic cardiopathy (1), hypertension (2); Without ZIKV-RNA detection: hypothyroidism (1), hypertension (2).
*[2] Coryza, sore throat or cough
*[3] Medications: with ZIKV RNA detection: levothyroxine (2), spiramycin (1), prednisone (1), sulfadiazine plus pyrimethamine (1), methyldopa plus metformin (1), methyldopa (1), acyclovir (1); Without ZIKV-RNA detection: clindamycin (1), levothyroxine (1), methyldopa (2).
*[4] Complications during pregnancy: With ZIKV-RNA detection: acute toxoplasmosis (2), rubella (2), gestational diabetes (1), HSV infection (1); Without ZIKV-RNA detection: gestational diabetes (2), rubella (2), syphilis (1)
*[5] US = ultrasound: with adverse outcomes: retro-ovulate hematoma (1), Oligohydramnios (2)
*[6] MRI = Magnetic resonance imaging: With adverse outcomes: placental thickening (1), asymmetrical thyroid lobes (1), increased subtentorial measures plus pericardial effusions (1), right renal cyst in fetus (1), eccentric placental insertion of umbilical cord (1), swallowing failure and gastric distention (1).

Supplemental Table 5. Characteristics of 51 newborn infants according to ZIKV RNA detection at birth

	ZIKV RNA detected (N=18)			No ZIKV RNA detected (N=36)			
	No. of Responses	**No. of Positives or (%) or IQR Median**		**No. of Responses**	**No. of Positives or Median**	**(%) or IQR**	***p*-value**
Birth							
Gestational age at birth (wks)	18	38	(37 - 38)	36	38	(37 – 38.5)	0.86
Premature (<37 wks gestation)	18	3	(17)	36	5	(14)	0.78
Male sex	18	11	(61)	36	19	(63)	0.56
Caesarean section delivery	14	11	(79)	21	18	(86)	0.58
Apgar score (median)							
At 1 min	13	9	(9 - 9)	20	9	(9 - 9)	0.70
At 5 min	13	10	(10 - 10)	20	10	(9 - 10)	0.26
Anthropometric Measurements							
Head circumference							
cm	17	35	(33.5 – 35.8)	36	35	(34 – 35.8)	0.85
percentile*[1]	17	91	(76 - 96)	36	85	(76 - 97)	0.99
Microcephaly *[2]	54	0	(0)	36	0	0	-
Weight							
kg	18	3.008	(2.745 – 3.421)	36	3.163	(2.930 – 3.420)	0.27
Percentile*[2]	18	51	(33 - 82)	36	68	(45 - 83)	0.26
small for gestational age*[2]	18	0	(0)	36	0	(0)	NA
Length							
cm	18	48	(47 - 49)	36	48	(46.8 - 49)	0.94
percentile*[2]	18	41	(34 - 63)	36	48	(15 - 73)	0.85
Clinical Evaluation							
Abnormal neurological evaluation	18	0	0	36	0		NA
Abnormal ophthalmological exam*[3]	8	1	(13)	14	1	(7)	0.67
Abnormal OAE/AABR*[4]	11	3*	(27)	23	3	(13)	0.31
Radiological Evaluations							
Abnormal exam	14	4	(29)	24	3	(13)	0.21
Cranial US*[5]	14	4	(29)	24	3	(13)	0.21
Cranial MRI*[6]	2	0	(0)	1	0	(0)	NA

ZIKV Diagnostic Testing							
RT-PCR+	18	18	(100)	33	0	(0)	00
Serum	18	14	(78)	30	0	(0)	00
Serum Ct	14	36.5	(36-37)	0	-		
Urine	17	4	(24)	29	0	(0)	0.006
Urine Ct	4	36.5	(31 - 37)	0	-	-	-
First trimester infection	18	2	(11)	36	0	(0)	-
Secondinfection	18	8	(44)	36	0	(0)	NA
Third trimester infection	18	8	(44)	36	0	(0)	NA
MAC-ELISA+ ZIKV	11	0	(0)	5	0	(0)	NA
Hospitalization							
Days	16	2	(2.5 - 3)	21	2	(2 – 5.5)	0.61
NICU admission	18	1	(6)	36	4	(11)	0.50

*[1] (<-2 SD HC)
*[2] (<10th weight percentile)
*[3] Abnormal ophthalmological exam: unilateral chorioretinitis
*[4] OAE = otoacoustic exam: one case confirmed by automated auditory brainstem response (AABR)
*[5]US = ultrasound: With adverse outcomes: lenticulostriate vasculopathy (2), subependymal cysts (3), choroidal cyst (1), bilateral cranial bleed (1)
*[6] MRI = magnetic resonance imaging

Supplemental Table 6. Virological outcomes in newborns exposed to ZIKV during pregnancy

Research Code	Pregnancy trimester in infection	Pregnancy week in birth	Molecular assay	ELISA assay	OBS
			Zika qPCR from umbilical cord umbilical cord blood and/or urine(ct value)	ZIKV NS1 (BioFront)	
4	3rd	38	Umbilical cord blood negative(ct 39.46)	Low positivity	
5	3rd	38	Urine positive(ct 31.1)	NP*	Umbilical cord blood not available
6	2nd	37	Umbilical cord blood negative (ct 39.9)	Negative	
7	2nd	38	Umbilical cord blood positive (ct 37.7)	Low positivity	
9	3rd	37	Umbilical cord blood positive (ct 36.81)	Negative	
11	2nd	39	Umbilical cord blood positive (ct 36.55)	Positive	
14	3rd	37	Umbilical cord blood negative (ct 39.4) and urine positive (ct 36.36)	NP	
16	3rd	36	Umbilical cord blood negative (ct 38.22)	Low positivity	
18	3rd	39	Urine positive (ct 34.98)	NP	Umbilical cord blood not available

20	1st	36	Umbilical cord blood positive (ct 35.81)	NP	Umbilical cord blood not available
21	2nd	37	Umbilical cord blood negative (ct 38.13)	NP	
22	2nd	39	Urine positive (ct 37.72)	NP	Umbilical cord blood not available
23	3rd	39	Umbilical cord blood positive (ct 37.65)	Low positivity	
24	3rd	39	Umbilical cord blood negative (ct inde-terminate)	NP	
26	2nd	39	Umbilical cord blood positive (ct 37.14)	Low positivity	
31	2nd	38	Umbilical cord blood negative (ct inde-terminate)	NP	Umbilical cord blood not available
34	1st	32	Umbilical cord blood positive (ct 36.76)	NP	
36	2nd	37	Umbilical cord blood positive (ct 35.62)	NP	Umbilical cord blood not available
37	3rd	39	Umbilical cord blood negative (ct 38.45)	Positive	
38	3rd	38	Umbilical cord blood positive (ct 35.51)	Low positivity	
39	2nd	39	Umbilical cord blood negative (ct 38.72)	Low positivity	

40	2^{n}	39	Umbilical cord blood positive (ct 37.47)	Positive
41	3rd	38	Umbilical cord blood positive (ct 36.42)	Negative
102	1st	28	Umbilical cord blood positive (ct 35.99)	Low positivity
107	3rd	39	NP	NP
114	3rd	38	Umbilical cord blood (ct 36.31) and urine (ct 36.57) positive	Low positivity

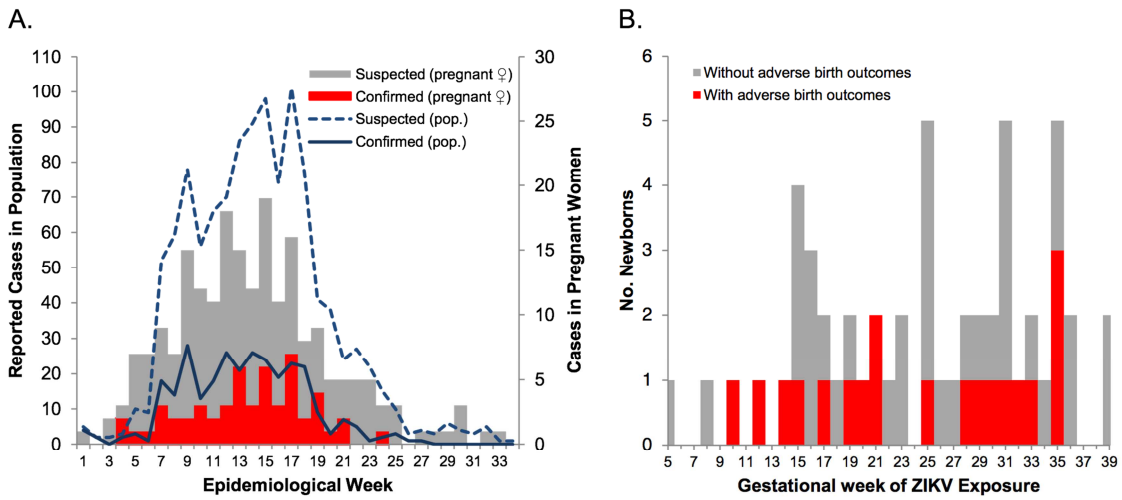
* NP: non performed

Supplemental Table 7. Results of ZIKV RT-PCR in newborns exposed to ZIKV during pregnancy

ZIKV RT-PCR	Blood positive	Blood negative	Non tested
Urine positive	3	0	0
Urine negative	10	28	4
Non tested	4	1	4

FIGURES

Figure 1. Suspected and confirmed cases of Zika virus infection according to epidemiological week, gestational week of ZIKV exposure, and birth rate of ZIKV-infected pregnant women in the city of São José do Rio Preto, São Paulo, Brazil, in 2016



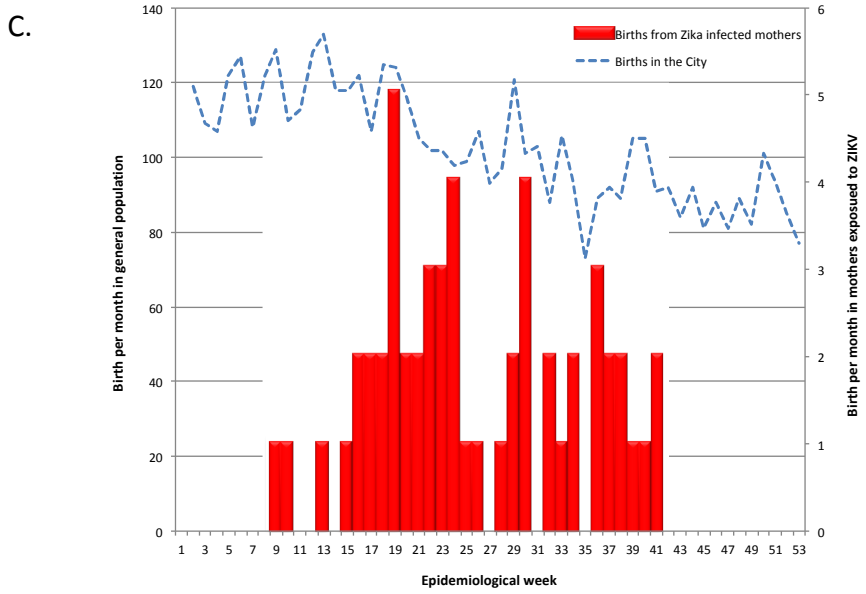
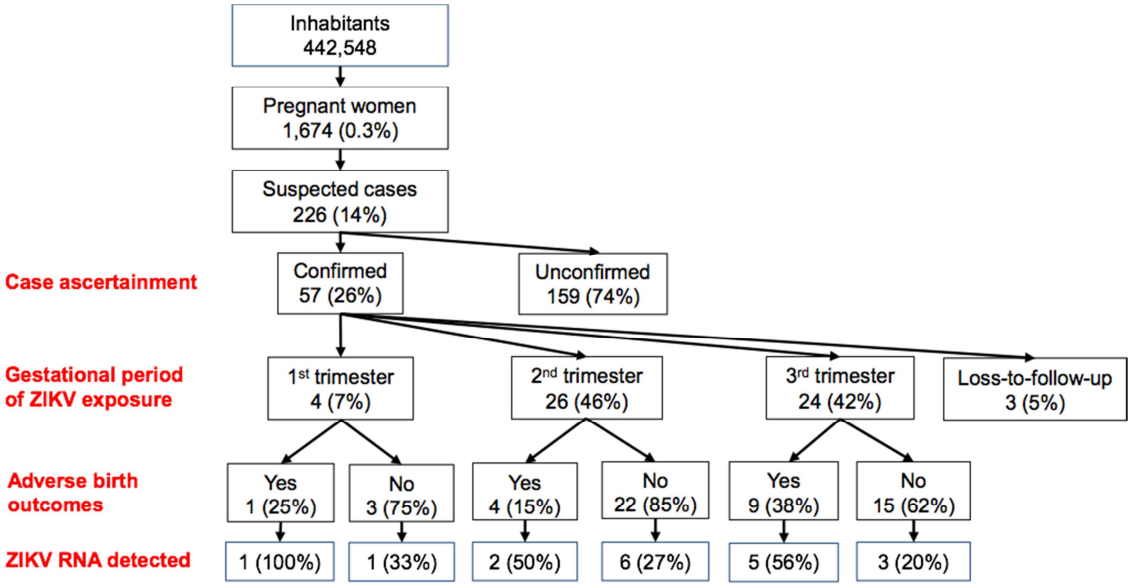


Figure 2. Characteristics of maternal cohort, Zika infection and adverse outcomes in the city of São José do Rio Preto, São Paulo, Brazil, in 2016



SUPPLEMENTAL FIGURE

Supplemental Figure 1. Phylogenetic analysis of ZIKV detected in pregnant women during the 2016 outbreak in the Brazilian city of São José do Rio Preto