Revista da Sociedade Brasileira de Medicina Tropical
Journal of the Brazilian Society of Tropical Medicine
Vol.:52:e20190105: 2019
doi: 10.1590/0037-8682-0105-2019

Major Article

Descriptive study of suspected congenital Zika syndrome cases during the 2015–2016 epidemic in Brazil

***José Henrique Francisco Roma*[1],[2], *Rachel Cruz Alves*[1], *Vanessa Siano da Silva*[1], *Marcio José Ferreira*[1], *Claudinéia de Araújo*[1] *and Juliana Helena Chavéz Pavoni*[1]**

[1]. Curso de Graduação em Medicina, Instituto de Ciências Exatas e Naturais, Universidade Federal de Mato Grosso, Rondonópolis, MT, Brasil.
[2]. Programa de Pós-graduação em Clínica Médica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, SP, Brasil.

Abstract

Introduction: Clinical and epidemiological data on suspected congenital Zika syndrome (CZS) cases from southern Mato Grosso (MT) in Brazil during the Zika virus (ZIKV) outbreak in 2015–2016 were evaluated. **Methods:** This is a descriptive case series study of newborns whose mothers were suspected cases of ZIKV infections during their pregnancies. The medical records of all the suspected CZS cases (mothers and newborns) treated by the specialized ambulatory service from June 2015 to August 2016 were analyzed. **Results:** Twenty suspected CZS cases were included in these analyses. They were categorized into four groups based on the clinical and laboratory findings: confirmed cases (n=1), highly probable cases (n=13), moderately probable cases (n=5), and somewhat probable cases (n=1). The mothers tested negative for STORCH (syphilis, toxoplasmosis, other infections, rubella, cytomegalovirus infection, and herpes simplex) and other important congenital infections; however, specific ZIKV tests were not performed during the study period. Microcephaly was observed in the majority of these newborns, and all the patients showed altered cranial computed tomography image findings. Extracranial abnormalities such as arthrogryposis, and otological and ophthalmological manifestations were also observed. **Conclusions:** Although ZIKV was not confirmed to cause the congenital malformations, this study demonstrated that the clinical and epidemiological findings associated with a STORCH exclusion strengthened the CZS diagnosis. The suspected cases in MT occurred simultaneously with the first CZS cases reported in Brazil, suggesting ZIKV circulation in the study region during the same period.

Keywords: Congenital Zika Syndrome. Epidemiology. Microcephaly. Mato Grosso. Zika virus.

INTRODUCTION

The Zika virus (ZIKV) is a mosquito-borne RNA virus belonging to the *Flavivirus* genus of the *Flaviviridae* family. This virus was initially isolated in 1947 from rhesus macaques in the Zika forest of Uganda. Subsequently, the ZIKV was associated with an acute febrile disease in humans with mild symptoms and no major intercurrences with other arbovirus diseases, such as dengue fever and yellow fever[1,2]. The ZIKV was first detected in Brazil during March 2015 as patients in the state of Bahia presented with dengue fever-like symptoms[1]. The autochthonous circulation and transmission of the ZIKV were confirmed in the country during April and May of the same year[3,4].

The most commonly observed symptoms described for a ZIKV infection include fever, maculopapular rash, arthralgia, and conjunctivitis[2]. During the epidemic outbreak in Brazil, the most commonly observed signs and symptoms were exanthema followed by headache, fever, arthralgia, myalgia, lymphadenopathy, and joint swelling. Conjunctivitis and retro-orbital eye pain were also reported[5]. During the ZIKV epidemic in Brazil, which occurred in 2015 and 2016, a notable increase in microcephaly cases was observed in newborns. Moreover, pregnant women who were symptomatic for ZIKV infections especially in the northeastern region of Brazil inferred the association between this infection and the appearance of neurological abnormalities in their newborns[6,7].

In addition to epidemiological evidence, laboratory findings were also associated with different imaging study patterns, reinforcing ZIKV infection as a causative agent for these congenital abnormalities. Additionally, the ZIKV genome was identified in samples collected from patients with microcephaly

Corresponding author: Dra. Juliana Helena Chavéz Pavoni.
e-mail: julianachavez@yahoo.com.br
Orcid: 0000-0003-2568-9601
Received 18 March 2019
Accepted 30 May 2019

and other malformations during pregnancy and after delivery, and this was extensively documented[8-10]. The size and severity of the outbreak and its link to congenital transmission led the World Health Organization to declare the infection as a public health emergency of international importance in February of 2016[11].

Recent studies have shown that microcephaly is not the only condition caused by congenital Zika syndrome (CZS). Even though it is the most dramatic finding, numerous neurological and motor abnormalities such as arthrogryposis, delayed neuropsychomotor development, intrauterine growth restriction, otological and ophthalmological manifestations, and Guillain-Barré syndrome can also occur[12,13].

Given this context, the objective of this study was to evaluate the clinical and epidemiological data obtained from newborn babies with suspected CZS in the southern Mato Grosso (MT) region of Brazil during the 2015–16 ZIKV outbreak.

METHODS

This descriptive case series study focuses on a group of newborn babies with suspected CZS. Their mothers were likely to be infected by ZIKV during their pregnancies, which occurred during the 2015–16 Brazilian epidemic. The newborn babies were accompanied by the Specialized Ambulatory Service (SAS) of the Rondonópolis municipality in MT from June 2015 to August 2016. All the medical records of the suspected cases were evaluated.

During the outbreak, pregnant women who presented with ZIKV infection symptoms were referred to the Municipal Health Division, which conducted a rigorous pregnancy follow-up. All the newborn babies with suspected CZS were referred to the SAS. At the health center, these babies were followed up by a pediatric physician specializing in infectious diseases as part of the clinical routine established by the Brazilian Ministry of Health protocols. The differential diagnosis was based on the clinical findings of CZS characteristics, family history, and laboratory tests for the main congenital infections affecting the central nervous system (CNS), as well as ultrasound, magnetic resonance imaging, and computed tomography (CT) findings.

Initially, 41 medical records were included in this study. Case selection was based on the following inclusion criteria: CZS suspicion or a microcephaly diagnosis confirmed by an ultrasound examination during pregnancy or at birth, with findings suggestive of a congenital infection. The cases were defined as probable CZS according to the protocol for surveillance and response to the occurrence of microcephaly and/or CNS Alterations of the Brazilian Ministry of Health[7].

The CZS cases that were included were classified into four groups (adapted from França et al.)[14]:

1. Confirmed cases, as defined by laboratory evidence of a ZIKV infection via an immunological examination (immunoglobulin M, IgM) or ZIKV genome detection using reverse transcriptase – polymerase chain reaction (RT-PCR) in a maternal or newborn sample.
2. Highly probable cases, as defined by neuroimaging findings suggestive of CZS, associated with negative STORCH laboratory results (syphilis, toxoplasmosis, others such as varicella-zoster and parvovirus B19, rubella, cytomegalovirus infection, and herpes simplex).
3. Moderately probable cases, as defined by neuroimaging findings suggestive of CZS and incomplete or nonexistent STORCH testing.
4. Somewhat probable cases, as defined by neuroimaging findings with little detail and a negative STORCH diagnosis or nonexistent STORCH testing.

When considering other possible CNS malformation causes, the following exclusion criteria were defined: positive serological STORCH results associated with a negative diagnosis for the ZIKV in a maternal or newborn sample, the diagnosis of a genetic syndrome identified during prenatal care or after birth, medical records with missing data, newborn prenatal and perinatal complications, and exposure to licit or illicit drugs or teratogenic substances during pregnancy.

Ethical considerations

All data described in this report were collected from medical records, and this study was approved by the Ethics Committee of the Research of the Federal University of Mato Grosso in Brazil (ECR 87100418.6.0000.8088).

RESULTS

Forty-one newborn babies from the Rondonópolis maternity ward were referred to the SAS owing to suspicion of CZS from June 2015 to August 2016. The medical follow-ups were performed as indicated previously, and all the data were registered in the SAS medical records. After considering the inclusion and exclusion criteria, twenty of the forty-one medical records were included in the data analyses. Among the excluded cases two were diagnosed with genetic syndromes, three had confirmed congenital toxoplasmosis based on serological testing, and the sixteen remaining patients either had incomplete medical records without imaging evaluations or laboratory test results, or due to lack of medical follow-ups at the healthcare center.

A ZIKV laboratory diagnosis was not fully available in Brazil during the beginning of the epidemic. Hence, a case classification into four CZS groups was proposed based on the clinical and laboratory findings. In our study, only one suspected case was confirmed through positive findings for IgM antibodies against the ZIKV, and this case corresponded to Group One (confirmed cases). The highly probable CZS case group had thirteen newborn babies who were included in Group Two. Five children were classified into Group Three, i.e., they had moderately probable CZS. Finally, only one patient was considered as somewhat probable CZS case (Group Four) (**Tables 1 and 2**).

The mothers' sociodemographic characteristics, obstetric histories, and prenatal care data were self-reported and analyzed (**Table 1**). In the cohort, there were several young mothers, with a mean age of ~24.35 years old [standard deviation (SD) 4.82 years]. The youngest mother was 18 years old, while the oldest was 30 years old. With regard to skin color, the majority of the mothers was brown (fifteen mothers). The mean family income was ~1.45 times the Brazilian minimum wage (BMW), which corresponds to R$880 (Brazilian reals) or approximately $233.57 (US dollars). The lowest income reported was 0.6 times the BMW, while the highest was 3.4 times the BMW. Four of the mothers reported the continuous use of

TABLE 1: Epidemiological, clinical, and prenatal data, along with laboratory findings from mothers and complications during pregnancy.

Classifications Cases	Patient n°	Mother Age (years)	Race/Color	Family Income/ Basic Salary	Schooling Level(years)	Continuous Drugs Use	Prenatal appointments	Nº of Pregnancies	Live Births	Abortions	X-Ray Trim Exp	Iron/Folic Acid Supl	Diabetes Gestational	Skin Rash /Trimester	STORCH									Complications on Pregnancy
															Syp	Toxop		RubV		CMV		HerpV		
																IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	
Confirmed	1	29	Wh	2.2	12	-	20	2	2	-	-	Y	N	1st	**N**	N	N	**N**	**P**	-	-	-	-	Placental abruption
Highly Probable	2	20	Bw	1	12	-	10	1	1	-	-	Y	N	-	**N**	N	N	**N**	**P**	N	P	**N**	**N**	-
	3	18	Bk	1	9	-	9	2	2	-	-	Y	N	-	**N**	N	P	**N**	**P**	N	P	**N**	**N**	-
	4	23	Bw	1.5	9	-	6	6	4	2	-	Y	N	-	**N**	N	N	-	-	-	-	-	-	Anemia
	5	23	Bw	1	5	Insulin	10	5	5	-	-	Y	Y	1st	**N**	N	P	**N**	**P**	-	-	-	-	Urinary tract infection, gestational diabetes, hypertension, hypothyroidism
	6	27	Bw	1.4	12	-	9	4	3	1	-	Y	N	-	**N**	N	P	**N**	**N**	-	-	**N**	**N**	Urinary tract infection, placental abruption
	7	17	Bw	-	9	-	9	1	1	-	3rd	Y	N	-	**N**	N	N	-	-	-	-	-	-	Urinary Tract Infection, Oligohydramnios
	8	18	Wh	1.8	9	-	5	1	1	-	-	Y	N	2nd	**N**	N	N	**N**	**N**	-	-	**N**	**N**	-
	9	30	Bw	-	5	-	6	2	1	1	-	Y	N	2nd	**N**	N	N	**N**	**P**	N	P	-	-	-
	10	17	Bw	1.1	9	-	8	1	1	-	-	Y	N	3rd	**N**	N	N	-	-	N	P	-	-	-
	11	30	Bw	1	5	-	5	7	5	2	1st	Y	N	2nd	-	-	-	-	-	-	-	-	-	Urinary Tract Infection, Anemia, Oligohydramnios
	12	30	Bw	1	5	-	5	7	5	2	1st	Y	N	2nd	-	-	-	-	-	-	-	-	-	Urinary Tract Infection, Anemia, Oligohydramnios
	13	27	Bw	2.3	12	Acetylsalicylic acid	-	3	2	1	-	Y	N	2nd	**N**	N	N	**N**	**N**	-	-	**N**	**N**	Urinary Tract Infection, Oligohydramnios
	14	30	Bw	1.6	16	-	10	2	1	1	-	Y	N	1st	**N**	N	P	-	-	-	-	-	-	-
Moderately Probable	15	24	Bw	3.4	12	-	7	2	2	-	-	-	N	1st	**N**	N	P	-	-	N	P	-	-	Urinary Tract Infection
	16	28	Yw	2.3	9	-	6	4	4	-	-	Y	N	1st	**N**	N	N	**N**	**P**	N	P	**N**	**P**	Anemia
	17	19	Bw	1.4	12	Ranitidine, Alprazolan, Fenoterol	5	2	1	1	-	Y	N	1st	**N**	N	P	-	-	N	N	-	-	Oligohydramnios
	18	22	Bw	1	5	-	9	2	2	-	-	Y	N	1st	-	-	-	-	-	-	-	-	-	-
	19	27	Bw	3.4	9	Ergotamine	-	2	2	-	-	Y	N	-	**N**	N	N	**N**	**N**	N	N	**N**	**N**	Placental Insufficiency
Somewhat Probable	20	28	Bw	0.6	5	-	6	3	3	-	-	Y	N	-	**N**	N	P	-	-	-	-	-	-	Urinary Tract Infection, Oligohydramnios, Umbilical Alteration

Wh: White; **Bk:** Black; **Yw:** Yellow; **Bw:** Brown; **Basic Salary:** R$ 880,00; **X-Ray Trim Exp:** X-Ray Trimester Exposure; **Iron/Folic Acid Supl:** Iron/Folic Acid Supplementation; **N:** Negative; **P:** Positive; **Syp:** Syphilis; **CMV:** Cytomegalovirus; **HerpV:** Herpes Virus; **RubV:** Rubella Virus; **Toxop:** Toxoplasmosis; **IgG:** Immunoglobulin G; **IgM:** Immunoglobulin M.

TABLE 2: Clinical and imaging data, laboratory findings, and CZS classification of newborns.

Classifications Cases	Patient n°	Sex	GA at Birth (w)	Childbirth	HC at Birth (cm)	HC Escore-Z	CT alterations	MRI Alterations	Transf. Cranial US	Abd US	ECG	EEG	Neonatal HS	Ophthal Findings	ZikV		STORCH								
																	Syp	Toxop		RubV		CMV		HerpV	
															PCR	IgM		IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
Confirmed	**1**	F	39	C-s	32	- 0.83	Y	-	-	-	-	Altered	Normal	Normal	-	P	**N**	N	N	**N**	**-**	N	-	**-**	**-**
Highly Probable	**2**	M	38	C-s	28	- 4.01	Y	-	Y	Normal	Altered	-	Altered	Normal	N	-	**N**	N	N	**N**	**P**	N	N	**N**	**N**
	3	M	40	Vd	31	- 2.77	Y	-	-	-	-	Altered	Normal	Normal	-	-	**N**	N	P	**N**	**P**	N	P	**N**	**P**
	4	F	40	-	31	- 2.22	Y	Y	-	Normal	Normal	Altered	Normal	Normal	-	-	**N**	N	P	**N**	**P**	N	P	**N**	**P**
	5	M	37	C-s	34	0.79	Y	Y	-	Normal	Normal	Altered	Normal	Normal	N	-	**N**	N	P	**N**	**P**	N	P	**N**	**P**
	6	F	34	C-s	27	- 2.95	Y	Y	Y	Normal	Altered	-	Normal	Normal	-	-	**N**	N	P	**N**	**P**	N	P	**N**	**P**
	7	F	36	C-s	27	- 3.74	Y	-	-	-	Normal	Altered	Normal	Normal	N	-	**N**	N	P	**N**	**N**	N	P	**N**	**P**
	8	F	39	C-s	32	- 1.28	Y	-	-	Normal	Normal	-	Normal	-	-	-	**N**	N	P	**N**	**P**	N	P	**N**	**P**
	9	M	39	C-s	30	- 3.13	Y	-		-	Altered	Altered	Normal	Strabismus	-	-	**N**	N	N	**N**	**P**	N	P	**N**	**P**
	10	M	34	Vd	27	- 2.82	Y	Y	Y	Normal	Normal	-	Altered	-	-	-	**N**	N	P	**N**	**N**	N	P	**N**	**P**
	11	M	33	C-s	29	- 1.35	Y	-	-	-	Normal	-	Normal	Normal	-	-	**N**	N	P	**N**	**P**	N	P	**N**	**P**
	12	M	33	C-s	24	- 4.25	Y	-	Y	-	Altered	-	Normal	Normal	-	-	**N**	N	P	**N**	**P**	P	P	**N**	**P**
	13	M	36	C-s	29	- 2.69	Y	-	Y	Normal	Normal	-	Altered	Normal	-	-	**N**	N	P	**N**	**P**	N	P	**N**	**P**
	14	F	38	C-s	30	- 2.56	Y	Y	-	Normal	Normal	Altered	Normal	Normal	-	-	**N**	N	P	**N**	**P**	N	P	**N**	**P**
Moderately Probable	**15**	M	38	C-s	28	- 4,01	Y	-	-	Normal	Normal	-	Altered	Normal	-	-	**N**	N	P	**N**	**P**	N	P	**-**	**-**
	16	F	39	C-s	31	- 2.14	Y	-	-	Normal	-	-	Normal	-	N	-	**-**	N	N	**N**	**P**	N	P	**N**	**P**
	17	F	35	C-s	26	- 3.64	Y	-	-	-	Normal	Altered	Altered	-	N	-	**-**	N	P	**N**	**P**	N	P	**N**	**P**
	18	M	39	Vd	31	- 2.41	Y	-	-	Normal	Altered	-	Normal	Isotropy	-	-	**-**	N	P	**N**	**P**	N	P	**-**	**-**
	19	F	37	C-s	31	- 0.96	Y	-	-	-	Normal	-	Normal	-	N	-	**N**	-	-	**N**	**N**	-	-	**-**	**-**
Somewhat Probable	**20**	F	37	C-s	32	- 0.52	Y	-	-	-	-	-	Normal	-	-	-	-	N	P	**N**	**P**	N	P	**N**	**P**

CZS: Congenital Zika Syndrome; **GA:** Gestational Age; **W:** Weeks; **HC:** Head Circumference; **Escore-Z:** Newborn Biometry Intergrownth-21th standard/reference; **Vd:** Vaginal delivery; **C-s:** Cesarean section; **Y:** yes; **CT:** Cranial Tomography; **MRI:** Magnetic Resonance Imaging; **Transf. Cranial US:** Transfontanellar Cranial Ultrasound Alteration; **Abd US:** Abdominal Ultrasound; **ECG:** Echocardiogram; **EEG:** Electroencephalogram; **N:** Negative; **P:** Positive; **Syp:** Syphilis; **CMV:** Cytomegalovirus; **HerpV:** Herpes Virus; **RubV:** Rubella Virus; **Toxop:** Toxoplasmosis; **IgG:** Immunoglobulin G; **IgM:** Immunoglobulin M; **Neonatal HS:** Neonatal Hearing Screening.

drugs/medications during pregnancy. Only four mothers were reported to be primiparous. Eight of the mothers had at least one previous abortion, and three of the mothers were exposed to x-rays during pregnancy. Thirteen of the women presented with maternal complications during pregnancy and the most frequently seen complications were urinary tract infections (eight), oligohydramnios (six), maternal anemia (four), placenta alterations (three), and gestational diabetes (one) (**Table 1**).

During the study period, serological or RT-PCR tests specific to the ZIKV were not used to diagnose in all the mothers during or following pregnancy. All 41 mothers referred to the SAS had associations with ZIKV infections during their pregnancies, with or without clinical signs. Of the twenty babies included in the study, thirteen mothers reported clinical symptoms of ZIKV infection especially cutaneous rashes during their pregnancies (**Table 1**).

When analyzing the newborn data, all the clinical and laboratory data were registered in the medical records by the SAS medical staff. The data analyses of this information (**Tables 2 and 3**) showed an equal gender distribution among the CZS cases: ten female and ten male cases. The mean gestational age at birth was ~37.05 weeks (SD 2.25 weeks), with the lowest at 30 weeks and the highest at 40 weeks. Most patients (80%) were born via cesarean sections (16/20). Half of the births occurred at the end of 2015, during November (five births) and December (five births). The mean birth weight among the newborns was 2,458 g (SD 0.62 g). Nine of the newborns had low birth weights (< 2,500 g), and one had a very low birth weight (< 1,500 g) (**Table 3**). The occurrence of microcephaly was considered to be a hallmark of CZS in this study, and it affected the majority of newborns (**Table 3**). The mean head circumference (HC) at birth was ~29.5 cm (SD 2.48 cm), the lowest and highest being 24 cm and 34 cm, respectively. The HC z-score was calculated according to the standards/references of the International Fetal and Newborn Growth Consortium for the 21st Century newborn biometry, which considered the sex, gestational age at birth, height, and weight (**Table 2**). The mean HC z-score was -2.35 (SD 1.93); the lowest was -4.25, and the highest was 0.79 (**Table 2**). In most patients, the microcephaly diagnosis (mean z-score of < -2.0) occurred after birth, and six of these cases had severe microcephaly (mean z-score of < -3.0) (**Tables 2 and 3**).

Specific ZIKV laboratory analyses were not performed in all patients. Only seven patients were tested for ZIKV; one of them had positive IgM serology results, and this was the only laboratory-confirmed case. The other six serum samples were submitted for RT-PCR testing, and all the samples were found to be negative for the ZIKV genome.

All newborns included in this study underwent a cranial CT scan, and all the patients exhibited alterations in the image analyses. The main imaging findings and frequencies are described in **Table 3**. Electroencephalogram analyses were only performed in eight patients, and all cases presented abnormal tracing with dyssynchronous activity, characteristic graphs of cerebral irritability, and nonspecific pathological slowing (**Table 2**).

The extracranial examinations were performed according to the protocol of the Brazilian Ministry of Health. Therefore, abdominal ultrasounds were conducted in ten patients, all of whom presented normal results. Sixteen of the patients underwent echocardiography, three of them had patent oval foramen, and two had interatrial communications. Neonatal hearing screenings were performed in all patients, and five of them presented with hearing losses ranging from moderate to severe. Fourteen of the newborn babies underwent ophthalmological assessments, and some abnormalities were observed in two patients (**Table 2**). The other extracranial malformations are summarized in **Table 3**.

DISCUSSION

The impact of the ZIKV epidemic in Brazil is mainly related to the occurrence of CZS, which leads to profound neurodevelopmental impairments in the affected children. Our study described data from the first suspected cases of congenital alterations related to maternal ZIKV infections in the southern region of MT in west central Brazil in November of 2015, revealing fifty-four suspected cases concentrated within the southern region[15,16]. The clinical and epidemiological findings presented in this study support the simultaneous introduction of the ZIKV and the occurrence of CZS cases in the northeastern[14], central western, and other regions of Brazil based on the date of birth of the first suspected case in this region in June of 2015. The mother reported a skin rash in May 2015, but had no travel history during her pregnancy. Thus, the maternal ZIKV infection symptoms were observed at approximately 26–27 weeks of pregnancy, while CZS was present at birth inferring that the maternal infection was an autochthone case in our study. Although this case was not confirmed by molecular or serological testing, it was considered to be a highly probable case because the STORCH results were negative, and the case exhibited imaging findings, such as calcifications, cerebral atrophy, and ventriculomegaly. These data strengthened the diagnosis of CZS.

A nonuniform profile in the geographical distribution of CZS cases throughout the national territory was observed. From 2015 to 2016, 2,176 suspected CZS cases were reported in Pernambuco, which represented 21.3% of the Brazilian cases. At the same time, only 319 cases were reported in MT (3.1%)[17]. Therefore, an unusually higher number of CZS cases were concentrated in the northeast when compared to the central western region, which might be explained by the higher *Aedes aegypti* (the principal ZIKV vector) infestation risk related to increased poverty and an elevated demographic density[18]. The increase in the principal vector number, which is associated with high demographic indices, could facilitate the spread of the ZIKV. Moreover, poverty contributes to the vector proliferation, malnutrition, and poor health which together can affect the immunity and increase the risk of ZIKV infections and CZS cases.

The social data analysis of the mothers who self-reported demonstrated that they earned less than two times the BMW family income and lacked proper education reflecting a fragile

TABLE 3: Epidemiological and clinical data classification based on the presence of microcephaly.

	Normal HC[a] (n=6) nº positive/nº tested	Microcephaly[a] (n=8) nº positive/nº tested	Severe Microcephaly[a] (n=6) nº positive/nº tested
Classification of Cases			
Confirmed	1/6	-	-
Highly probable cases	3/6	6/8	4/6
Moderate probable cases	1/6	2/8	2/6
Somewhat probable cases	1/6	-	-
Sex			
Male (n=10)	2	4	4
Female (n=10)	4	4	2
Gestational age of newborn			
At term, 37 at 41 weeks and 6 days	5/6	5/8	3/6
Preterm, < 37 weeks	1/6	3/8	3/6
Post term, ≥ 42 weeks	-	-	-
Weight for gestational age			
Normal	6/6	8/8	4/6
Small for gestational age	-	-	2/6
Birthweight			
<1500 g	-	-	1
1500 – 2499 g	3	3	3
≥ 2500 g	3	5	2
Mother self-reported rash during pregnancy (n=20)			
No rash	2/6	3/8	2/6
First Trimester	2/6	3/8	2/6
Second Trimester	2/6	1/8	2/6
Third Trimester	-	1/8	-
Zika Virus Laboratorial Test in Newborn			
RT-PCR (n=6)	-/2	-/1	-/3
ELISA IgM (n=1)	1/1	-	-
Brain abnormal findings in CT or MRI (n=20)	6/6	8/8	6/6
Abnormal findings in TU (n=7)	-/1	3/4	2/2
Abnormal findings in Ophthal Evaluation (n=14)	-/3	1/7	1/5
Abnormal findings in NHS (n=19)	-/5	2/8	3/6
Main cranial imaging findings			
Calcifications	5/6	7/8	6/6
Malformation of cortical development, including lissencephaly	1/6	8/8	2/6
White substance hypodensity	2/6	1/8	-/6
Ventriculomegalia	1/6	6/8	4/6
Agenesis/dysgenesis of corpus callosum	-	5/8	1/6
Subarachnoid Spaces Prominence	-	1/8	1/6
Hydrocephalus	1/6	1/8	-/6
Myelination Failure	1/6	-	-
Craniofacial Disproportion	4/6	8/8	6/6
Main extracranial findings			
Anemia	3/6	1/8	2/6
Arthrogryposis	1/6	-	-
Auricular System Alteration	-	1/8	-
Circulatory System Alteration	1/6	-	2/6
Jaundice	3/6	3/8	6/6
Sickle cell trait	1/6	-	-

[a]measured at birth; **HC:** Head Circumference; **CT:** Computed Tomography; **MRI:** Magnetic Resonance Imaging; **RT-PCR:** Reverse Transcriptase-Polymerase Chain Reaction; **NHS:** Neonatal Hearing Screening; **TU:** Transfontanela Ultrasound.

socioeconomic background. Only four mothers reported the use of drugs and fetal development was not affected in these reported cases. The most commonly seen maternal complication during pregnancy was the presence of oligohydramnios (six cases), which was also described as having a possible relationship with a ZIKV infection in others studies[19].

The majority of the suspected cases in our study was not confirmed by specific serological or molecular diagnostic testing because no standard or commercial tests were available during ZIKV introduction in Brazil. Only one child in this study had a laboratory confirmation of a ZIKV infection using an enzyme-linked immunosorbent assay (ELISA) IgM detection. An ELISA

test can exhibit a cross reaction with other arboviruses, such as those causing dengue fever and yellow fever; however, the CZS diagnosis in this case was determined by the clinical and epidemiological features[20]. According to their medical records, RT-PCR was performed using the serum of six newborns, and none of the samples tested positive for the viral genome. Although the persistent detection of ZIKV RNA has been described in the cerebrospinal fluid and blood samples of an infant with CZS[21], the negative results observed in our study may be explained by certain limitations, such as the kinetics of viremia, viral RNA degradation during sample processing and storage, and the limited RT-PCR sensitivity. The data obtained from the medical records of the other newborns in our study showed that the specimens were not submitted for molecular investigations, probably due to the late maternal ZIKV infection period, the unavailability of a diagnostic facility, or even neglecting the ZIKV as a causative agent at the time of birth.

In this emerging CZS epidemic scenario, without confirmatory and ZIKV-specific diagnostic tools, this study adopted a classification based on epidemiological data, clinical features, and the exclusion of positive STORCH results obtained from the medical records of the children and their respective mothers. This classification is similar to the one adopted by França et al.[14], and it might be useful for further retrospective analyses, such as the one proposed by this study. Except for only one confirmed case, all patients in this study were classified as having highly, moderately, and somewhat probable CZS cases.

The patients who did not undergo specific confirmatory tests and those who had negative RT-PCR results were classified as highly probable cases with the exclusion of congenital malformations due to STORCH, genetic, and teratogenic drugs. When the STORCH causes were not excluded, the moderately probable case classification was adopted, because even when presenting imaging findings suggestive of CZS associated with ZIKV symptoms during pregnancy (four to five mothers), it is not possible to exclude other congenital infectious diseases that are also endemic to the study region.

According to the Brazilian Ministry of Health guidelines[7], the highly and moderately probable cases in this study were defined as *Confirmed Cases of Congenital Infection without Etiological Identification.* This classification includes newborns or stillbirths with clinical findings suggestive of a maternal infection, maternal exanthema, or unspecified fever, with inconclusive or negative results for the STORCH and ZIKV tests. The significant 134% increase in microcephaly reports in November and December 2015 and the microcephaly occurrences (14 cases) in the study reinforce our classification focused on CZS based on epidemiological and clinical data[15].

The initial clinical evaluation of the only somewhat probable case revealed an absence of microcephaly and little detailed neuroimaging findings, which included periventricular white matter hyposensitivity without calcifications. Moreover, this newborn presented incomplete STORCH results and no maternal skin rash was observed during pregnancy. Although this newborn was considered to have suspected CZS, more detailed information about congenital alterations should be determined in order to confirm this diagnosis.

According to CZS data, most of our patients presented morphological CNS alterations, including microcephaly (2 SDs below the mean) and severe microcephaly (3 SDs below the mean), cortical and subcortical junction calcifications, and other encephalic anomalies, which are followed by ocular failing, hearing loss, and craniofacial disproportion[22]. Even though microcephaly was initially characterized as the main finding of CZS, new reports have shown other CNS deformities that are not visible in the absence of microcephaly[13]. Some of the patients in our study did not present with microcephaly, although encephalic injury was reflected by microcalcifications, lissencephaly, ventriculomegaly, white substance hypodensity, myelination defects, and corpus callosum dysgenesis, which corroborates early reports. It is important to highlight that all these forms of CNS impairment are commonly observed in CZS but are not pathognomonic, and they can be observed in other congenital syndromes[20]. In addition, isolated HC measurement is an ambiguous parameter for determining the presence of microcephaly; other CNS imaging findings associated with craniofacial disproportion should be also considered. This was observed in the only confirmed CZS case (patient one, **Table 2**), in which the HC measurement was considered to be at the inferior limit of normality, but significant craniofacial disproportion was observed, leading to the clinical characterization of CZS. Other highly probable cases showed similar data (patients five and eight, **Table 2**). Moreover, the study cases presented other important clinical findings, such as low birthweights, arthrogryposis[23,24], circulatory system alterations, irritability, jaundice, anemia, and sickle cell traits.

In conclusion, the evaluation of the clinical and epidemiological data associated with the adopted classification strengthened the CZS diagnosis in the study cases, even without confirmatory maternal ZIKV infection results. Furthermore, these cases occurred simultaneously with the first reported CZS cases in Brazil, suggesting ZIKV circulation in the southern region of MT in 2015. Inexpensive diagnostic tools, vaccine development, and vector control are essential for preventing future outbreaks and reducing the effect of CZS on newborns and children. Furthermore, medical and psychological support for mothers and their affected children is indispensable.

ACKNOWLEDGEMENTS

Authors are grateful to Aparecida Carvalho da Silva, technical director and Aline Spanevello Alvares, nurse in chief of SAS, Rondonópolis. Mariuva Valentim Chaves, municipal health in chief and Ednaldo Santos de Souza, health counsellor for technical support and data viability.

Conflict of interest

The authors declare that they have no conflict of interest.

Financial support

This work did not receive any funding support.

REFERENCES

1. Campos GS, Sardi SI, Bandeira AC. Zika Virus Outbreak, Bahia, Brazil. Emerg Infect Dis. 2010;21(10):1885-86.

2. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009;360(2):36-43.
3. Zanluca C, Melo VCA, Mosimann ALP, Santos GIV, Santos CN, Luz K. First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz. 2015;110(4):569-72.
4. Ministério da Saúde. Confirmação do Zika Vírus no Brasil [Internet]. Brasília, DF: Ministério da Saúde; 14 de Maio 2015 [atualizado 2015 Maio 14; citado 2018 Setembro 18]. Available from: http://portalms.saude.gov.br/noticias/svs/17702-confirmacao-do-zika-virus-no-brasil.
5. Cerbino-Neto J, Mesquita EC, Souza TML, Parreira V, Wittlin BB, Durovni B, et al. Clinical Manifestations of Zika Virus Infection, Rio de Janeiro, Brazil, 2015. Emerg Infect Dis. 2016;22(7):1318-20.
6. Ministério da Saúde. Ministério da Saúde confirma relação entre vírus Zika e microcefalia [Internet]. Brasília, DF: Ministério da Saúde; 30 de Novembro de 2015 [atualizado 2016 Janeiro 21; citado 2018 Agosto 30]. Available from: http://www.blog.saude.gov.br/index.php?option=com_content&view=article&id=50399&catid=566&Itemid=50155.
7. Ministério da Saúde. Protocolo de vigilância e resposta à ocorrência de microcefalia [Internet]. Brasília, DF: Ministério da Saúde (Brasil). Available from: http://portalsaude.saude.gov.br/images/pdf/2016/janeiro/22/microcefalia-protocolo-de-vigilancia-e-resposta-v1-3-22jan2016.pdf.
8. Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? Ultrasound Obstet Gynecol. 2016;47(1):6-7.
9. 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(10):951-8.
10. Aragão MFV, Linden VVD, 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.
11. World Health Organization (WHO). WHO Director-General summarizes the outcome of the Emergency Committee regarding clusters of microcephaly and Guillain-Barré syndrome [Internet]. Geneva, SWI: World Health Organization. 2016 [cited 2018 July 7]. Available from: http://www.who.int/mediacentre/news/statements/2016/emergency-committee-zika-microcephaly/en/.
12. 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(24):2321-34.
13. Ventura CV, Maia M, Dias N, Ventura LO, Belfort R Jr. Zika: neurological and ocular findings in infant without microcephaly. The Lancet. 2016;387(10037):2502.
14. França GV, Schuler-Faccini L, Oliveira WK, Henriques CM, Carmo EH, Pedi VD, et al. Congenital Zika virus syndrome in Brazil: a case series of the first 1501 livebirths with complete investigation. The Lancet. 2016;388(10047):891-7.
15. Ministério da Saúde (MS). Secretaria de Vigilância em Saúde. Situação epidemiológica de ocorrência de microcefalias no Brasil. Brasília: MS; 2015. 34p.
16. Secretaria de Saúde de Mato Grosso. Monitoramento dos Casos de Microcefalia em Mato Grosso [internet]. Cuiabá, MT: Secretaria de Saúde de Mato Grosso. 2016 [citado 2018 Agosto 15]. Available from: http://www.saude.mt.gov.br/arquivo/5144.
17. Ministério da Saúde. Monitoramento integrado de alterações no crescimento e desenvolvimento relacionadas à infecção pelo vírus Zika e outras etiologias infecciosas. Brasília, DF: Ministério da Saúde; 2017 [citado 2019 Janeiro 18]. Available from: http://portalarquivos2.saude.gov.br/images/pdf/2017/fevereiro/27/2017_003.pdf.
18. Alirol E, Getaz L, Stoll B, Chappuis F, Loutan L. Urbanization and infectious diseases in a globalized world. Lancet Infect Dis. 2011;10(2):131-41.
19. Carvalho FH, Cordeiro KM, Peixoto AB, Tonni G, Moron AF, Feitosa FE, et al. Associated ultrasonographic findings in fetuses with microcephaly because of suspected Zika virus (ZIKV) infection during pregnancy. Prenat Diagn. 2016;36(9):882-7.
20. Moreira-Soto A, Cabral R, Pedroso C, Eschbach-Bludau M, Rockstroh A, Vargas LA, et al. Exhaustive TORCH Pathogen Diagnostics Corroborate Zika Virus Etiology of Congenital Malformations in Northeastern Brazil. mSphere. 2018;3(4) pii:e00278-18.
21. Brito CAA, Henriques-Souza A, Soares CRP, Castanha PMS, Machado LC, Pereira MR, et al. Persistent detection of Zika virus RNA from an infant with severe microcephaly - a case report. BMC Infect Dis. 2018;18(1):388.
22. Moore CA, Staples JE, Dobyns WB, Pessoa A, Ventura CV, Fonseca EB, et al. Characterizing the pattern of anomalies in congenital Zika syndrome for pediatric clinicians. JAMA Pediatr. 2017;171(3):288-95.
23. Ribeiro BNF, Muniz BC, Gasparetto EL, Ventura N, Marchiori E. Congenital Zika syndrome and neuroimaging findings: what do we know so far? Radiol Bras. 2017;50(5):314-22.
24. Leyser M, Fernandes A, Passos P, Pupe C, Matta AP, Vasconcelos MM, et al. Microcephaly and arthrogryposis multiplex congenita: the full-blown CNS spectrum in newborns with ZIKV infection. J Neurol Sci. 2017;372:73-4.