Article

Zika virus infection in a cohort of pregnant women with exanthematic disease in Manaus, Brazilian Amazon

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Abstract: The epidemic transmission of Zika virus (ZIKV) in Brazil has been identified as a cause of microcephaly and other neurological malformations in babies of ZIKV-infected women. This study provides a descriptive analysis, since the onset of symptoms to the delivery, of a cohort who were registered as having ZIKV infection in pregnancy, from November 2015 to December 2016. Suspected cases were registered at a referral center for infectious and tropical diseases in Manaus, in the Brazilian Amazonian region. A total of 834 women with suspected ZIKV in pregnancy were included, of whom 91.4% had confirmed pregnancy. Reverse-transcriptase polymerase chain reaction (RT-PCR) confirmed ZIKV infection in 42.2% of the cohort. In 35.2% of the cohort, ZIKV was the sole infection identified. Severe adverse pregnancy outcomes (abortion, stillbirth, or microcephaly) were observed in both RT-PCR ZIKV-positive (4.96%) and ZIKV-negative (2.15%) cases. Women with suspected ZIKV infection were much more likely to have adverse pregnancy outcomes if they were symptomatic during the first trimester of pregnancy (odds ratio 10.5; 95% confidence interval 4.0–27.0; $p<0.001$). Among pregnant women with suspected ZIKV infection, the occurrence of symptoms in the first trimester is associated with an especially high risk of severe adverse pregnancy outcomes.

Keywords: Amazonian region, exanthematic disease in pregnancy, Torch syndrome, ZIKV infection, abortion, stillbirth, Microcephaly, preterm delivery, low birth weight

1. Introduction

Since 2015, the transmission of Zika virus (ZIKV) in Brazil has been associated with neurological alterations and malformations in babies exposed during pregnancy [1-6]. Although this association has been the subject of many studies, several of its most important features are not fully understood.

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For example, considerable uncertainty remains regarding the sizes of the effects of exposure during pregnancy on the following events: maternal infection, maternal symptoms, fetal infection, fetal loss, and the clinically-varied sequelae that occur in newborns and children in their first years of life. One of the main obstacles to understanding the effects of ZIKV is the difficulty of diagnosing the disease during the acute event. Consequently, ZIKV diagnosis is often made in symptomatic persons or in patients receiving care at some point after the onset of symptoms. In the present study, our research group has described the main characteristics and pregnancy outcomes of a cohort of women in the Amazonian region of Brazil who were registered as having suspected ZIKV infection during the period of peak ZIKV transmission mainly in 2016.

2. Materials and Methods

This study describes a cohort of notified-as-suspected cases of ZIKV in pregnancy. The patients in the cohort were followed up from the onset of symptoms until delivery during a period of 58 weeks, which lasted from the 47th epidemiological week of 2015 to the last epidemiological week of 2016. Patients were attended to at the Doctor Heitor Vieira Dourado Foundation for Tropical Medicine (FMT-HVD), a referral center for infectious diseases that is in Manaus, in the state of Amazonas, Brazil. FMT-HVD is neither an antenatal clinic nor a maternity center.

Study Population

During the period of epidemic transmission, pregnant women were regarded as a high-priority group in efforts diagnose ZIKV infection quickly and with high specificity using reverse transcription polymerase chain reaction (RT-PCR). Blood and urine samples were collected from symptomatic women of childbearing age, who also received ambulatory care.

Definitions

Pregnancy was confirmed using a beta human chorionic gonadotropin (HCG) test, ultrasonography (USG), or clinic examination. Stage of pregnancy was calculated from the first day of the last regular menstruation period, by USG performed in the first trimester of pregnancy, or otherwise determined from the maturity of the newborn baby. The first trimester was defined as lasting from 0 to 13.3 weeks of amenorrhea, the second trimester was defined as lasting from 13.4 to 26.6 weeks of amenorrhea, and the third trimester was defined as occurring thereafter.

Any interruption of pregnancy before the 22nd week of pregnancy was recorded as an abortion. Any interruption of pregnancy occurring from the 22nd to the 37th week of pregnancy was recorded as a preterm birth. Low birth weight was defined as a weight of less than 2500 g at birth. Microcephaly was defined as a head circumference more than 2 standard deviations (SDs) below the expected mean for the same sex and gestational age. The pregnancy outcome was defined as being severe if abortion, stillbirth, or microcephaly occurred. The pregnancy outcome was defined as being moderate if low birth weight or preterm delivery occurred.

A case was defined as suspected ZIKV if the patient had a macular or papular rash with two or more other symptoms, such as fever, conjunctival hyperemia without secretion, pruritus, or arthralgia of the hands or feet. A case was defined as confirmed ZIKV if RT-PCR showed positive results for ZIKV. The definitions of suspected and confirmed ZIKV cases follow those used by the Ministry of Health of Brazil.[7]

RT-PCR results were classified into five categories: ZIKV RT-PCR positive cases, ZIKV RT-PCR negative cases, ZIKV RT-PCR with indeterminate results, ZIKV RT-PCR that was “under analysis” (analysis no completed), and ZIKV RT-PCR not tested. All cases were included and followed until delivery.

Patient care

FMT-HVD is a referral center for tropical and infectious diseases in the state of Amazonas in Brazil. For this reason, it is one of the institutions that is most sought out by members of the population,

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especially in epidemic situations such as ZIKV transmission. FMT-HVD is also one of the most important education and research institutions in tropical medicine and infectious diseases in the Brazilian Amazonian region.

Because of known associations of ZIKV infection with teratogenesis and malformations in the fetus, pregnant women sought out diagnostic confirmation and procedures that they should follow. The patients were received by the emergency services room and were subsequently evaluated by the epidemiological surveillance service, which referred patients to outpatient care with the research team. In most cases, the pregnant women underwent outpatient screening and thereafter were attended to by the epidemiological surveillance service and outpatient care.

Sample collection mostly occurred when the pregnant women received their first care at the institution, although outpatient care was provided at a variety of times, ranging from the same day to within a week of receipt of first care. During the first or a subsequent instance of outpatient care, all the patient's demographic and clinical data were recorded on a form and a new laboratory test (for TORCH) was required by an infectologist belonging to our research team; a new contact occurred four or five weeks later. Because the evaluation at FMT-HVD was not a replacement for antenatal care, all pregnant women were referred to a high-risk prenatal service, which was created for this purpose by the prefecture of Manaus.

Confirmatory tests for ZIKV during gestational acute infection [8] were performed at the Central Laboratory of Public Health (LACEN) in Manaus using the reverse transcriptase reaction, and followed by real-time polymerase chain reaction (RT-qPCR) in serum and urine as well as immunoenzymatic tests for Chikungunya. Collection of serum samples in the first five days of symptom onset was strongly encouraged, as was collection of urine samples in the first eight days of symptom onset. Tests for Dengue virus and Parvovirus B19 virus infection were performed by the Virology Lab at the FMT-HVD, using commercial immunoglobulin M serological capture kits. The detection of etiological agents of TORCH Syndrome was mainly performed at the Central Laboratory of the FMT-HVD; however, if the patient had recent test results from a public or private-network laboratory, new tests were not performed.

Ethic approval and consent to participate

Written informed consent was obtained from each patient for the study, with the signature of the Free and Informed Consent Form (ICF), under the approval of the ethics committee obtained from the Doctor Heitor Vieira Dourado Foundation for Tropical Medicine (FMT-HVD) Ethical Committee (CAAE 60168216.2.0000.0005) approved number 1'806.030. The study was conducted in accordance with the Declaration of Helsinki.

Statistical analysis

All the data were stored in an Excel spreadsheet (Microsoft, Redmond, USA) and analyzed using Epi Info 7 (Centers of Disease Control and Prevention, Atlanta, USA) and Minitab 17 (Minitab, State College, USA). Chi-square tests (McNemar's tests) were used to compare discrete data, and z-scores were used to compare continuous data. *P* values less than 0.05 were regarded as statistically significant.

3. Results

3.1 Case Incidence in Manaus

According to the Foundation of Health Surveillance (FVS, Manaus), 1286 cases of suspected ZIKV in pregnant women were registered in Manaus from November 2015 until January 2017, a period of almost 60 epidemiological weeks. Five hundred of the 1286 suspected cases were confirmed by RT-PCR. According to the Information System of Natality (SINASC), there were 47.791 live newborn babies during the same period, meaning ZIKV infection had probably affected almost 3% of pregnancies and was confirmed to have affected almost 1% of the total (incidence index, 10.4 cases per 1000 liveborn babies).

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An ambulatory clinic for infectious diseases in pregnancy at the FMT-HVD attended to 64.9% and 67.8% of the notified and confirmed cases in the municipality of Manaus, respectively. The patient cohort of this study consists of the cases registered at the FMT-HVD. Blood and urine sample were collected from the patients, and the presence of positive RT-PCR ZIKV findings in either sample was used to confirm ZIKV infection.

3.2 Temporal Distribution of Cases

The temporal distribution of cases at the FMT-HVD clinic is shown in Figure 1 for a period of 58 epidemiological weeks, which matches the period described by the FVS very closely. During the 58-week period, 834 cases of suspected ZIKV in pregnancy were registered by the FMT-HVD.

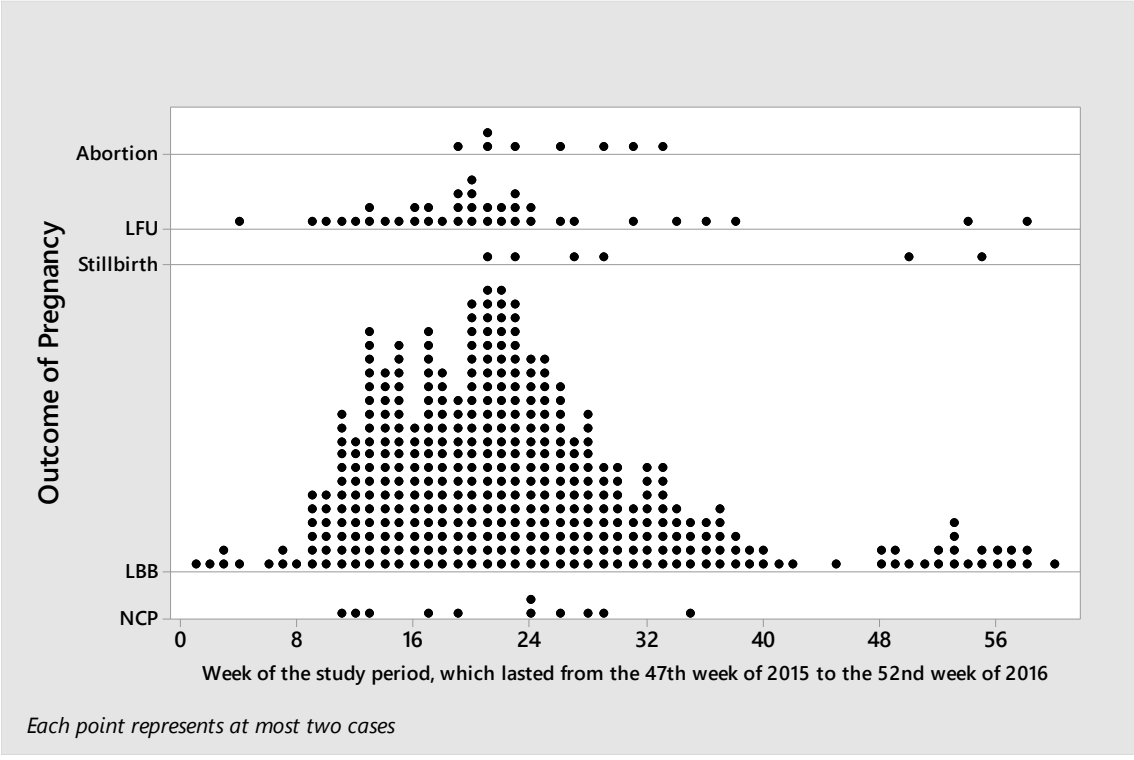

Figure. 1: Distribution of registered cases of suspected or confirmed Zika virus infection in pregnancy, during the period lasting from the 47th epidemiological week of 2015 to the 52nd epidemiological week of 2016. Each point indicates the time of symptom onset, as stratified according to gestational outcome (Abortion; LFU: lost to follow-up; Stillbirth; LBB: live born baby; NCP: no confirmation of pregnancy). The reported cases are from the Doctor Heitor Vieira Dourado Foundation for Tropical Medicine (FMT-HVD; Manaus, Brazil).

3.3 Clinical Courses

The Figure 2 summarizes the RT-PCR ZIKV test results and the outcomes of pregnancies. In 12 (1.44%) of the cases, the pregnancy was not confirmed. Additionally, loss to follow-up occurred in 60 (7.19%) cases.

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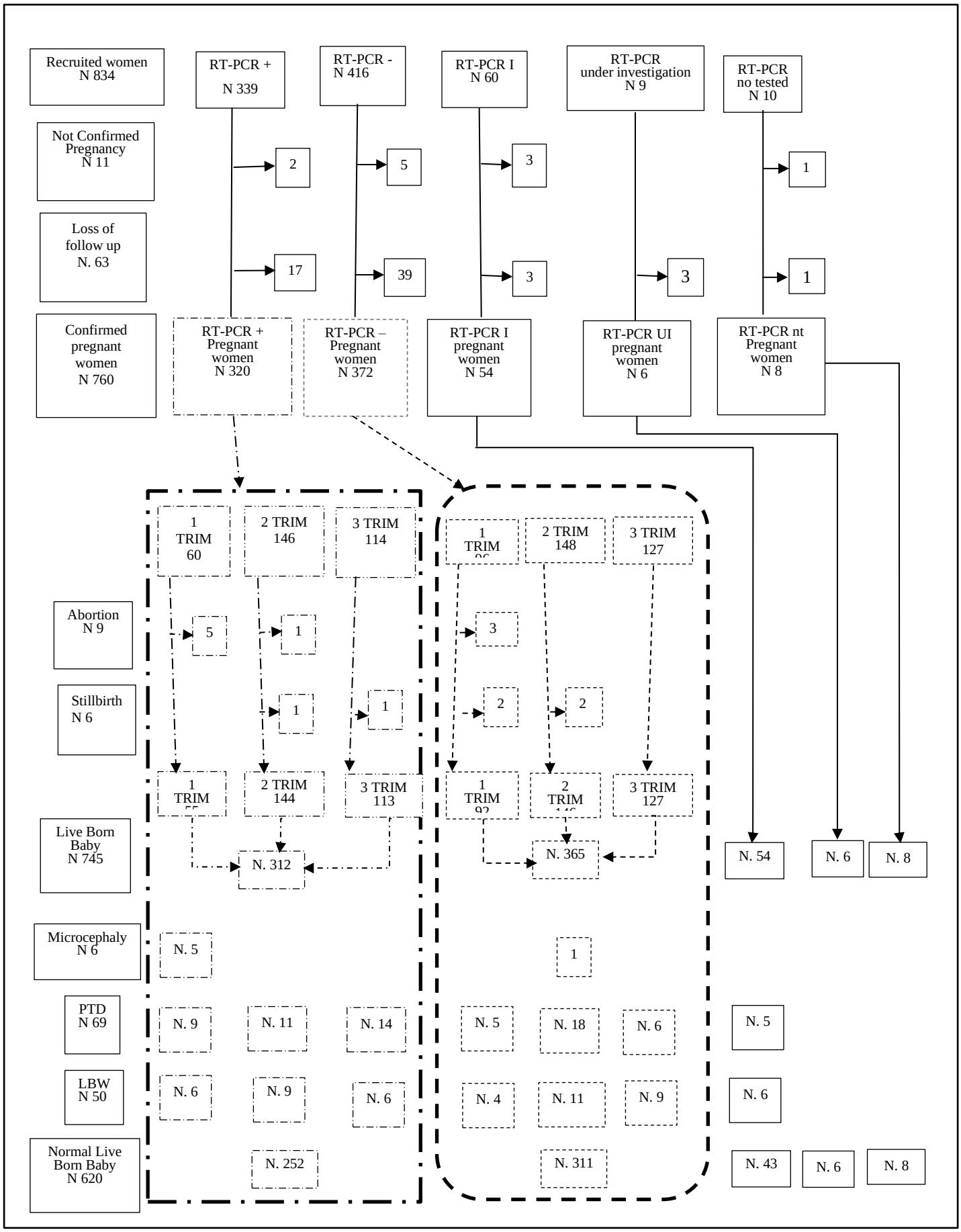

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Figure 2: Recruitment, follow up, and pregnancy outcomes in women registered as having been exposed to Zika virus infection in pregnancy. The reported cases are from the Doctor Heitor Vieira Dourado Foundation for Tropical Medicine (FMT-HVD; Manaus, Brazil). RT-PCR: reverse transcription polymerase chain reaction; I: indeterminate; UI: under investigation; NT no tested; PTD: preterm delivery; LBW: low birth weight; TRIM: trimester of pregnancy at onset of symptoms.

Case Characteristics and Presentation

The characteristics of the women with suspected ZIKV in pregnancy are summarized in Table 1. The women were most likely to be younger adults (age, 18 to 29 years), to be married or in a stable union, to have a high school education, to have at least one previous pregnancy, and to be in the first half of pregnancy **(Table 1)**.

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Table 1. Characteristics of a cohort of pregnant women with exanthematic disease during the period when Zika virus transmission was most intense in Manaus, according to the results of RT-PCR ZIKV testing.

Characteristic	RT-ZIKV+ (*n* = 339)	RT-ZIKV– (*n* = 416)	RT-ZIKV results indeterminate (*n* = 60)	RT-ZIKV under analysis (*n* = 9)	Not tested (*n* = 10)
MATERNAL AGE AT INFECTION					
Maternal age (yrs), mean ± SD	27.013 ± 6.428	27.619 ± 6.32	27.619 ± 7.479	28.95 ± 6.99	27.9 ± 7.73
Teenagers, *n* (%)	25 (7.40%)	25 (6.02%)	6 (10.0%)	0 (0.0%)	1 (10.0%)
Not adolescents	313 (92.6%)	390 (93.98%)	54 (90%)	8 (100.0%)	9 (90.0%)
<18 years old	25 (7.40%)	25 (6.02%)	6 (10.0%)	0 (0.0%)	1 (10.0%)
18 to 29 years old	203 (60.06%)	239 (57.59%)	33 (55.00%)	5 (62.5%)	5 (50.0%)
> 29 to 35 years old	82 (24.26%)	106 (25.54%)	10 (16.67%)	1 (12.5%)	2 (20.0%)
> 35 years old	28 (8.28%)	45 (10.84%)	11 (18.33%)	2 (25.0%)	2 (20.00%)
MARITAL STATUS					
Single	154 (45.43%)	177 (42.55%)	31 (51.67%)	3 (33.33%)	4 (40.00%)
Married/stable union	175 (51.62%)	219 (52.64%)	27 (45.00%)	5 (55.56%)	6 (60.00%)
Separated/divorced	2 (0.59%)	8 (1.92%)	1 (1.67%)	0 (0.00%)	0 (0.00%)
Widow	0 (0.00%)	1 (0.24%)	0 (0.00%)	0 (0.00)	0 (0.00%)
Ignored	8 (2.36%)	11 (2.64%)	1 (1.67%)	1 (11.11%)	0 (0.00%)
SCHOOLING					
Without schooling	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Fundamental	49 (14.45%)	51 (12.26%)	9 (15.0%)	2 (22.22%)	2 (20.00%)
High school	193 (56.93%)	208 (50.00%)	30 (50.00%)	5 (55.56%)	4 (40.00%)
Higher education	90 (26.55%)	139 (33.41)	19 (31.67)	1 (11.11%)	4 (40.00%)
No information	7 (2.06%)	18 (4.33%)	2 (3.33%)	1 (11.11%)	0 (0.00%)
GYNECOLOGICAL HISTORY					
Mean parity	2.40 ± 1.47	2.31 ± 1.41	2.35 ± 1.30	2.67 ± 1.37	3.00 ± 1.41
Primigravida	31.33	32.07	30.77	16.67	14.29
Gestational age (weeks) at the infection					
Mean ± SD (weeks)	22.59 ± 8.87	21.86 ± 9.93	23.82 ± 9.63	17.67 ± 9.4	16.88 ± 9.01
≤ 22 weeks	51.19	54.43	36.84	71.43	66.67

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> 22 weeks					
1st. Trimester	69	116	11	3	4
2nd. Trimester	150	159	23	5	3
3rd. Trimester	118	135	23	0	2
ANTENATAL CARE					
Antenatal consultations, mean ± SD	3.57 ± 0.70	3.57 ± 0.67	3.67 ± 0.55	3.17 ± 1.17	3.75 ± 0.46
Birth weight, mean ± SD	3248.8 ± 575.1	3302.0 ± 503.0	3212.6 ± 650.6	3540 ± 487	3306 ± 379
Mean ± SD Pregnancy age at birth (weeks), mean ± SD	38.67 ± 2.16	38.78 ±1.89	38.77 ± 2.29	39.33 ± 1.21	39.00 ± 1.51
CLINICAL CONDITION					
Days from the onset of symptoms to sample collection, mean ± SD	2.83 ± 3.39	3.99 ± 4.46	3.65 ± 3.45	9.44 ± 10.21	5.67 ± 3.50
Fever, %	44.96	53.82	57.45	25.00	88.89
Rash, %	99.30	94.34	100.00	100.00	87.50
Pruritus, %	93.21	90.65	100.00	80.00	87.50
Headache, %	51.07	63.10	56.76	100.00	100.00
Odynophagia, %	6.93	7.92	2.94	0.00	0.00
Eye burning, %	49.32	47.73	45.95	50.00	50.00
Ocular pruritus, %	16.15	16.79	4.55	0.00	33.33
Conjunctivitis, %	50.68	44.95	47.06	50.00	33.33
Hand arthralgia, %	65.80	59.40	58.33	50.00	71.43
Foot arthralgia, %	57.64	55.65	42.86	50.00	57.14
Other arthralgia, %	14.12	20.95	9.09	0.00	50.00
Hand edema, %	55.96	36.71	38.24	0.00	40.00
Foot edema, %	50.46	38.54	41.18	0.00	40.00
Asthenia/adynamia, %	40.19	42.93	51.43	0.00	40.00
Myalgia, %	50.89	57.85	44.74	0.00	25.00
Vomiting, %	17.45	26.91	8.57	0.00	16.67
Diarrhea, %	18.78	19.35	12.12	50.00	20.00
Lymphadenopathies, %	8.37	6.25	3.23	0.00	0.00
Vaginal bleeding, %	5.66	6.80	0.00	0.00	0.00
Other bleeding, %	6.64	7.43	9.09	0.00	0.00

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Uterine contractions, %	21.26	18.69	16.67	0.00	50.00
Epidemiological contact, %	68.86	59.72	70.83	0.00	33.33
OUTCOME OF PREGNANCY, *n*	320	372	54	6	8
Abortion, %	1.88	0.81	0	0	0
Stillbirth, %	0.63	1.08	0	0	0
Microcephaly in LBB, %	1.60	0.27	0	0	0
Severe outcome, *n*	13	8	0	0	0
Preterm delivery and/or low birth weight, *n*	40	35	7	0	0
No undesirable outcome observed at birth, *n*	267	329	47	6	8

RT-PCR: reverse transcription polymerase chain reaction; ZIKV: Zika virus; SD, standard deviation

Table 2. Summary of symptoms for isolated infections detected in a cohort of pregnant women who had exanthematic disease during the period when Zika virus transmission was most intense in Manaus

Symptom	ZIKV	DENV	Syphilis	HIV	Toxoplasmosis	CMV	HERPES	PARVOV	None	Total
Eye burning	50.30%	66.67%	100.00%	0.00%	0.00%	100.00%	50.00%	37.50%	48.33%	49.41%
Hand arthralgia	67.42%	45.45%	100.00%	50.00%	66.67%	100.00%	41.18%	25.00%	63.72%	63.31%
Foot arthralgia	56.82%	45.45%	0.00%	50.00%	33.33%	100.00%	35.29%	25.00%	58.37%	55.68%
Asthenia adynamia	39.24%	27.27%	0.00%	33.33%	0.00%	100.00%	50.00%	50.00%	42.56%	41.01%
Headache	54.70%	64.29%	50.00%	50.00%	66.67%	0.00%	66.67%	62.50%	64.32%	60.43%
Conjunctivitis	52.98%	72.73%	100.00%	0.00%	50.00%	100.00%	50.00%	25.00%	44.44%	48.56%
Uterine contractions	21.79%	0.00%	0.00%	0.00%	50.00%	0.00%	18.75%	14.29%	19.89%	20.00%
Diarrhea	19.14%	10.00%	0.00%	33.33%	0.00%	0.00%	23.53%	25.00%	18.54%	18.83%
Odynophagia	4.24%	0.00%	0.00%	0.00%	0.00%	0.00%	5.88%	12.50%	7.73%	5.99%
Hand edema	54.55%	50.00%	0.00%	0.00%	0.00%	100.00%	43.75%	37.50%	36.36%	44.17%
Foot edema	49.39%	50.00%	0.00%	0.00%	0.00%	100.00%	37.50%	37.50%	38.78%	43.00%
Rash	99.14%	100.00%	100.00%	75.00%	100.00%	100.00%	100.00%	100.00%	94.70%	96.71%
Fever	47.32%	66.67%	100.00%	50.00%	33.33%	100.00%	55.56%	44.44%	55.13%	52.38%
Lymphadenopathies	6.54%	11.11%	0.00%	0.00%	0.00%	0.00%	12.50%	0.00%	4.97%	5.90%
Myalgia	50.58%	63.64%	100.00%	66.67%	33.33%	100.00%	37.50%	50.00%	58.05%	54.65%

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Pruritus	93.81%	100.00%	100.00%	75.00%	66.67%	100.00%	100.00%	100.00%	90.71%	92.39%
Ocular pruritus	15.00%	11.11%	0.00%	0.00%	0.00%	0.00%	7.14%	16.67%	18.03%	15.58%
Vaginal bleeding	5.56%	0.00%	0.00%	33.33%	0.00%	0.00%	0.00%	0.00%	6.22%	5.54%
Vomiting	16.77%	30.00%	50.00%	66.67%	0.00%	0.00%	6.25%	12.50%	25.70%	21.58%

ZIKV: Zika virus; DENV: Dengue virus; CMV: cytomegalovirus: HERPES: herpes virus.

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When the symptoms of all the isolated infection were analyzed, rash and pruritus were found to be the most frequent (Table 2). Different infections did not appear to be associated with any large differences in the frequencies of symptoms (Table 2), but low case counts for most of the symptoms makes interpretation of these results difficult. Symptom frequencies were also analyzed according to RT-PCR ZIKV test results, as shown in Table 1.
After initial presentation at the FMT-HVD, the women returned to the ambulatory clinic as soon as possible, at which point TORCH infection status was evaluated, clinical data were collected, and information about antenatal care was recorded.

TORCH Syndrome
ZIKV infection (40.65%) was the most prevalent of the detected infections, but cases of dengue virus (4.8%), syphilis (0.84%), HIV (0.84%), herpes 1 and 2 (5.88%), and parvovirus (3.24%) infection were also detected, as well as some additional infection types and cases of multiple simultaneous infections. In 51.44% of the patients, no infection was found (Fig 3). The time that had elapsed between the onset of symptoms and sample collection was lower in the RT-PCR positive patients than for the RT-PCR negative patients (mean ± SD 2.75 ± 2.93 vs 4.0 ± 4.46 days, $p<0.001$). There were two cases of rubella, a vaccine preventable disease.

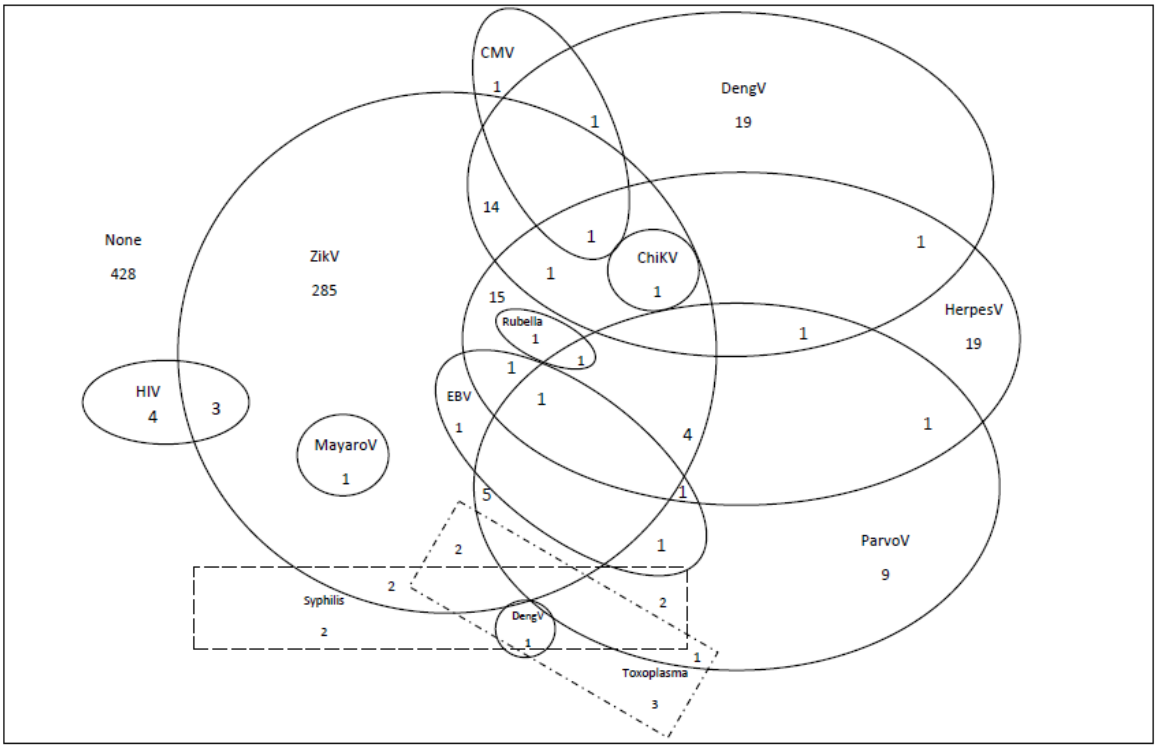

Figure 3. Distribution of serological markers of infections and coinfections in a cohort of women in Manaus who had exanthematic diseases registered as Zika virus infection in pregnancy. ChikV: Chikungunya virus; CMV: cytomegalovirus; DengV: Dengue virus; EBV: Epstein-barr virus; HerpesV: herpes virus; HIV: Human immunodeficiency virus; MayaroV: Mayaro virus; ParvoV: Parvovirus B19; ZikV: Zika virus

Pregnancy Outcomes
In the patient cohort, the most severe adverse outcomes of pregnancy were abortion (10 cases, 1.2%), stillbirth (7 cases, 0.84%), and microcephaly (7 cases, 0.84%). Although severe adverse outcomes were most common among RT-PCR ZIKV-positive patients, they were also observed in other groups of patients. For example, stillbirth was most common among RT-PCR ZIKV-negative patients. Both in cases of abortions and stillbirths, as well as of ZIKV-negative microcephaly, no other etiological agent was evidenced except for one abortion case with positive results for toxoplasmosis and parvovirus.

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Risk factors for severe adverse outcomes of pregnancy were also evaluated. Onset of symptoms in the first trimester of pregnancy had the greatest statistical significance as a risk factor, with an odds ratio for severe adverse outcomes of 10.5 (95% CI, 4.0-27.0) (Table 3). Other undesirable outcomes, such as low birth weight and preterm delivery, are summarized in Fig 2.

Table 3. Risk factors associated with a severe pregnancy outcome in a pregnant woman with exanthematic disease.

Risk factor	Severe outcome	No severe outcome	OR	95% CI	*p*
Teenager	0	45			
Single	10	294	1.5	0.59 - 3.93	> 0.05
Basic education	4	89	1.8	0.58 - 5.64	> 0.05
First pregnancy	5	183	1.41	0.45 - 4.39	> 0.05
First trimester	**17**	**140**	**10.46**	**4.04 - 27.03**	**< 0.001**
RT-PCR ZIKV+	15	267	2.3	0.96 - 5.53	> 0.05

Table of risk factors associated with a severe pregnancy outcome (abortion, stillbirth and microcephaly) in a cohort of pregnant women with exanthematic disease during the period of the most intense transmission of Zika virus in Manaus. December 2015 to January 2017. OR, odds ratio; CI, confidence interval; PCR: polymerase chain reaction; PCR: polymerase chain reaction; ZIKV: Zika virus.

4. Discussion

Although we found that confirmed ZIKV infection was associated with elevated rates of severe adverse pregnancy outcomes, our results do not allow us to establish a greater adverse effect of ZIKV infection on pregnancy outcomes, since the study patients in whom ZIKV infection could not be confirmed do not constitute an adequate control group. Even though abortion and microcephaly were most common among RT-PCR ZIKV-positive patients, they also occurred among RT-PCR ZIKV-negative patients, and stillbirth was most common among the RT-PCR ZIKV-negative patients. The mean duration between the onset of symptoms and sample collection for the PCR test was longer in the RT-PCR ZIKV-negative group. This could partially explain the observed findings, since false negative test results may occur if a sample is collected outside the period of viremia. Additionally, other factors associated with suspected ZIKV infection may also have caused adverse pregnancy outcomes.

The proportion of suspected cases of ZIKV in pregnancy that tested negative in the current study is similar to that observed by Brasil et al.[4,6] in Rio de Janeiro, but is lower than that observed by Nogueira et. al.[9] in São José do Rio Preto. This suggests that there are limits to the diagnostic ability of the test used to confirm cases. (In a study of women from French territories in the Americas, Hoen et al.[10] also analyzed a pregnant cohort, but their report does not allow to know the proportion of negative PCR findings for symptomatic cases.) Together with these prior studies, the results of our study raise the question, "If these symptomatic patients did not have ZIKV infection, then what did they have?" In the Rio de Janeiro cohort, 42% of women with negative RT-PCR ZIKV findings had confirmed Chikungunya infections. Neither the Sao José do Rio Preto study, nor the study of French Guiana estimated the frequency of adverse outcomes in symptomatic pregnant women with negative ZIKV test findings. Further, neither of these studies examined non-ZIKV causes of the symptoms that were presented by the patients.

Relatively few patients were lost to follow-up in this study, which could be attributed to pregnant women in the study area considered diagnostic testing and care to be very important due to fear of the ZIKV epidemic and microcephaly. This temptation may be attributable to the extent of interest in the epidemic (including in the media), which may have led individuals to seek out a diagnosis at a time when diagnostic testing and care was only being offered to pregnant women.

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Additionally, our group attempted to reduce losses to follow-up by contacting all the women, since data on births and fetal deaths in other municipalities is unavailable.

5. Conclusions

In conclusion, our results show that the frequency of severe adverse pregnancy outcomes was high among women infected with ZIKV during the period of peak ZIKV transmission in Manaus. The study findings indicate that infection may be especially devastating when symptoms present during the first trimester of pregnancy. In pregnant women with suspected ZIKV infection, most cases of adverse pregnancy outcomes can be attributed to ZIKV infection. However, in some cases, it has not been possible to confirm that ZIKV infection was the underlying cause, probably due to limitations in the diagnostic test itself.

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Ethic approval and consent to participate

Written informed consent was obtained from each patient for the study, with the signature of the Free and Informed Consent Form (ICF), under the approval of the ethics committee obtained from the Doctor Heitor Vieira Dourado Foundation for Tropical Medicine (FMT-HVD) Ethical Committee (CAAE 60168216.2.0000.0005) approved number 1'806.030.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.