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Original article

Zika virus infection in pregnant travellers and impact on childhood neurodevelopment in the first two years of life: A prospective observational study

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ABSTRACT

Background: The emergence of Zika virus (ZIKV) represents a threat with consequences on maternal and children's health. We aimed to assess the clinical and epidemiological characteristics of pregnant women returning from ZIKV affected areas, and the effects of maternal ZIKV infection on birth outcomes and children's health.
Methods: This was a hospital-based prospective observational study conducted at the Hospital Clínic of Barcelona and Hospital Sant Joan de Déu, Barcelona, Spain, from January 2016 to February 2020.
Results: One hundred and ninety-five pregnant women who had travelled to ZIKV affected areas during pregnancy were recruited. Four women (2.1%) had a confirmed ZIKV infection, 40 women (20.5%) a probable infection, and 151 (77.4%) were negative for ZIKV. Among the ZIKV confirmed cases, a pregnant woman suffered a miscarriage, highly plausible to be associated with ZIKV infection. Brain cysts and microcalcifications were detected in 7% of fetuses or infants from women with confirmed or probable ZIKV infection. Neurodevelopmental delay in the language function was found in 33.3% out of the 21 children evaluated.
Conclusions: These findings contribute to the understanding of ZIKV prevalence estimates, and the impact of maternal ZIKV infection on pregnancy outcomes and children's health. Results highlight the importance of long-term surveillance in pregnant travellers and their children.

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Abbreviations

ABR	Auditory brainstem responses
CDC	Centers for Disease Control and Prevention
CHIKV	Chikungunya virus
CZS	Congenital Zika Syndrome
DENV	Dengue virus
HCB	Hospital Clínic Barcelona
HSJD	Hospital Sant Joan de Déu
MRI	Magnetic Resonance Imaging
RT-PCR	Reverse transcription polymerase chain reaction
US	Ultrasound
WHO	World Health Organization
ZIKV	Zika virus

1. Introduction

The emergence of Zika virus (ZIKV) represents a global threat of dramatic consequences on maternal, fetal and children's health. Much of the concern around ZIKV is explained by its tropism for the human nervous system [1]. The link between infection in pregnancy and microcephaly was suggested as a result of the increase in the number of microcephaly cases in Northeastern Brazil in September 2015 [2,3]. ZIKV spread to almost all countries in the Americas, resulting in warnings for pregnant women to avoid travelling to endemic areas [4]. Similarly to Dengue virus (DENV), ZIKV is endemic in all tropical areas globally [5]. Poor pregnancy outcomes associated with ZIKV infection include miscarriages, intrauterine growth restriction, and perinatal death [2,3,6]. The first trimester represents the period of highest fetal risk associated with maternal ZIKV infection [7–10]; infections in the third trimester do not represent an increase in the incidence of structural fetal anomalies [11]. ZIKV is also responsible for a wide range of fetal anomalies and defects, including brain abnormalities with or without microcephaly, neural tube defects, other malformations, eye abnormalities and central nervous system dysfunction, described as Congenital Zika Syndrome (CZS) [12–14]. Infants born with CZS are at an increased risk for motor, language and cognitive developmental delays [15,16], vision loss, epilepsy [17], and growth decline compared to healthy children [16,18]. However, beyond severe structural brain defects and microcephaly, there are other consequences arising over time [19]. Postnatal defects, such as postnatal microcephaly, neurodevelopmental delays, ocular abnormalities, and autism spectrum disorder have been reported in infants *in uterus* exposed to ZIKV [18–22]. Recent studies have found language and cognitive delays in normocephalic children with RT-PCR confirmed ZIKV neonatal [23], or maternal [19] infection. This evidence has led to an increasing concern to unveil the neurodevelopmental consequences of the infection on ZIKV-exposed children further beyond birth, as certain milestones can only be assessed after the first years of life [1,12,19,23,24], and stresses the importance of long-term postnatal follow up [19,25]. Detection of neurodevelopmental delays in the first years of life will warantee prompt stimulation and treatment.

However, the magnitude of the risk, the full spectrum of the syndrome, and particularly, the potential long-term sequelae both of symptomatic and asymptomatic maternal ZIKV infections has not been completely described yet [12,26]. Imported ZIKV cases among travellers have been diagnosed in non-endemic countries. Until 2018, European countries reported 2474 confirmed cases of ZIKV infection [27]. Spain, has a long history of migration from the Americas resulting in a high number of migrant travellers. This has been associated with a substantial number of ZIKV imported cases [28], including the first case of ZIKV-related microcephaly in Europe [29]. From December 2015 to June 2018, the Spanish Ministry of Health registered 517 ZIKV cases, among which 358 were laboratory confirmed, and half of them among women of reproductive age [30,31].

Since the beginning of the ZIKV epidemic, the number of long-term longitudinal studies conducted in children with antenatal ZIKV exposure has been scarce [19,23]. We aimed to describe the clinical and epidemiological characteristics of pregnant women returning from ZIKV affected areas and to assess the prevalence of ZIKV infection in pregnancy in a non-endemic area, and its effects on pregnancy outcomes and children's neurodevelopment during the first two years of age.

2. Methods

2.1. Study design and participants

This was a multicenter hospital-based prospective observational study of ZIKV infection in pregnant women conducted from January 2016 to February 2020 in Barcelona, Spain, a non-endemic area for ZIKV. The study was conducted at the Hospital Clínic of Barcelona (HCB) – Maternal-Fetal and International Health Departments –, and the Hospital Sant Joan de Déu (HSJD) – Maternal-Fetal and Pediatric Infectious Diseases Units –, both tertiary healthcare facilities and referral hospitals in Catalonia for ZIKV surveillance [32].

Women attending study hospitals and meeting the following inclusion criteria were invited to participate in the study and were screened for ZIKV: 1) potential exposure defined by history of travel to a ZIKV affected country according to the World Health Organization (WHO) during current pregnancy or two months before pregnancy, regardless of the presence of symptoms, or whose sexual partner had travelled to those areas in the previous six months; and 2) willingness to follow antenatal care and deliver at the HCB or HSJD and to continue follow up of their children at HSJD in case of confirmation or suspicion of ZIKV maternal infection. Written informed consent was obtained prior to enrolment. Obstetric, clinical, vaccination, and travel history data were collected through standardized questionnaires.

Screening for ZIKV infection consisted of serological analysis on blood samples, and molecular diagnosis on blood and urine samples, depending on the time elapsed since exposure (less than 7 days and 20 days since onset of symptoms for blood and urine sample collection, respectively), according to regional guidelines [32]. Screening included serological testing for DENV and Chikungunya virus (CHIKV) infection. Study women confirmed as negative for ZIKV continued routine antenatal care.

2.2. Maternal-fetal follow up

Fetal ultrasonographic (US) assessment was performed every four weeks, with supplementary neurosonographic screening. US was performed by experienced examiners using high-resolution equipment (Voluson 730 Expert and E6 or E8, GE Healthcare, Kretz, Zipf, Austria). Gestational age was assessed by correction of last menstrual period according to crown-rump length measurement during first trimester scan (<14 weeks) [33], or by biparietal diameter and head circumference when results of first trimester scan were not available [34]. In cases with confirmed maternal ZIKV infection or with scans showing any brain abnormality, an amniocentesis for ZIKV RT-PCR assay and fetal brain magnetic resonance imaging (MRI) (1.5T GE Sigma Horizon, Echo speed, LX MRI scanner, Milwaukee, WI, USA) were offered.

At delivery, maternal, placental, cord, and neonatal blood, neonatal urine, as well as residues from the curettage (if applicable) were obtained for ZIKV molecular and/or serological analysis. Whole fresh placentas were processed within the first 4 hours after delivery for molecular analysis and histopathological evaluation. In case of prenatal suspicion of brain abnormalities, cerebrospinal fluid was drawn and screened for ZIKV screening by molecular methods. Breast milk samples were tested for ZIKV by RT-PCR within the 48 hours after delivery or during the post-partum visit.

2.3. Neonatal assessment and children's follow up

Neonatal assessment included the evaluation of congenital anomalies and anthropometric measurements (weight, length and head circumference). Transfontanelar US exam was performed after birth. MRI was only offered in case of abnormal postnatal US. Children's follow up was designed and adapted from the Centers for Disease Control and Prevention (CDC) guidelines for infants without birth defects born to mothers with laboratory evidence of infection [35]. Children were evaluated at months 1, 4, 6, 12, 18 and 24. Peripheral capillary blood samples were collected at 6 months of age for infant ZIKV serological screening. If a positive IgG was found, screening was repeated at 12 months of age. In each study visit, anthropometric measurements were obtained and compared to WHO growth reference charts. Auditory brainstem responses (ABR), and eye fundus assessments were conducted in months 1, 6 and 12. If pathological findings were found, exams were repeated. The psychomotor development evaluation guidelines by the Health and Social Security, Department of the Government of Catalonia, and the HOME scale (Home Observation for Measurement of the Environment) were followed to assess infant development [36]. Last study visit (months 18–30) included the Bayley Scales of Infant and Toddler Development®, Third Edition (Bayley-III®) test, to address children's psychomotor development (cognitive, sub-receptive language, expressive language, fine motor, and gross motor skills). A standardized socioeconomic questionnaire was administered to assess socio-demographic characteristics of the families and its association with children's neurodevelopment [37].

2.4. Laboratory evaluation and case definitions

Molecular diagnosis for ZIKV genome detection was performed using RT-PCR with a commercial test (Real Star® Zika RT-PCR kit, Altona Diagnostics). Antibodies against ZIKV (IgG and IgM) were detected by a commercial immunofluorescence test (Euroimmun, Lübeck, Germany). If positive results were found for ZIKV-IgM or IgG, samples were sent to the reference laboratory in Spain, Centro Nacional de Microbiología, Instituto de Salud Carlos III, for confirmation by microneutralization assays. Microneutralization titers of >1/32 were considered as indicative of presence of ZIKV antibodies [38]. A confirmed case of ZIKV infection was defined as a positive RT-PCR in serum or urine samples; a probable case as a positive serology for ZIKV with microneutralization titers >1/32; and a negative case as a negative serology for ZIKV or a positive serology with microneutralization titers <1/32 [38]. Antibodies against DENV (IgG and IgM) were tested by a commercial assay (Panbio ELISA, Alere, Australia), and against CHIKV using IFA (Euroimmun, Lübeck, Germany).

2.5. Statistical analysis and data management

Data collection, cleaning, and management were performed using the OpenClinica open source software, version 3.1 Copyright© OpenClinica LLC and collaborators, Waltham, MA, USA, www.OpenClinica.com. Categorical data were summarized by frequencies and percentages. Continuous variables were presented as median, and ranges (minimum and maximum values). Missing values were not considered in the description of the data. The number of observations was reported in each variable description. Key variables under investigation included travel dates, date of sampling, date of onset of symptoms, duration of symptoms, mosquito bites during travel, history of yellow fever vaccination, number of previous dengue episodes among others. The analysis was carried out using Stata (StataCorp 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LP). Microcephaly was defined by a head circumference 2 SD below the mean or <3rd percentile according to sex and gestational age [39]. Prematurity was defined as a neonate born before 37 weeks of gestation according to WHO definition.

2.6. Ethics statement

Ethical approval for this study was granted by the Ethics Review Committee of the Hospital Clínic of Barcelona, Spain (CEIC) [Reg. No. HCB/2016/0250]. The study was conducted in accordance with the Good Clinical Practice Guidelines, and under the provisions of the Declaration of Helsinki and local rules and regulations.

3. Results

3.1. Study profile

From the 1st of January 2016 to the 31st of December 2017, a total of 195 pregnant women with history of potential exposure were recruited in the study and screened for ZIKV (Fig. 1). They represented 10.8% of the total number of pregnant women screened for ZIKV in Catalonia. The distribution of cases by epidemiological week according to the date of their ZIKV screening is presented in Supporting information (Fig S1). Four women (2.1%) had a confirmed ZIKV infection, 40 (20.5%) had a probable ZIKV infection and 151 (77.4%) were negative for ZIKV.

3.2. Demographic, clinical and travel characteristics of study women

Table 1 shows the demographic, clinical and travel characteristics of the study women according to ZIKV screening results. Median age of women with confirmed ZIKV infection was 26.5 years old [23, 39]. Most confirmed and probable cases were exposed to ZIKV during the periconceptional period (37/44), or the first trimester of pregnancy (31/44). Three out of four ZIKV confirmed cases and 19/40 (48%) of the probable cases were symptomatic, while 18% of negative cases reported symptoms compatible with ZIKV infection. Commonly reported signs and symptoms included rash, headache, fever, asthenia, and myalgia. More than 50% (77/148) of pregnant women had previous exposure to DENV with detectable IgG antibodies, and nearly 15% to CHIKV (12/93). Furthermore, 6.8% of women screened (10/146) had positive results for anti-DENV IgM and 3.4% (3/88) for anti-CHIKV IgM. Detailed information on pregnant women with confirmed ZIKV infection is shown in Table 2, and data on probable cases in Table 3.

3.3. Pregnancy and infant outcomes

One of the four pregnant women with confirmed ZIKV infection suffered a miscarriage likely associated with infection occurring in the first eight week of gestation. The woman persisted with viremia for 28 days after obstetrical curettage [40]. Isolation of ZIKV from the karyotype cell culture showed active viral replication in embryonic cells from the miscarriage specimen [40]. Two women with confirmed ZIKV infection had fetal US and neurosonographic assessments with normal results, and in one woman a subtle fetal unilateral periventricular hyperechogenicity was found in the neurosonographic evaluation at 32 weeks of gestation, which evolved into a subependymal pseudocyst at 35 weeks. The newborn was born at term, asymptomatic, and ZIKV RT-PCR tests in serum, urine and cerebrospinal fluid samples were negative. Transfontanellar cerebral US at birth showed a small choroid plexus cyst (3 × 4.3 mm). No pathological findings were found in subsequent MRI performed at six months of age. Additionally, two fetuses (5%) out of the forty pregnant women with probable ZIKV infection presented with findings in neuroimaging; thus, a total of 7% (3/42) fetuses or infants presented brain calcifications or periventricular cysts. One fetus had a periventricular hyperechogenic image in prenatal US, later described by MRI at 36 weeks of gestation as a subcortical dotted foci in the frontal region suggestive of microcalcification, with no pathological findings in the transfontanellar cerebral US at birth or the MRI at two months of age. Another infant, with normal prenatal US, presented with bilateral choroid plexus cysts (3 × 3mm) at 6 months of age detected by US and confirmed by MRI at 13 months of age. See Table 3 for further details on

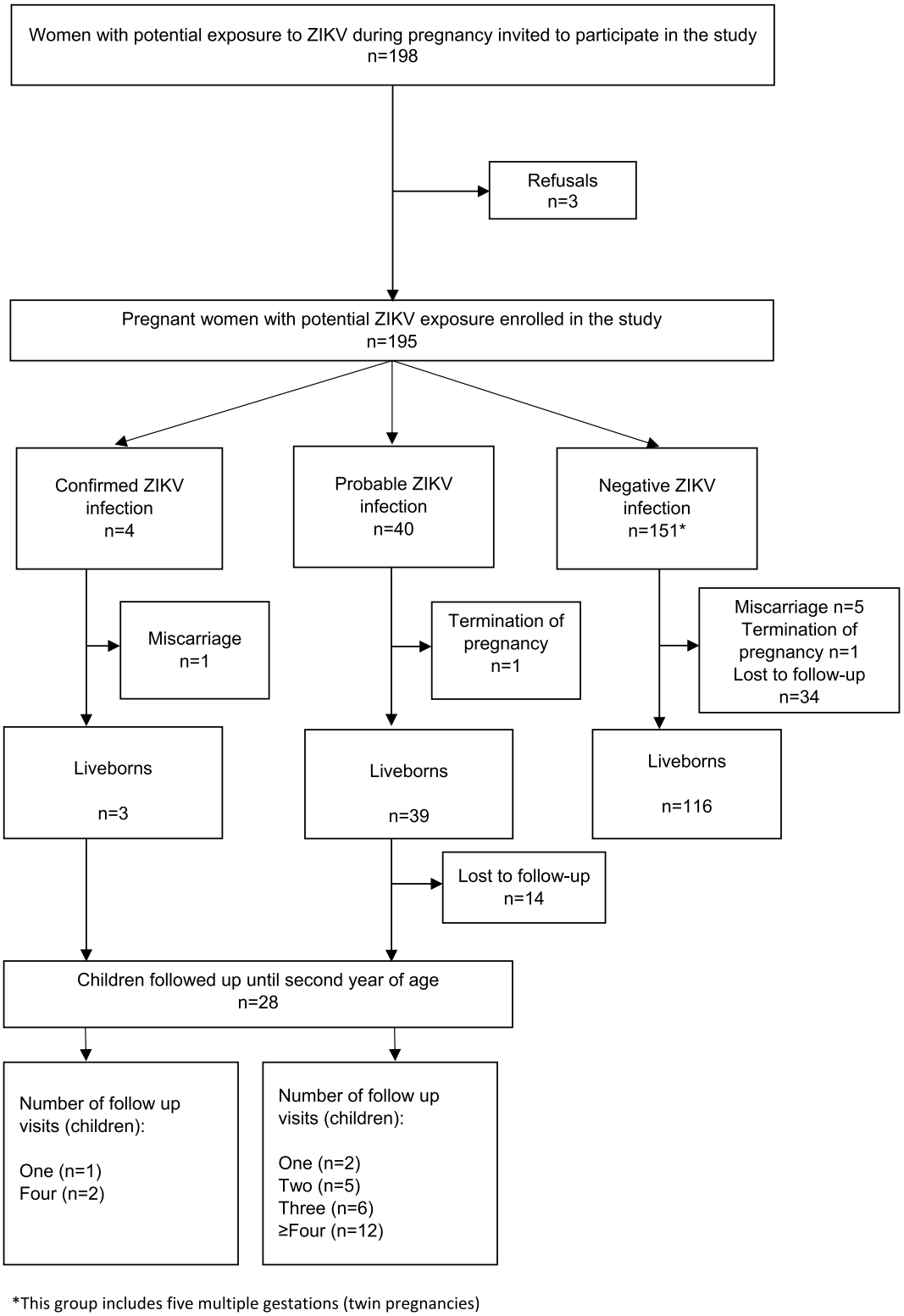

Fig. 1. Flow diagram of maternal enrolment in Barcelona, Spain, from January 2016 to December 2017, pregnancy outcomes, and children's follow up until February 2020.

clinical and laboratory characteristics of probable ZIKV cases. All breast milk samples were negative for ZIKV molecular diagnosis.

None of the newborns presented any apparent congenital anomaly associated with ZIKV infection. Congenital defects detected included two neonates with plagiocephaly, one neonate with left renal agenesis, immature sulcus pattern, and systolic murmur; one with mild signs of mineral bilateral vasculopathy with right predominance found by transfontanellar US; one neonate with deformed and low implantation

Table 1
Demographic, clinical and travel characteristics of study pregnant women at enrolment according to ZIKV infection status.

	Confirmed	Probable	Negative
Participants (N)	4	40	151
Age (years)§	26.5 [23, 39] [4]	27 [18, 41] [40]	33 [17, 49] [151]
Duration of symptoms (days) ¥	3 [2, 6] [3]	3 [1, 25] [19]	5 [1, 40] [26]
	N = 4 n (%)	**N = 40 n (%)**	**N = 151 n (%)**
Trimester at ZIKV exposure*			
Periconceptional	0 (0)	37 (93)	82 (55)
First	3 (75)	28 (70)	85 (57)
Second	2 (50)	20 (50)	70 (47)
Third	1 (25)	9 (23)	22 (15)
Gravidity			
Multigravidae	4 (100)	23 (57)	89 (59)
Country of exposure**			
Dominican Republic	3 (75)	12 (30)	14 (9)
Colombia	0 (0)	7 (18)	19 (13)
Brazil	0 (0)	3 (8)	23 (16)
Ecuador	1 (25)	1 (3)	17 (11)
Others	0 (0)	18 (45)	79 (53)
Symptomatic	3 (75)	19 (48)	26 (18)
Rash	3/3 (100)	12/19 (63)	10/26 (38)
Headache	1/3 (33)	13/19 (68)	9/26 (35)
Asthenia	1/3 (33)	10/19 (53)	12/26 (46)
Myalgia	1/3 (33)	8/19 (42)	6/26 (23)
Fever	0/3 (0)	11/19 (58)	16/26 (62)
Arthralgia	2/3 (67)	0/19 (0)	1/26 (4)
Conjunctivitis	0/3 (0)	4/19 (21)	1/26 (4)
>1 symptom reported	2/3 (67)	15/19 (79)	15/26 (58)
Laboratory results (n/N)			
Zika - RT-PCR	4/4	0/39	0/140
Zika - IgG	4/4	38/40	68/110
Zika - IgM	1/4	4/40	9/110
Dengue - IgG	3/3	34/34	40/75
Dengue - IgM	1/3	2/33	7/71
Chikungunya - IgG	0/0	7/18	5
Chikungunya - IgM	0/0	1/17	2

§ Median [Range] [n]; †We have 13 missing values for Negative group; ¥ and ∂ N correspond to symptomatic women (3, 19 and 26 for Confirmed, Probable and Negative group respectively); ∂ We have 2 and 7 missing values for the Probable and Negative group respectively; *Periconceptional period: before 14 days, Trimesters: 1st (3–12 weeks), 2nd (13–24), 3rd (25–40); **Other countries of exposure include: Peru, Honduras, Cuba, Mexico, Venezuela, Bolivia, Costa Rica among others; (n/N): number of positive patients and number of patients tested.

of auricular pavilions; one with sacral dimple; and one with cardiac interventricular communication (ventricular septal defect in echocardiography). Adverse perinatal outcomes detected included, two preterm neonates (6%) –one of them with prenatally detected bile lithiasis, one with mild hypoxic ischemic encephalopathy and respiratory distress, and one with paraphysiological jaundice and abdominal dysfunction. All neonates had negative results for ZIKV IgM antibodies and for RT-PCR in serum samples. Six out of seven neonates had positive results for ZIKV IgG. At six months of age, four out of six infants had positive IgG, and at 12 months of age, only one out of four infants had a positive IgG for ZIKV.

3.4. Neurodevelopmental assessment

Among the twenty-eight children that underwent clinical assessments during the first two years of age (cerebral US, ABR, eye fundus and psychomotor development evaluation), abnormal findings were found in two infants; one with physiological retinal hemorrhage in the eye examination at one month of age, and another one who showed a slightly altered V wave in the hearing assessment at four months of age.

Neurodevelopment was evaluated in twenty-one (50%) out of 42 study children. Cognitive, language and motor function domains were assessed in children between 12 and 36 months of age. Fig. 2 and Table 4 show individual scores on the Bayley-III scales for neurodevelopment assessment. One child (4.8%) scored below average (−1 to −2 SD, score 84–71) in the cognitive function, and seven (33.3%) in the language function; one of them performing well below the average (<-2 SD, scores < 70) . Motor function for all the children examined was within average score (±1 SD, score 85–115). One child presented a delay in the gross motor function, but the general score of the motor domain was within the average. All the parents of this group of children who underwent neurodevelopment evaluation were of migrant origin. One third of them, 33% of mothers and 28% of fathers, had a low level of education (they had only completed elementary education), and a significant proportion of them were unemployed (39% of mothers and 16% of fathers). Two children (9.5%) showed a performance above the average in the cognitive function.

4. Discussion

Of the women who travelled to ZIKV endemic areas, 2% had a laboratory confirmed ZIKV infection, and more than 20% were classified as probable cases. Women with confirmed or probable ZIKV infection were mainly exposed during the first trimester of pregnancy, and most of them were symptomatic. Among the ZIKV confirmed cases, one pregnant woman had a miscarriage highly plausible to be associated with ZIKV infection [40]. Brain findings in neuroimaging were detected in 7% of the fetuses born to women with confirmed or probable ZIKV infection, without apparent clinical translation into poor neurodevelopment outcomes in childhood. During the first year of life, children did not have pathological findings in neurodevelopment assessment; except for unspecific and mild findings, not apparently associated with ZIKV. Neurodevelopmental assessments performed during the second year of age unveiled a neurodevelopmental delay in 33.3% (7/21) of children, being the language function the most affected domain (with one children performing well below average, and six children with mild below average scores).

Regarding the risk of ZIKV infection among pregnant travellers, a large prenatal screening program in the USA showed that ZIKV infection was found in 5.3% of the pregnant women tested [41]. In this study, 2.1% of the women screened had a confirmed infection, but if probable cases are considered, the proportion of pregnant women affected by ZIKV would be 22.6%. However, the number of probable cases has to be interpreted with caution, since case definition relies on results from serology (IgM plus IgG) and microneutralization assays, which represents a different diagnosis approach than that used in the USA.

In our study, the proportion of asymptomatic women was lower (25% of women with confirmed infection, and 48% of probable cases) than in previous reports, where asympotamic infections were reported to occur in 80% of ZIKV cases [42,43]. This might be explained by the smaller number of women enrolled in this study. Also, our definition of confirmed ZIKV infection was more specific (presence of ZIKV RNA in serum by RT-PCR) than in other studies, as definitions in US rely on serological analysis based on IgM confirmation. In a prenatal ZIKV screening program in Dallas, USA, 83% of women with possible ZIKV infection did not report any symptoms [41] and data from the US Zika Pregnancy Registry [17] showed that 63% of pregnant women with possible ZIKV infection were asymptomatic. A study performed in Colombia showed that among pregnant women (n = 86) with confirmed ZIKV infection by RT-PCR the most prevalent symptoms were rash, in 79% of cases, 55.8% fever, 48.8% arthralgia and 23.3% of women presented with anaemia [44]. Children in our study were in-uterus exposed to ZIKV, but none of them was infected. A case of vertical transmission occurred, that resulted in an spontaneous abortion, which represents 2.3% out of the 44 confirmed and probable cases. Results are lower compared to a previously published study where maternal-fetal

Table 2
Description of case series of pregnant women with confirmed ZIKV infection and their infants.

Pregnancy								Delivery				
Age	GA	Country	Symptoms	Screening			Ultrasound	Pregnancy outcome	Placental findings	ZIKV screening		Sex, weight, HC
				ZIKV		DENV				M.	N.	
				RT-PCR*	**IgM/IgG**	**IgM/IgG**				**RT-PCR**	**RT-PCR, IgM/IgG**	
39	4–7	The D.R.	Rash and arthralgia	+	+/+	−/+	Periventricular choroid plexus cyst.	Live birth (40 + 0)	Choriamnionitis, scant Hofbauer cells, calcifications and fibrine depositions	Neg	Neg	Female, 2964 g, 34.5 cm
24	6–14	The D.R.	Rash, headache, arthralgias, myalgias and asthenia	+	+/+	NA	No pathological findings	Live birth (37 + 0)	Scant Hofbauer cells, calcifications, infarcted areas and fibrine depositions	Neg	Neg, Positive IgG in infants' blood	Male, 2800 g, 34 cm
29	20–25	Ecuador	None	+	+/+	−/+	No pathological findings	Live birth (40 + 6)	Choriamnionitis, calcifications and fibrine depositions	Neg	Neg	Female, 3310 g, 34 cm
23	7–10	The D.R.	Rash	+	+/+	+/+	Early pregnancy loss at 10 weeks of gestation	Miscarriage (10 + 6)	Moderate Hofbauer cells and fibrine depositions	Pos. in curettage	Infective virus isolated	NA

Units: Age (in years); Gestational age (GA) at time of exposure to ZIKV (in weeks); Pregnancy outcome includes gestational age in weeks + days; *RT-PCR results from serum samples, all urine samples tested negative for ZIKV. DENV: Dengue virus; GA: Gestational Age; HC: Head Circumference; Ig: Immunoglobulin; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; The D.R.: The Dominican Republic; M.: Maternal, N.: Newborn; NT: Micro-neutralization Test; NA: Not available; ZIKV: Zika virus.

transmission occurred in a quarter of exposed fetuses [45]. Data from the USA Zika Pregnancy Registry showed that 6% of infants born to pregnant women with possible ZIKV infection had birth defects potentially associated with the virus [8,46–48]. The proportion was higher when restricted to pregnant women with confirmed ZIKV (10%), and for infections in the first trimester (15%) [46]. Data from French Polynesia suggested that the risk of microcephaly was approximately 1% for cases of ZIKV infection occurring during the first trimester of pregnancy; other birth defects were present in 7% of infants from mothers with maternal symptomatic RT-PCR-confirmed ZIKV infection [49]. A modelling study based on data from Bahia, Brazil, suggested a risk between 1% and 13% respectively [50]. A recent literature review described vertical transmission of ZIKV to occur in around 20%–30% of pregnant women with ZIKV infection; spontaneous abortions occurred in 4%–7% of cases, and CZS in 5%–14% of cases (including 4%–16% risk of microcephaly) [51].

In our study, no birth defect potentially associated with the virus was detected among study infants. The subtle anomaly observed in the fetus of a mother with confirmed ZIKV did not translate into any clinical pathological finding or altered imaging after the first year of life. The child presenting a brain calcification in prenatal US had a normal neurodevelopment assessment at the second year of age; only showing a delay in the gross motor domain. It has been reported that 70%–80% of healthy infants born to mothers with confirmed ZIKV infection could develop medium and long-term sequelae [51]. Differences in frequency of birth defects between this and other studies might be explained by the number of pregnant women enrolled, and by the number of ZIKV confirmed and probable cases detected.

On the histopathological features of the placentas assessed in this study, these showed some, and in all cases mild, unspecific inflammatory changes suggestive of infection, such as chorioamnionitis, presence of Hofbauer cells, lymphocytic infiltration, fibrin deposits, infarcted areas, fibrosis and calcifications, which are consistent with findings from recent reports including evaluation of placental specimens [52].

We reported fetal brain calcifications in the prenatal US assessment of one out of 42 pregnant women, and brain periventricular cysts in two fetuses/infants, both unspecific findings with no confirmed clinical translation. Different studies have reported abnormal neuroimaging findings among non-infected children exposed to ZIKV prenatally [10,53]. One of these studies showed 10% of children with normal neurological evaluation presenting with structural anomalies on neuroimaging, being calcifications the most common finding [10]. A cohort study of 28 pregnant women with confirmed ZIKV infection by RT-PCR found two women with fetal brain calcifications and highlighted the need to include careful assessment of possible fetal anomalies by US exams [54].

In line with recent evidence [17,18], children in our study performed lowest in the language function, with 33.3% of children affected; however, only one children showed a extremely low score in the language domain. Recent studies have described language and cognitive delays in normocephalic children infected with ZIKV (31% and 14%, respectively) [23], in healthy at birth non-infected children born to women with confirmed ZIKV infection (31%–35% and 3.4%–9%, respectively) [19,55]; and in healthy at birth ZIKV exposed non-infected children born to women with probable ZIKV infection. Neurodevelopment delay was reported in total score: self-care domain, communication, social cognition and mobility (assessed by the Warner Initial Developmental Evaluation of Adaptive and Functional Skills) [56]. One of this studies suggested that receptive language was the only domain that differed significantly among ZIKV exposed and non-exposed children [55]. On the contrary, a recent study did not find significant differences in the neurodevelopment of children prenatally exposed to ZIKV and unexposed controls [57]. Studies comparing children neurodevelopment in groups exposed and not exposed to ZIKV are scarce. The only two studies including a control group used different tools to measure development, Bayley-III Scales vs. Mullen Scales of Early Learning (MSEL), and had a small sample size [55,57]. Further studies including longer-term children's follow up are needed to unveil if delay in language, or in any other domains, among infants who were healthy at birth is significantly associated with prenatal exposure to ZIKV infection. Most families in the study were of low socio-economic status, low educational level, and unemployed. Data on childhood neurodevelopment needs to be interpreted with caution as demographic characteristics could act as confounders of its association with prenatal exposure to ZIKV. Neurodevelopment delay in early life has been associated with low maternal socioeconomic status [53].

The prospective characteristics of this study enrolling both symptomatic and asymptomatic pregnant women, the wide variety of samples collected, the use of RT-PCR methods, and the extended follow up of children including neurodevelopmental assessment over the first two years of life make this study one of the few of its class. Even so, this study has several limitations. For laboratory ascertainment of ZIKV infection, the study followed regional guidelines for ZIKV screening during

Table 3
Description of case series of pregnant women with probable ZIKV infection and their infants.

Pregnancy								Delivery	
Age	GA	Country	Symptoms	Screening				Pregnancy outcome	Sex, weight, HC
				ZIKV		DENV	CHIKV		
				IgM/ IgG	**NT**	**IgM/ IgG**	**IgM/ IgG**		
19	P	Bolivia	Rash, headache, arthralgia, myalgia, fever, and asthenia	−/+	NA	−/+	NA	Live birth (40 + 4)	Male, 3580 g, 34 cm
35	P-T1	The D.R.	Rash, headache, arthralgia, myalgia, and asthenia	+/+	+	−/+	−/−	Voluntary interruption of pregnancy (13 + 4)	NA
33	P-T1	Brazil	Rash, headache, arthralgia, myalgia, and asthenia	−/+	+	+/+	−/−	Live birth (40 + 3)	Male, 3280 g, 36 cm
36	P-T1	Colombia	Fever and asthenia	−/+	+	−/+	NA	Live birth (37 + 0)	Male, 3070 g, 35 cm
38	P-T3	Colombia	Rash	−/+	+	−/+	−/−	Live birth (40 + 2)	Male, 3280 g, 36 cm
31	P-T2	The D.R.	Rash	−/+	+	−/+	NA	Preterm live birth (36 + 5)	Female, 2660 g, 33 cm
41	P-T1	Colombia	Fever, asthenia, and arthritis	−/+	+	−/+	−/−	Live birth (37 + 1)	Female, 3230 g, 34.5 cm
32	P-T1	The D.R.	Headache, asthenia, and arthralgia	−/+	+	−/+	−/−	Live birth (39 + 4)	Male, 3450 g, 35 cm
19	P-T2	The D.R.	None	NA	+	NA	NA	Live birth (41 + 0)	Male, 3410 g, 36 cm
27	T1-T2	The D.R.	Rash	−/+	+	NA	NA	Live birth (37 + 6)	Male, 2960 g, 35 cm
21	P-T2	Venezuela	None	−/+	+	−/+	−/+	Live birth (41 + 1)	Female, 3060 g, 34 cm
21	P-T2	Colombia	None	−/+	+	−/+	−/+	Live birth (40 + 2)	Female, 2850 g, 33 cm
25	P-T1	The D.R.	Fever, headache, and asthenia	−/+	+	−/+	−/−	Live birth (37 + 5)	Male, 2540 g, 33 cm
27	P-T3	Colombia	Fever, rash, headache, arthralgia, myalgia, red eyes, and asthenia	+/+	+	−/+	−/−	Live birth (38 + 6)	Male, 3080 g, 35 cm
29	P-T2	The D.R.	None	−/+	+	−/+	−/−	Live birth (39 + 3)	Male, 3076 g, 35.5 cm
21	P-T2	Brazil	None	−/+	+	NA	NA	Live birth (39 + 4)	Female, 3360 g, 37 cm
31	P	Honduras	None	−/+	+	−/+	NA	Live birth (41 + 3)	Male, 3550 g, NA
23	P-T1	The D.R.	Fever, rash, headache, arthralgia, myalgia, red eyes, and asthenia	−/+	+	−/+	NA	Live birth (39 + 3)	Male, 3242 g, 33 cm
29	P	Honduras	Fever, and headache	−/+	+	−/+	NA	Live birth (40 + 3)	Male, 3730 g, 36 cm
35	P	Honduras	Fever, headache, arthralgia, and myalgia	−/+	+	−/+	NA	Live birth (40 + 0)	NA
24	P-T1	Honduras	None	−/+	+	−/+	−/+	Live birth (38 + 4)	Male, 3120 g, 34 cm
28	T3	The D.R.	None	−/+	+	NA	NA	Live birth (38 + 4)	Male, 3430 g, 34.5 cm
20	P-T2	The D.R.	None	−/+	+	NA	NA	Live birth (38 + 6)	NA
26	P-T2	Colombia	None	−/+	+	NA	NA	Live birth (40 + 5)	Male, 3140 g, 35.5 cm
33	P	Thailand	None	−/+	+	−/+	−/−	Live birth (39 + 4)	Male, 3392 g, 33 cm
27	P-T3	Equatorial Guinea	None	−/+	NA	−/+	NA	Live birth (39 + 5)	Male, 3330 g, 35 cm
23	P-T1	Brazil, Venezuela, Colombia and French Guiana	None	−/+	+	NA/+	−/+	Live birth (39 + 2)	Male, 2940 g, 34 cm
22	P	Honduras	None	−/+	+	−/+	NA	Live birth (39 + 6)	Male, 3320 g, 33 cm
28	P	Honduras	None	−/+	+	−/+	+/+	Live birth (40 + 3)	NA
26	P-T1	Ecuador	None	−/+	+	−/+	NA	Live birth (40 + 2)	Male, 3226 g, 34 cm
18	P-T2	The D.R.	Headache, and red eyes	−/+	+	−/+	NA	Preterm live birth (36 + 5)	Male, 2815 g, 33.5 cm
31	P-T3	Honduras	None	−/+	+	−/+	NA	Live birth (40 + 1)	Male, 3820 g, 36 cm
32	T2-T3	Honduras	Rash	+/+	+	−/+	−/−	Live birth (41 + 0)	Male, 3800 g, 36.2 cm
22	P-T3	Venezuela	None	+/+	+	−/+	NA	Live birth (40 + 5)	Male, 3640 g, 35 cm
22	P-T2	Honduras	None	−/+	+	NA	NA	Live birth (38 + 0)	Male, 3420 g, 35 cm

(*continued on next page*)

Table 3 (*continued*)

Pregnancy								Delivery	
Age	GA	Country	Symptoms	Screening				Pregnancy outcome	Sex, weight, HC
				ZIKV		DENV	CHIKV		
				IgM/ IgG	**NT**	**IgM/ IgG**	**IgM/ IgG**		
20	P-T3	Honduras	None	NA/+	+	NA/+	NA/+	Live birth (40 + 2)	Male, 3900 g, NA
27	P	El Salvador	Fever, rash, headache, arthralgia, myalgia, and red eyes	−/+	+	−/+	NA	Live birth (38 + 6)	NA
31	P-T3	Venezuela	None	−/+	+	−/+	NA	Live birth (38 + 3)	Female, 3400 g, 36 cm
26	P	The D.R.	Fever, rash, and headache	−/+	+	−/+	NA	Live birth (39 + 3)	NA
29	P	Venezuela	Fever, rash, headache, arthralgia, myalgia, and asthenia	−/+	+	NA	−/−	Live birth (NA)	Female, 3020 g, 34.5 cm

Units: Age (in years); Gestational age (GA) at time of exposure to ZIKV (in weeks); Pregnancy outcome includes gestational age in weeks + days; CHIKV: chikungunya virus; cm: centimeters; DENV: Dengue virus; g: grams; GA: Gestational Age; HC: Head Circumference; Ig: Immunoglobulin; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; The D.R.: The Dominican Republic; M.: Maternal, N.: Newborn; NT: Micro-neutralization Test; NA: Not available; NT: Microneutralization assay; P: Periconceptional period; T: Trimester of pregnancy (T1, T2 or T3; the number represents the order in trimesters); ZIKV: Zika virus.

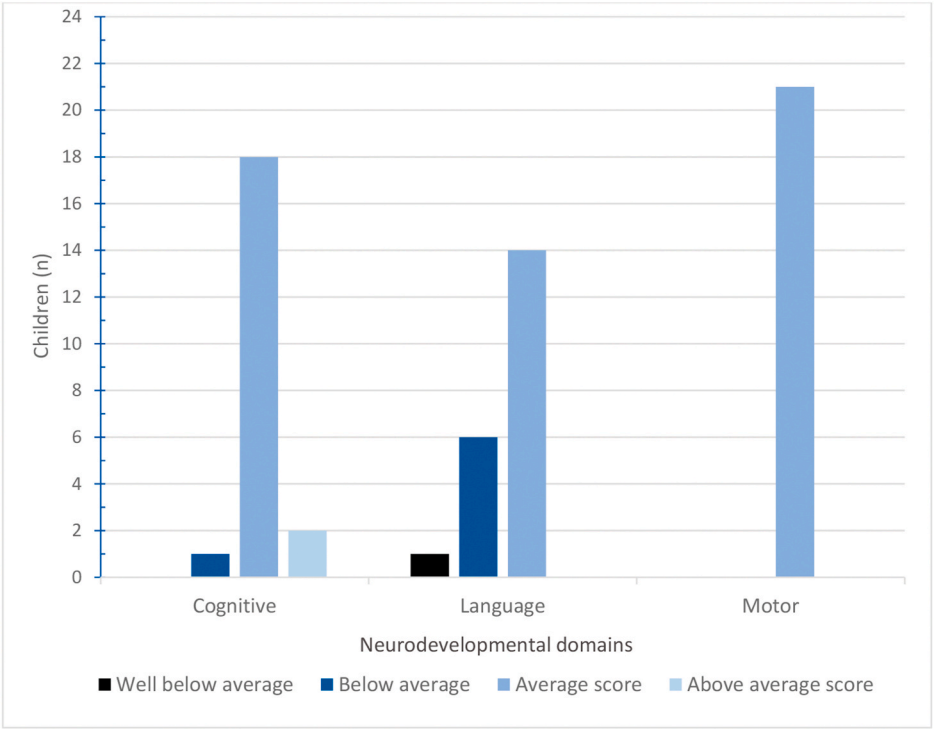

Fig. 2. Individual Scores on the Bayley-III Scales assessment in 21 children between the ages of 12–36 months of age for cognitive, language, and motor functions (Bayley Scales of Infant and Toddler Development, third edition). Scores for cognitive, language, and motor functions on the Bayley Scales of Infant and Toddler Development, third edition (Bayley-III) are shown. Standard scores on the Bayley-III scales range from above average (1–2 SD, score 116–130), average (−1 to 1 SD, score 85–115), below average (1 to– 2 SD, score 84–70) and well below average (<-2 SD, scores < 70).

gestation, which included determination of IgM and IgG antibodies for ZIKV and confirmation by microneutralization assays at the national referral center. This approach may represent a limitation because 1) the microneutralization assay was not performed for other flaviviruses, 2) this diagnostic approach differs from those used in other settings, predominantly based on IgM determination, and 3) a considerable proportion of women included in the group of probable cases might be actual ZIKV confirmed cases. The ongoing obstacles to serologic-based screening and diagnosis require an urgent development of rapid, sensitive, specific and widely available tests for detection of viral antigens [58], to avoid misclassification and eventually inadequate management of cases. In addition, the low number of ZIKV confirmed cases did not allow us to assess associations between ZIKV infection and poor pregnancy outcomes and its risk factors. As pregnant women found to be negative for ZIKV infection did not have follow up evaluations, according to study protocol, comparisons in pathological findings in the US, MRI and neurodevelopment assessments at birth could not be performed. Additionally, high lost to follow up rates during childhood limited sample size for neurodevelopmental assessment. Lastly, other variables than the socioeconomic data, could have had an effect on the neurodevelopmental assessment, such as maternal alcohol and tobacco use, or intercurrent infections, that we could not consider. Regardless of the limitations, to our knowledge, this is the first prospective observational study on the risk of ZIKV maternal infection and subsequent adverse outcomes in their children among pregnant travellers conducted in a non-endemic area in Europe.

5. Conclusions

This prospective observational study shows that pregnant women returning from ZIKV affected areas are at risk of acquiring the infection and presenting harmful consequences on their offspring. It also corroborates

Table 4
Neurodevelopmental evaluations of 21 children exposed to ZIKV while in uterus using the Bayley Scales of Infant and Toddler Development-Third Edition (BSID-III).

	Neurodevelopmental delay, n (%)					
BSID-III domain	Mean score (SD)	Range	Moderate delay (score 55–70) < -2SD	Mild delay (score 71–84) -1SD/-2SD	Average score (score 85–115) 1 SD	Above average (score 116–130) 1–2 SD
Cognitive	101.0 (24.4)	[80, 125]	0	1 (5%)	18 (86%)	2 (10%)
Language	90.3 (22.6)	[68, 112]	1 (5%)	6 (29%)	14 (67%)	0
Motor	100.4 (23.3)	[85, 115]	0	0	21 (100%)	0

the limitations and challenges of current screening and diagnostic tools for the ascertainment of ZIKV infection. Language delay was the domain most affected in the long-term neurodevelopmental assessment, though socio-demographic characteristics may confound its potential association with ZIKV prenatal exposure. These findings contribute to our understanding on ZIKV prevalence estimates and its impact on pregnancy outcomes and children's health. This stresses the importance of epidemiological surveillance of ZIKV in pregnancy, and children's long-term follow up.

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Declaration of competing interest

Authors have no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2021.101985.

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