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Association between Zika virus and foetopathy: a prospective cohort study in French Guiana. Preliminary report.

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Zika virus, congenital Zika virus syndrome, ultrasound, congenital infection, microcephaly.

Abstract:

Objectives The main objectives of the present study were to establish the existence of significant differences in the incidence of central nervous system (CNS) anomalies (including microcephaly), signs of congenital infection, or foetal loss between Zika virus (ZIKV)–infected and non-infected pregnant women in western French Guiana.

Methods This prospective cohort study was conducted between January 1st and July 15th 2016. We evaluated the clinical and foetal ultrasound (US) examinations of 301 pregnant women with biological confirmation of ZIKV infection and 399 pregnant women who were negative for ZIKV infection.

Findings Overall, the total number of foetuses with CNS involvement in the infected group was higher than in the control group (9.1% vs. 4.3%; RR=2.11; 95% CI [1.18, 4.13]). Anomalies of the corpus callosum and the presence of cerebral hyperechogenicities were also found to be significantly more common in the affected group. There was an increased risk of microcephaly (1.66%) in the affected group compared with the control group (0.25%), although this difference was not significant, unlike other signs of CNS and non-CNS foetal infection. When the mother was infected during the first or second trimester, there was a greater risk of more severe CNS involvement, the presence of more signs of infection and intrauterine foetal demise (IUFD). The vertical transmission rate in the exposed group was 10.9%

Interpretation ZIKV infection during pregnancy is associated with a significant risk of foetal CNS involvement and IUFD, particularly when the infection occurs during the first and second trimesters. Microcephaly was not present in every case of congenital Zika virus syndrome that we observed. Until more is known about this disease, it is paramount to evaluate suspected cases by detailed neurosonographic examination on a monthly basis, paying particular attention to the corpus callosum and the presence of hyperechogenic foci.

Funding This study was not funded.

Introduction

The Zika virus (ZIKV) epidemic first affected countries in the Pacific Rim, and has since spread to the Americas and Asia[1, 2].

The association between ZIKV and the increased risk of foetal insults, particularly those affecting the brain, was not observed until evidence from Brazil indicated this possible link.[3–6]

Ever since the original report from Olivera de Melo *et al.*,[4] it has been clear that the increase in the number of children born with microcephaly and severe brain lesions[7] is caused by the virus. This report and others that followed showed that affected foetuses exhibit a more complex syndrome with brain calcifications, ventriculomegaly, callosal and infratentorial anomalies, and sometimes ocular anomalies, hydramnion, arthrogryposis, brainstem anomalies and intrauterine growth restriction (IUGR) [8–14].

The first locally transmitted cases of ZIKV were diagnosed in French Guiana in December 2015. Since February 2016, all pregnant women undergo three serology tests for ZIKV during pregnancy and up to the time of delivery, which are conducted by the National Reference Center (NRC) for Arboviruses for French departments in the Americas located at the Pasteur Institute in French Guiana. When the mother is symptomatic, the infection is confirmed by positive RT-PCR tests of the blood or urine[15] and/or by detection of anti-ZIKV antibodies. As of July 6, 2016, ZIKV infection has been confirmed in 744 pregnant women.[16] These patients undergo monthly ultrasonographic follow-up in a foetal diagnosis unit. Almost the entire population of western French Guiana is managed in a single centre, enabling a population-based study.

The main objective of this prospective cohort study was to determine whether there is a significant difference in the prevalence of CNS anomalies and signs of foetal infection between ZIKV-infected patients and those found to be ZIKV negative throughout pregnancy, in order to help to confirm the hypothesis that ZIKV infection during pregnancy is the cause of

the foetal insults. The secondary objective was to describe the prenatal ultrasound (US) findings characteristic of congenital Zika virus syndrome (CZVS).

Methods

This observational prospective cohort study compared US findings in ZIKV seropositive and seronegative pregnant patients attending the prenatal diagnostic unit of the Centre Hospitalier de l'Ouest Guyanais (CHOG) between January 1st and July 15th 2016.

All patients with singleton and bichorionic twin pregnancies who were referred to the unit for US evaluation during pregnancy and who were willing to participate in the study after being informed about its purpose were asked to sign an informed consent form. Pregnant patients were defined as ZIKV positive either by a positive RT-PCR result (using the RealStar®Zika Kit, Altona Diagnostics GmbH, Hamburg, Germany) from blood and/or urine samples or by anti-ZIKV antibody detection using an in-house (NRC) MAC- and GAC-ELISA.

The timing of the infection was established based on the appearance of clinical signs and symptoms or, in their absence, by the laboratory test results. Seronegative patients remained as such for the entire pregnancy and after delivery. Additional samples, including placenta, amniotic fluid, and foetal samples, were assayed by RT-PCR to look for the virus in the materno-fetal compartment.

Data regarding demographic characteristics, medical parameters, and possible risk factors for congenital diseases were obtained at the beginning of the study and included in the analysis.

Both groups were followed using the same protocol, which included monthly US examinations from 11-14 weeks up to the end of pregnancy, with standardized biometric measurements and anatomic survey, paying special attention to the brain anatomy, as recommended by national and international medical societies.[17,18] All foetal US examinations were performed by only two sonographers using GE E8 and E10 Voluson machines (General Electric, Milwaukee,

USA) with abdominal and transvaginal transducers. Before the study commenced, both of these investigators performed 50 examinations together to standardize the procedure, with the purpose of minimizing intra-observer variability.

A foetus was considered to be likely to have microcephaly when the head circumference (HC) was smaller than two standard deviations (SD) below the norm, according to the CFEF nomograms, and was considered to have severe microcephaly when the HC was smaller than three SD below the norm.

Abnormal biometry of the brain structures was defined using well-established criteria.[17,18] Sulcation and gyration; the presence of calcifications; periventricular or cortical hyperechogenicity; and the presence of non-CNS signs of infection or neurologic involvement were also evaluated.

The presence of extended brain involvement or a single CNS finding with more than three non-CNS signs of infection was defined as severe disease. The cases were classified as *confirmed* following confirmation of the presence of the virus in the materno-fetal compartment without any other suspected etiology from the prenatal and postnatal evaluations, and *suspected* when the clinical and US findings were evocative of CZVS but the presence of ZIKV in the materno-fetal compartment was not confirmed.

When one of the investigators suspected an anomaly, the images were submitted for consideration by the second investigator, and a multidisciplinary consultation was offered for further evaluation or to consider termination of pregnancy, if requested. When the US findings led to suspicion of CZVS, an amniocentesis was offered. ZIKV serology and RT-PCR analyses were performed for all newborns at delivery using cord blood, as well as RT-PCR analysis of the urine. A postmortem examination was performed in all cases of termination due to foetal abnormality. The incidence of intrauterine foetal demise (IUFD) was compared between the two groups (from the second trimester of pregnancy).

Statistical analysis was performed using XLStat (Addinsoft, Paris, France). Comparisons were performed using Chi-squared, Yates correction, and Fisher tests for expected counts of >5, 3-5 and <3, respectively. The t-test was used for quantitative variables and a value of $p<0.05$ was considered significant. The study protocol was approved by the Institutional Review Board of the CHOG.

Role of the funding source

This study was not funded, and so no funding source had any role in the study design; in the collection, analysis, and interpretation of the data; in the writing of the report; or in the decision to submit the paper for publication.

Results

Study population

During the study period, 1690 pregnant women underwent laboratory screening for ZIKV infection, and 498 (29.5%) were found to be positive by RT-PCR and/or serological analysis. Three hundred and one of these patients (60.4%) were followed by the CHOG prenatal diagnosis unit, and represent the ZIKV positive cohort. Fifty-two of these patients (17.3%) presented with clinical symptoms of ZIKV infection (fever, pruritus, erythema, conjunctivitis, arthralgia, or myalgia) at 6 to 32 weeks of gestation, and 10 (3.3%) had prolonged viraemia throughout pregnancy. Over the same period of time, 399 women with a negative serology throughout their pregnancy also agreed to monthly US follow-up. A flowchart of the recruitment process and pregnancy outcomes is shown in Figure 1.

The vertical transmission rate in the exposed group was 10.9% (12 out of 110, 95% CI [5.9-18.1]), as determined by positive RT-PCR results from the amniotic fluid (3/12) or positive RT-PCR or serology results at birth (9/98).

Foetal ultrasound findings

Overall, CNS anomalies were more common in the ZIKV positive group than in the ZIKV negative group: 27 (9.0%) vs. 17 (4.3%), for a 2.11 increase in the relative risk (95% CI [1.18-4.13]). Details regarding the specific anomalies and signs of infective feotopathy related to the presence of ZIKV and the time of infection are presented in Table 2. A detailed description of the nine (3.0%) patients with confirmed or suspected CZVS is presented in Table 3. The CNS iconography of a confirmed CZVS is presented in Figure 2. In our patients, CNS anomalies were diagnosed as early as 4 weeks after maternal infection. It worth mentioning that, while five (1.66%) of the ZIKV positive patients presented with foetuses with a HC smaller than 2SD less than the norm, the HC was in the severe microcephaly range for only one these foetuses. These patients became infected in the first and second trimesters. In the control group, only one foetus (0.25%) had suspected microcephaly (RR=6.63; 95% CI [0.78-57.83]).

Anomalies of the corpus callosum and the presence of any form of hyerechogenicity were also associated with maternal ZIKV infection. In contrast, ventriculomegaly, infratentorial anomalies and abnormal gyration were not found to be significantly increased when compared with the controls, nor were other signs of infective feotopathy, amniotic fluid abnormalities, or being small for gestational age (Table 2).

IUFD occurred more often in the exposed group (RR=3.98; 95% CI [1.09-15.17]), and was primarily due to maternal infection in the first and second trimesters of pregnancy.

Discussion

The severe effects of ZIKV infection on the CNS of foetuses has been demonstrated in case reports[4,5] and case series[8,9,11] of affected foetuses, but not in large prospective cohorts comparing pregnancies in ZIKV positive and ZIKV negative mothers. Our study confirms the hy-

pothesis that infection with ZIKV during pregnancy increases the relative risk of foetal CNS involvement 2-fold.

ZIKV and microcephaly

According to initial reports, the prevalence of microcephaly in Brazil,[19] French Polynesia,[20] and Colombia[14] rose rapidly during the Zika epidemics.[19] The European Centre for Disease Prevention and Control calculated that there was a 20-fold increase in the number of reported cases of microcephaly at the beginning of the epidemics (99.7 per 100,000 live births).[21] During the first period of the Zika crisis in Brazil, all newborns with a HC less than 1SD below the norm were suspected of having microcephaly, creating the false impression that as many as 16% of these newborns were microcephalic.[22] Following changes in the definition of microcephaly, and based on a complete examination of more than 50% of the cases, the incidence of reported microcephaly declined steadily.[23,24] In a study of a group of newborns with suspected microcephaly or CNS involvement, found that microcephaly was not invariably present in those with CNS involvement, and cases of severe microcephaly were even less frequent.[24] A preliminary report from Colombia found 20 (1.3%) foetuses with a HC smaller than 2SD below the norm, and four (0.3%) with severe microcephaly out of 1484 confirmed cases of maternal infection during pregnancy.[25] A retrospective report from French Polynesia shows similar results, with a 0·8% rate of foetal malformations and a 0.52% rate of microcephaly.[20] Although the incidence of microcephaly in our study (1.66%) is close to that reported in the Colombian study, there was no significant increase when compared with the control group (Table 2), and the overall rate was lower than the expected 2.3% in the general population.[26]

Our findings show that foetal microcephaly does not occur in every case of ZIKV infection, and when present, most likely represents only "the tip of the iceberg".[4]

ZIKV and foetal insults

The secondary objective of the present study was to present a description of US findings in patients with CZVS. The incidence of callosal anomalies and the presence of hyperechogenic foci were found to be significantly increased in ZIKV positive patients when compared to ZIKV negative patients. These findings are common in other foetal infections, and particularly in cases with severe cytomegalovirus (CMV) brain involvement. Other findings described for CZVS,[5,10,11] although present in some of our cases, were not significantly increased in the ZIKV positive cohort (Table 2). The significantly increased risk of IUFD in our study population is similar to reports from Brazil and Colombia.

Time of infection

Our study confirms previous findings regarding the increased risk of transmission and foetal involvement during the first and second trimester of pregnancy; in fact, all of the patients presenting with severe foetal involvement or IUFD were infected during this period of pregnancy. Third trimester infection resulted in findings that appeared to be clinically non-specific for ZIKV congenital infection, such as large placentas, amniotic fluid disturbances, and IUGR. It is important to remember that, as with other intrauterine TORCH infections, subclinical disease at birth may result in late appearance of symptoms, particularly hearing, vision, and neurodevelopmental deficits. Although Franca *et al.* described patients with CNS anomalies who were diagnosed only after delivery following maternal infection with ZIKV in the third trimester. [24]

Maternal symptoms and prolonged viraemia

We found an apparent increased risk of severe or complex foetal involvement when maternal symptomatology was reported; 66.7% of severe cases were identified following the appearance of clinical disease in the mother (Table 3). These results are similar to those from a recent Brazilian.[24] In our cohort, we identified a twin pregnancy with IUFD in the first foetus

and a severe CNS disorder in the second foetus that was associated with prolonged maternal viraemia (Table 3, case 6). At least one case of CZVS has already been linked to prolonged maternal viraemia, possibly due to viral replication in the foetus or placenta.[7,27]

Vertical transmission

The incidence of materno-fetal transmission is not well known for ZIKV, but has been studied for other arboviruses; generally, transmission in early pregnancy occurs rarely but causes severe disease (embryopathies), whereas transmission in late pregnancy occurs more frequently and causes less severe effects.[28-30]

The 10.9% vertical transmission rate reported here represents the average rate for the entire pregnancy. This estimate may vary depending on the trimester during which the mother became infected, as is the case with other materno-fetal infections.

Strengths and limitations of the present study

To the best of our knowledge, this is the first study comparing a large cohort of ZIKV positive patients with a similar group of patients known to remain ZIKV seronegative throughout pregnancy. Since an average of four US examinations were performed for each patient using excellent technical equipment following adequate certification of only two investigators, the possible biases were reduced as much as possible.

The most important limitation of the study is the fact that the results are still preliminary, as many patients have not yet delivered. This has most likely resulted in the false negative diagnosis of patients with less severe disease or those who will go on to develop symptoms later in pregnancy. The possible limitations of current laboratory tests for ZIKV may have introduced misclassifications into the study; the sensitivity and negative predictive value of the serological results are controversial, following cases of the contamination of foetal fluids and tissues by mothers with negative RT-PCR and serology.[29] Importantly, some patients with an

initial negative serological result can convert to IgG anti ZIKV positive serological status following infection by other arboviruses, and not necessarily by ZIKV.

Conclusion

ZIKV infection during pregnancy is associated with a significant risk of foetal CNS involvement and IUFD, particularly when the infection occurs during the first and second trimesters. The estimated vertical transmission rate is 10.9 %.

Contrary to general understanding, the increase in the risk of microcephaly in the exposed group was at the limit of significance. Our results, being preliminary, should be interpreted with caution. Until more information is obtained, it is paramount to evaluate suspected cases by detailed neurosonographic monthly examinations, paying particular attention to the corpus callosum and the presence of hyperechogenic foci.

We expect that postnatal follow-up of this cohort will provide a better understanding of the early and late effects of prenatal ZIKV infection, and a thorough knowledge of vertical transmission in connection with the trimester of infection.

Declaration of interests

The authors have no conflicts of interest to declare.

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The corresponding author (LP) had full access to all the data collected as part of the study and had final responsibility for the decision to submit for publication.

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Table 1 Baseline Characteristics of ZIKV infected and non-infected groups

		ZIKV infected	ZIKV non-infected	*p*
Total number of patients		301	399	
Maternal age, mean (min - max)		28.1 (15.5 - 44.4)	27.9 (14.5 - 45.2)	0.66
Gestations, mean (min - max)		4.3 (1 - 15)	4.1 (1 - 16)	0.06
Parity, mean (min - max)		3.5 (0 - 11)	3.4 (0 - 12)	0.39
Twin pregnancies, n (%)		3 (1)	4 (1)	1
T21 screening test >1/250, n (%)		11 (3.6)	18 (4.5)	0.79
Number of USs performed, mean (min - max)		4·7 (1 - 7)	4·4 (1 - 6)	0·78
Risk Factors	Vascular, n (%)	18 (6.0)	23 (5.8)	0.90
	Severe anaemia, n (%)	9 (2.9)	13 (3.3)	0.84
	Valproate, n (%)	1 (0.3)	0 (0)	0.43
	Alcohol consumption, n (%)	6 (2.0)	9 (2.3)	0.81
	Lead poisoning, n (%)	6 (2.0)	11 (2.7)	0.52
Co-infection	CMV, n (%)	1 (0.3)	1 (0.3)	1
	Toxoplasmosis, n (%)	1 (0.3)	2 (0.5)	1
	Chicken pox, n (%)	2 (0.7)	3 (0.8)	1
	HIV, n (%)	2 (0.7)	3 (0.8)	1
	Coxsackie virus, n (%)	1 (0.3)	0 (0)	0.43
History of aneuploidy or risk of syndrome re-currence, n (%)		1 (0.3)	2 (0.5)	1

CMV, cytomegalovirus; HIV, human immunodeficiency virus; US, ultrasound; ZIKV, Zika virus.

Table 2 CNS anomalies and signs of foetal infection in the infected and non-infected groups according to trimester of infection

	ZIKV infected patients				ZIKV non-infected patients n=399	Relative Risk (95% CI)	*p*
	1st tri-mester n=80	2nd tri-mester n=96	3rd tri-mester n=125	Total n=301			
CNS anomalies, total	10 (12.50)	13 (13.54)	4 (3.20)	**27 (8.97)**	**17 (4.26)**	**2·11 (1.18-4.13)**	**0.01**
Severe microcephaly HC < -3SD	1 (1.25)	0 (0.00)	0 (0.00)	**1 (0.33)**	**0 (0.00)**	**Inf (0.034-inf)**	**0.43**
Microcephaly, HC < -2SD	2 (2.50)	3 (3.12)	0 (0.00)	**5 (1.66)**	**1 (0.25)**	**6.63 (0.78-57.83)**	**0.07**
Callosal anomalies	4 (5.00)	8 (8.33)	3 (2.40)	**15 (4.98)**	**9 (2.26)**	**2.21 (1.08-5.26)**	**< 0.05**
Posterior fossa anomalies	4 (5.00)	3 (3.12)	1 (0.80)	**8 (2.66)**	**4 (1.00)**	**2.65 (0.81-9.05)**	**0.09**
Ventriculomegaly >10 mm	2 (2.50)	3 (3.12)	0 (0.00)	**5 (1.66)**	**1 (0.25)**	**6.63 (0.78-57.83)**	**0.07**
Abnormal gyration	2 (2.50)	2 (2.08)	0 (0.00)	**4 (1.33)**	**1 (0.25)**	**5.30 (0.6-48.2)**	**0.09**
Cerebral hyperechocenicity	5 (6.25)	10 (10.41)	0 (0.00)	**15 (4.98)**	**5 (1.25)**	**3.98 (1.48-11.49)**	**< 0.01**
Non-CNS involvement							
Intestinal hyperechogenicity	6 (7.50)	6 (6.25)	0 (0.00)	**12 (3.99)**	**10 (2.51)**	**1·59 (0.69-3.80)**	**0.26**
Liver/spleen echogenicity	2 (2.50)	2 (2.08)	0 (0.00)	**4 (1.33)**	**3 (0.75)**	**1.78 (0.40-8.06)**	**0.71**
Tachyarrhythmia	0 (0.00)	0 (0.00)	1 (0.80)	**1 (0.33)**	**2 (0.50)**	**0.66 (0.01-12.77)**	**1**
Abnormal amount of AF	2 (2.50)	12 (12.50)	4 (3.20)	**18 (5.98)**	**13 (3.26)**	**1.84 (0.91-3.92)**	**0.08**
Placentomegaly	2 (2.50)	6 (6.25)	1 (0.80)	**9 (2.99)**	**6 (1.50)**	**1.99 (0.71-5.74)**	**0.20**
IUGR	8 (10.00)	15 (15.62)	9 (7.20)	**32 (10.63)**	**28 (7.02)**	**1.51 (0.93-2.69)**	**0.09**
Ocular anomalies	1 (1.25)	1 (1.04)	1 (0.80)	**3 (1.00)**	**1 (0.25)**	**3.98 (0.42-38.74)**	**0.32**
Cardiomyopathy	1 (1.25)	1 (1.04)	0 (0.00)	**2 (0.66)**	**3 (0.75)**	**0.88 (0.07-7.75)**	**1**
Genitourinary tract anomalies	3 (3.75)	2 (2.08)	1 (0.80)	**6 (1.99)**	**8 (2.01)**	**0.99 (0.34-2.88)**	**0.79**
Akinesia	0 (0.00)	2 (2.08)	0 (0.00)	**2 (0.66)**	**1 (0.25)**	**2.65 (0.24-29.47)**	**0.58**

	ZIKV infected patients				ZIKV non-infected patients n=399	Relative Risk (95% CI)	*p*
	1st tri-mester n=80	2nd tri-mester n=96	3rd tri-mester n=125	**Total n=301**			
IUFD	5 (6.25)	4 (4.16)	0 (0.00)	**9 (2.99)**	**3 (0.75)**	**3.98 (1.09-15.17)**	**< 0.05**

Data are shown as n (%). AF, amniotic fluid; CI, confidence interval; CNS, central nervous system; HC, head circumference; FHR, foetal heart rate; IUFD, intrauterine foetal death; IUGR, intrauterine growth restriction; SD, standard deviation; SGA, small for gestational age; ZIKV, Zika virus.

Table 3 Description of foetuses with confirmed and suspected CZVS

Case	Trimester of maternal disease/ symptomatology	Maternal laboratory tests	Foetal laboratory tests	CNS findings	Non-CNS findings	Follow-up	CZVS
1	1st trimester Symptomatic	**14w** IgM/IgG anti-ZIKV positive	**22w** amniotic fluid ZIKV RT-PCR positive	- HC< -2SD - Cerebellar hypoplasia/large CM - Ventriculomegaly w/irregular ventricular walls - Periventricular hyperechogenicity - Callosal hypoplasia w/calcifications - Parenchymal calcifications	- Ocular hyperechogenicity - Liver calcification	Ongoing pregnancy (30w) Not interested in further follow-up	Confirmed
2	1st trimester Symptomatic	**15w** IgM anti-ZIKV positive **23w** IgM/IgG anti-ZIKV positive	**32w** amniotic fluid ZIKV RT-PCR positive **32w** IgG/IgM anti-ZIKV positive in foetal CSF	- 19w Severe microcephaly (HC < -3SD - Cerebellar hypoplasia/large CM - 32w Severe ventriculomegaly - Cortical hyperechogenicities - Parenchymal and periventricular calcifications	- Severe bilateral microphtalmia - Severe IUGR - Fetal akinesia - Hydramnios	Delivery at 32w HC = 31cm Weight = 1590g	Confirmed
3	2nd trimester Asymptomatic	**23w** IgM/IgG anti-ZIKV positive	**35w** amniotic fluid ZIKV RT-PCR positive	- Parenchymal hyperechogenicities	- Club foot - Placentomegaly	IUFD at 40w Suspected foetal akinesia	Confirmed
4	1st trimester Asymptomatic	**8w** IgM/IgG anti-ZIKV positive **23w** IgG anti-ZIKV positive	**23w** amniotic fluid ZIKV RT-PCR negative IgG anti-ZIKV in neonate blood	- 20w Cystic dilatation of the cisterna magna - Cortical hyperechogenicities - Severe vermian hypoplasia - Pericallosal echogenicities	- Intestinal hyperchogenicity	Delivery at 38w HC= 32.5cm Weight= 3080g	Suspected
5	1st trimester Symptomatic	**16w** IgM/IgG anti-ZIKV	Refused amniocentesis IgM / IgG anti-ZIKV in neonate blood	- 22w Periventricular hyperechogenicity - Irregular ventricular walls - Parenchymal and periventricular calcifications		Delivery at 41w HC = 32cm Weight = 2950g	Suspected
6 Twins	2nd trimester Symptomatic	**25w** IgM/IgG anti-ZIKV positive & serum RT-PCR ZIKV positive	**27w** amniotic fluid ZIKV RT-PCR negative IgG anti-ZIKV positive in foetal blood	Foetus A - 28 w Severe ventriculomegaly w/irregular ventricular walls - Thin, hypoplastic corpus callosum	- Intestinal hyperchogenicity - Hydramnios	29w Fetus A: TOP, ZIKV not found in foetal tissues 24w Fetus B: IUFD	Suspected
7	2nd trimester Symptomatic	**39w** IgM/IgG anti-ZIKV positive	Refused amniocentesis	- 37w HC = -2SD - Callosal hypoplasia - Gyration anomalies	- Placentomegaly - IUGR	Delivery at 39w HC = 32cm	Suspected

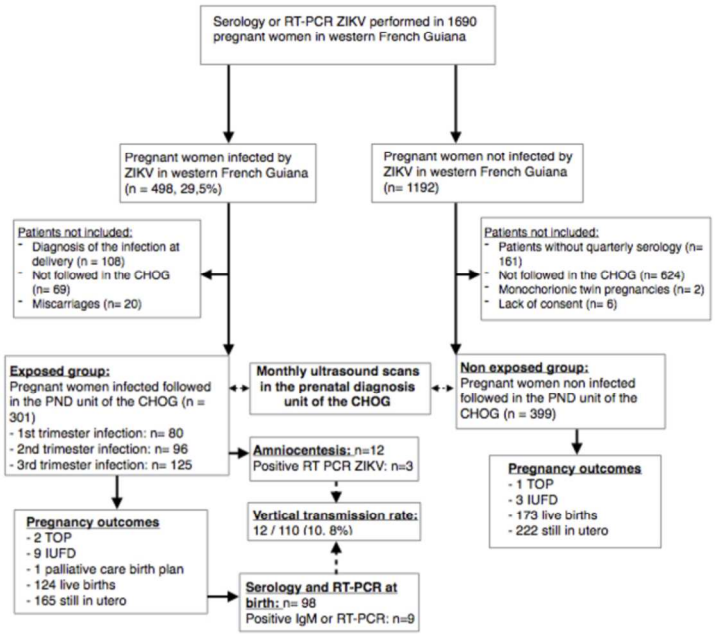
						Weight = 2995g	
8	2nd trimester Asymptomatic	**27w** IgM/IgG anti-ZIKV positive	Refused amniocentesis	- 29w Lentostriatal vasculopathy	- IUGR - Placentomegaly - Oligoamnios	Delivery at 39w HC = 32cm Weight = 2645g	Suspected
9	1st trimester Symptomatic	**15w** IgM anti-ZIKV positive	ZIKV RT-PCR negative in the placenta and foetal tissues	- HC < -2SD	- NIHF	IUFD at 19w	Suspected

CM, cisterna magna; CNS, central nervous system; CSF, cerebrospinal fluid; CZVS, congenital Zika virus syndrome; HC, head circumference; IUFD, intrauterine foetal death; IUGR, intrauterine growth restriction; NIHF, non-immune hydrops fetalis; PROM, premature rupture of membranes; RI, resistance index; SD, standard deviation; TOP, termination of pregnancy; W, weeks of amenorrhea; ZIKV, Zika virus.

Figure legends

Figure 1 Study flowchart. CHOG, Centre Hospitalier de l'Ouest Guyanais; IUFD, intrauterine foetal demise; PND, prenatal diagnosis; TOP, termination of pregnancy; ZIKV, Zika virus.

Figure 2 Confirmed congenital Zika virus syndrome (case 1).



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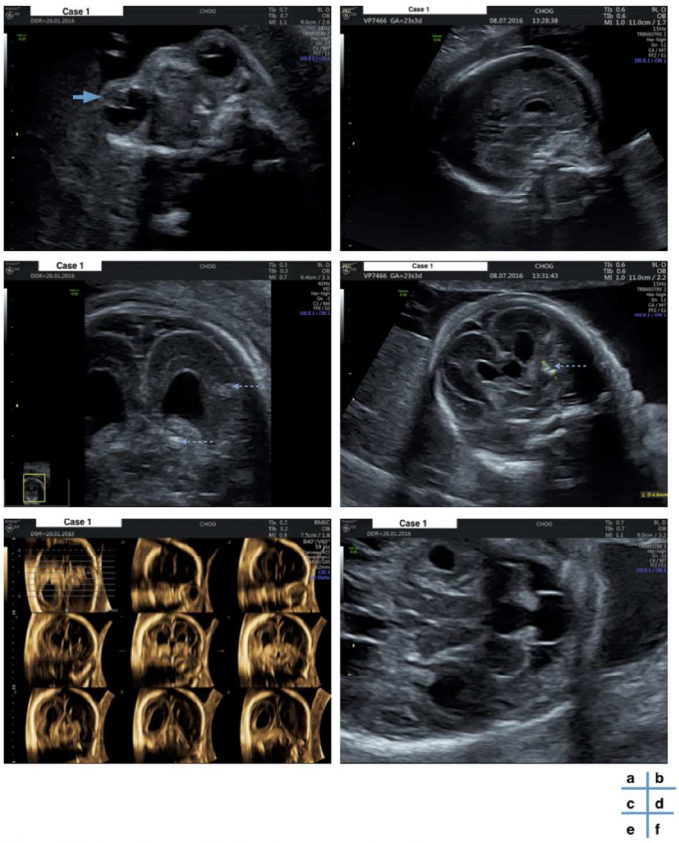

Figure 2 Confirmed congenital Zika virus syndrome (case 1)

Lens hyperechogenicity(→), 21w (a); dysgenesis of the corpus callosum, 23w (b); dilated frontal horns with hyperechogenic periventricular halo and periventricular calcifications (⇢),21 w (c), 23w (d); ventriculomegaly and thin cerebral cortex, 21 w (e), dilatation of the cisterna magna, 21 SA (f)

Figure 2 Confirmed congenital Zika virus syndrome (case 1)

Lens hyperechogenicity(—>), 21w (a); dysgenesis of the corpus callosum, 23w (b); dilated frontal horns with hyperechogenic periventricular halo and periventricular calcifications (- - >),21 w (c), 23w (d); ventriculomegaly and thin cerebral cortex, 21 w (e), dilatation of the cisterna magna, 21 SA (f)

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