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Diagnosis and outcomes of pregnant women with Zika virus infection in two municipalities of Risaralda, Colombia: Second report of the ZIKERNCOL study☆

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ABSTRACT

Background: Zika virus (ZIKV) infection has emerged as a significant threat for pregnant women and newborns in populations living in or visiting Latin America. We previously reported a preliminary analysis in Sucre, Colombia, as the first group of pregnant women with RT-PCR-confirmed ZIKV (***ZIK****a en* ***E****mbarazadas y* ***R****ecién* ***N****acidos en* ***COL****ombia*, ZIKERNCOL).

Methods: In this second report, findings of the first 86 pregnant women from La Virginia and Dosquebradas (municipalities), Risaralda, Colombia, with RT-PCR-confirmed ZIKV infection are reported. Clinical, demographical and obstetrical findings are described.

Results: All women reported ZIKV symptoms during pregnancy: 79.1% rash, 55.8% fever, among others. In addition to ZIKV, RT-PCR was positive for dengue in 18.6%; 45.3% Dengue IgM+; 5.8% RT-PCR positive for chikungunya; 3.6% Chikungunya IgM+. STORCH screening in mother: 11.6% IgG + anti-*Toxoplasma gondii*, 6% IgG + anti-rubella, 4.7% IgG + CMV. The rest of STORCH tests were negative. Microcephaly was observed in 2.4% of the newborns. No calcifications or other CNS alterations were detected. One newborn had cleft palate and one had bilateral renal ectopy.

Conclusions: The rate of microcephaly in our cohort was consistent with other studies. Pregnant women in endemic areas should be followed and tested according to standard protocols, and asymptomatic ZIKV infection should be considered. Long-term follow-up of children is required in the congenital Zika syndrome (CZS) assessment.

☆ This study was previously presented in part at the 27th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Vienna, Austria, April 22–25, 2017 (Session: EP072A Late-breaker: what else is being discussed out there? – Moderated ePoster: EP0381E) as well at the XVIII Pan-American Congress of Infectious Diseases (API), Panama City, Panama, May 16–20, 2017 (Oral presentation).

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1. Introduction

Zika virus (ZIKV) infection has had a significant impact in terms of morbidity and long-term consequences in over 48 countries in the region of the Americas from 2015 till 2018 [1]. Though epidemics in the region were declared concluded, transmission still occurs in many countries [2], including Colombia [3]. Thus, the real magnitude of its effects on the population remain to be defined, especially for those suffering the Congenital Zika Syndrome (CZS) and its wide spectrum of complications [4–6].

Despite multiple studies in Brazil and other countries in the Americas, there are still many questions that should be addressed regarding the impact of ZIKV during pregnancy as well as on newborns and children exposed to this arboviral infection [7]. Duration of viral persistence in mother and child [7], transmission risk associated with breastfeeding [8,9], extent of motor and cognitive impairments, incidence of epilepsy [10,11], and the range of visual complications [10–13] are just some examples of the multiple potential aspects and sequelae of ZIKV infection during pregnancy that warrant further investigation.

Although over 100,000 cases were diagnosed in Colombia just during 2015–2016 (around 10% confirmed by RT-PCR), there is a lack of studies about ZIKV in pregnancy [3,14–18]. About 20,000 cases of ZIKV (one-third with RT-PCR confirmation) in pregnant women were reported in that period, with 291 cases linked to microcephaly [6,19]. In 2017, almost 2000 additional cases were reported, with around 13% of them being pregnant, including 39 cases of microcephaly and CZS. Thus, ZIKV transmission and its adverse health effects are an ongoing problem. For 2018, 481 cases have been reported through June 2, 28.5% of them in pregnant women [5].

In this setting, we previously reported a preliminary analysis of the first group of pregnant women from the Colombia (Sucre) with RT-PCR-confirmed ZIKV (***ZIK****a en* ***E****mbarazadas y* ***R****ecién* ***N****acidos en* ***COL****ombia*, ZIKERNCOL) [3,19]. The present study sought to identify the main clinical, sociodemographic and maternal-fetal complications in patients with ZIKV infection in two hospitals in the municipalities of La Virginia and Dosquebradas, department of Risaralda, Colombia, in 2016.

2. Methods

2.1. Study design and setting

A retrospective cohort study was carried out in a population of 86 pregnant women with ZIKV infection who attended medical consultation at Hospital San Pedro y San Pablo (La Virginia municipality) and Hospital Santa Monica (Dosquebradas municipality), primary care institutions, department Risaralda, Colombia, during 2016. Suspected ZIKV cases were recruited based on symptoms and the diagnosis was then confirmed by RT-PCR.

Risaralda is a department in the Coffee-Triangle region of Colombia, which includes three departments (Caldas, Quindio and Risaralda) (first administrative territory level) and 53 municipalities (second administrative territory level). La Virginia and Dosquebradas (urban areas) are two of them, with 32,114 and 200,829 inhabitants, respectively (2016) and with prone ecoepidemiological conditions for arboviral diseases, including entomological studies done by our group identifying *Aedes aegypti* and *A. albopictus* in these territories [20–22]. Both municipalities border Pereira, the capital of Risaralda (957,250 habitants in 2016), and other endemic areas of ZIKV, chikungunya (CHIK) and dengue (DENV) [22–24].

2.2. Inclusion criteria

- Patients who met the definition of suspected case of ZIKV according to the criteria of the National Institute of Health (INS), Bogotá, Colombia: rash with or without pruritus, and/or fever.
- Patients who had been confirmed as positive (RT-PCR), during 2016, at the two sites.

Table 1
Sociodemographic and maternal findings in pregnant women with ZIKV infection in two municipalities of Risaralda, Colombia, 2016.

Variables	n	%
Sociodemographic variables		
Sites		
La Virginia	46	53.5
Dosquebradas	40	46.5
Age (years)		
< 15	4	4.7
> 35	2	2.3
Age (mean ± SD) (years)	24.12 ± 5.5	
Maternal Variables		
Prenatal Control		
Yes	61	70.9
No	25	29.1
Number of Pregnancies		
First (primigravid)	40	46.5
Second	29	33.7
Third	11	12.8
Fourth	2	2.3
Fifth	3	3.5
Sixth	1	1.2
Deliveries		
1	40	46.5
2	28	32.6
3	12	14.0
4	1	1.2
5	2	2.3
6	2	2.3
Gestational trimester		
First	20	23.3
Second	32	37.2
Third	34	39.5
Weeks of gestation (mean ± SD)	22.6 ± 10.3	
Spontaneous abortions		
0	72	83.7
1	11	12.8
2	2	2.3

SD = Standard deviation.

2.3. Variables

Information was obtained on sociodemographic data of the patients, maternal variables (obstetric history) (Table 1), clinical and laboratory findings (Table 2), fetal-newborn findings (Tables 3 and 4).

2.4. Statistical analysis

All data were recorded in a predesigned format, tabulated and the results analyzed statistically by StataIC 14 (64-bit), licensed for Universidad Tecnologica de Pereira. Descriptive statistics were used, quantitative variables were presented with mean (± standard deviations, SD), as well qualitative with proportions (%). Relative risk (RR) was calculated for any adverse maternal and newborn outcomes. P values considered significant if < 0.05, 95% confidence intervals (95% CI) were calculated.

2.5. Ethical considerations

As a retrospective study, access to clinical charts was authorized by the Hospital Boards and Directors. All study activities were approved by the Universidad Tecnologica de Pereira Ethics Committee.

3. Results

In this cohort of 86 women, the mean age was 24 years-old (± 5.5).

Table 2
Clinical and laboratory findings presented in pregnant women with ZIKV.

Findings	n	%
Clinical		
Rash	68	79.1
Fever	48	55.8
Headache	46	53.5
Arthralgia	42	48.8
Myalgia	40	46.5
Malaise	39	45.3
Anemia	20	23.3
Asthenia	16	18.6
Conjunctivitis	12	14.0
Retroocular pain	8	9.3
Lumbar pain	5	5.8
Lower limb edema	3	3.5
Pelvic pain	2	2.3
Required Hospitalization	13	15.1
Comorbidities		
Thyroid disorder	9	10.5
Obesity	2	2.3
Gestational Diabetes	1	1.2
Arterial Hypertension	1	1.2
Vital signs		
Heart Rate (> 100 per minute)	15	17.4
Respiratory Rate (> 22 per minute)	6	6.9
Temperature (mean ± SD) (ºC)	36.49 ± 0.74	
Systolic blood pressure (mean ± SD) (mmHg)	109.9 ± 9.90	
Diastolic blood pressure (mean ± SD) (mmHg)	99.9 ± 8.8	
Laboratories		
Blood count		
Leukopenia	20	23.3
Neutrophilia	12	14.0
Thrombocytopenia	10	11.6
Leukocytosis	4	4.7
Leukocytes (mean ± SD) (cells/mL)	6300 ± 5345	
Hematocrit (mean ± SD) (%)	30.68 ± 11.16	
Hemoglobin (mean ± SD) (mg/dL)	10.25 ± 3.740	
Platelets (mean ± SD) (cells/mL)	220884 ± 109087	
Serological and molecular tests		
Toxoplasma gondii-IgG	10	11.6
Rubella-IgG	5	5.8
CMV-IgG	4	4.7
Dengue RT-PCR	16	18.6
Dengue-IgM	39	45.3
Chikungunya RT-PCR	5	5.8
Chikungunya-IgM	3	3.6
HIV Western-blot	3	3.6
VDRL/FTA-ABS	3	3.6
HBVsAg	1	1.2

SD = Standard deviation; RT-PCR = real time-polymerase chain reaction; CMV = Cytomegalovirus; HIV = Human Immunodeficiency Virus; VDRL = Venereal designed research laboratory; FTA-ABS = Fluorescent Treponemal Antibody Absorption; HBVsAg = Hepatitis B virus surface antigen.

The mean gestational age was 22.9 weeks (± 10.1) at enrollment, 46.5% being primigravidae, 16.3% had previous miscarriages and 46.5% had history of C-sections, among other characteristics. ZIKV infection occurred during the first trimester in 23.3%, 37.2% during second and 39.5% during the third (Table 1).

All patients reported ZIKV symptoms during pregnancy (as this was part of the inclusion criteria for this cohort), 79.1% had rash, 55.8% fever, 48.8% arthralgia, 23.3% anemia, 14% conjunctivitis, among other findings (Table 2). From the laboratory findings, 11.6% of the patients presented thrombocytopenia (Table 2).

In addition to ZIKV, RT-PCR was positive for dengue in 18.6%; 45.3% Dengue IgM+; 5.8% RT-PCR positive for chikungunya; 3.6% Chikungunya IgM+. Regard the STORCH: 11.6% IgG + anti-*Toxoplasma gondii*, 6% IgG + anti-rubella, 4.7% IgG + CMV. In addition, HIV, VDRL/FTA-ABS and HBVsAg were positive in 3.6, 3.6 and 1.2%, respectively (Table 2). Testing for other STORCH pathogens was negative (Table 2).

In the newborns, 9.3% presented head circumference below −1 SD of the WHO reference chart, and 2.4% below −2 SD (microcephaly), with no calcifications or other Central Nervous System (CNS) alterations detected (Table 3). Cleft palate was observed in one newborn, and bilateral renal ectopy was observed in another (Table 4). In total, 23.3% of all pregnancies were complicated by at least one adverse outcome (Table 4), with primigravidae women being at higher risk (RR = 3.045; 95%CI 1.018–9.105).

Table 3
Clinical findings of neonates from pregnant women with ZIKV.

Neonatal variables	n	%
WHO Head circumference percentile < −1 SD	8	9.3
WHO Head circumference percentile < −2 SD	2	2.4
APGAR at the first minute (points)		
7	6	7.0
8	13	15.1
9	3	3.5
APGAR at 5 min (points)		
9	2	2.3
10	21	24.4
Height (cm)		
45	1	1.2
46	2	2.3
47	2	2.3
48	8	9.3
49	8	9.3
50	2	2.3
51	2	2.3
Head circumference (mean ± SD) (cm)	34.47 ± 2.280	
Thorax perimeter (mean ± SD) (cm)	33.16 ± 1.410	
Birth weight (mean ± SD) (grams)	2468.4 ± 1249.4	

WHO = World Health Organization; SD = Standard deviation.

Table 4
Gestational and neonatal outcomes.

Outcome	n	%
Referral to other institution	7	8.1
Hypotension	7	8.1
Caesarean section	6	7.0
Leukocytosis	4	4.7
Threatened abortion	3	3.5
Premature rupture of membranes	2	2.3
Preeclampsia	2	2.3
Preterm birth	2	2.3
Microcephaly	2	2.3
Fetal suffering	1	1.2
Pneumonia	1	1.2
Fissured lip	1	1.2
Voluntary termination of pregnancy	1	1.2
Induction failed	1	1.2
Prolonged expulsion	1	1.2
Bilateral renal ectasia	1	1.2
Low birth weight	1	1.2
Preterm labor threat	1	1.2

4. Discussion

Zika virus infection still represents a significant cause of concern, especially in pregnant women [25–29]. Although most epidemics in the Americas region have ceased, transmission became endemic in ecoepidemiologically prone areas [24,30], such as is the case of La Virginia and Dosquebradas, Colombia, where our study took place [20,21]. During 2016, the populations in these urban areas, including pregnant women and their newborns, were heavily affected. As expected, microcephaly and CZS as consequence of ZIKV infection during pregnancy were observed at a relatively low rate, consistent with the recent literature [14,26,31–35]. Nevertheless, when this occurs, its impact on those few cases is high in terms of morbidity and long-term complications [5,36].

Studies have reported that acquiring ZIKV infection during the first trimester is associated with a higher risk of fetal complications [37]. First trimester ZIKV infections occurred in one-fifth of our cohort, and this subset of subjects accounted for all of the adverse fetal outcomes observed. Even more, the associated risk between being primigravidae with ZIKV infection and adverse pregnancy outcome was three times higher compared to multigravidae in our study. In a recent, larger study, also one-fifth of their ZIKV-infected pregnant women were primigravidae, but the association with adverse fetal outcomes was not assessed [35].

Our data confirmed that rash is the most common clinical manifestation of ZIKV infection in pregnant women as has been described in multiple studies [35,38–40]. In a similar study in Brazil, 72% of the pregnant women presented with rash as the main symptom [41]. Most studies have shown that fever is not a frequent finding in ZIKV infection. One cohort study of pregnant women with ZIKV infection showed that the fever (temperature $\geq 38\,^{\circ}C$) was present in less than one-third of the population [42], which is also consistent with our study, where less than 60% had documented fever. A similar matter would be considered for conjunctivitis, which appeared to be higher in non-pregnant women and adults in other studies [3,5,35,42,43].

Over 70% of our pregnant women attended the standard recommended antenatal visits, similar to other reports [26,35]. However, one study that followed 442 pregnant patients in the US with laboratory evidence of possible ZIKV infection demonstrated that adherence to testing and management guidelines was not perfectly consistent even among women accessing antenatal care [26]. Asymptomatic ZIKV infections during pregnancy resulting in birth defects have also been reported, highlighting the need for more studies, not only those based on passive surveillance, but also active and community and population-based [44–48]. Most studies and protocols, only measure ZIKV RNA once in the blood (viremia) in asymptomatic and symptomatic cases, but this could be prolonged and persistent over months (maybe > 1 year) [49,50] in mothers and newborns, requiring additional assessments given the potential implications [7–9,48].

Leukopenia, anemia and thrombocytopenia (including severe thrombocytopenia) were the most significant alterations in the blood count. Two patients with leukopenia associated with ZIKV infection have been previously reported [51]. Thrombocytopenia was described in a study in which 7 patients with it had ZIKV infection (and not dengue) [52]. In the context of atypical, severe, and even lethal ZIKV cases (non-pregnant women), thrombocytopenia (including severe, < 60,000 cells/mL) has been observed [52–55].

No reports yet were found with relationships between signs and symptoms with gestational complications in ZIKV infection. In the United States of America also reports described that fetuses and infants of pregnant women with asymptomatic ZIKV infection may be at risk of developing microcephaly, CZS and other birth defects [56]. Neurotropism of ZIKV has been demonstrated over the last 3 years, showing the suitability for such clinical manifestations [57–60]. Although, society was concerned about the potential impact of ZIKV infection in pregnancy, this did not necessarily affect the prevention of reproduction. A study carried out in Colombia, showed that there was little adherence to contraceptive recommendations [61]. Thus, enhanced surveillance and prevention methods are urgently needed.

The major limitation to our study was the relatively small cohort size resulting in inability to define all the factors associated with rare, adverse outcomes such as microcephaly. Despite this, we show the clinical findings and associated outcomes of ZIKV infection from pregnant women in two areas of Colombia. In addition to ZIKV infection, other infectious agents such as *Toxoplasma gondii*, rubella and CMV, among others regional or locally occurring (e.g. malaria, Chagas disease) infections, are still important exposures in endemics areas of the country and could lead to microcephaly and other CNS alterations (e.g. especially, brain calcifications) [62]. Also, in the context of co-circulation of other arboviral diseases such as chikungunya and dengue, coinfections can occur [1]. During the recent epidemics, especially in Colombia, multiple coinfections with these arboviruses have been reported [63,64], including in pregnant women [15]. In our study, less than a fifth of the pregnant women with ZIKV infection were coinfected with acute dengue and 6% with acute chikungunya. But, also recent infections potentially occurred among them before ZIKV, 45% and 4%, respectively. A very recent study in Colombia confirmed that coinfections would occur more than expected [65]. Among 157 patients in the Colombian-Venezuelan border, 7.6% were coinfected with dengue and chikungunya, 6.4% with dengue and ZIKV, 5.1% with chikungunya and ZIKV and 1.9% with dengue, chikungunya and ZIKV. This is fully apparent with ongoing health crisis such as those seen in Venezuela. There, people are forced to migrate due to political and socieconomical factors, and this has resulted in the re-introduction of infectious diseases such as malaria [66–68], arboviral diseases [69,70], tuberculosis [71,72] and even vaccine-preventable conditions, such as measles [73], mumps and whooping cough among others [74] into areas of Colombia where they were previously controlled. This has also severe implications for public health and travel medicine across the borders, including their considerations in pregnant women.

In conclusion, given the complex ecoepidemiological scenario, microcephaly and the CZS are still a matter of concern in endemic areas as well in pregnant women travelling to and returning from there. In this study, although a relatively low-frequency event, CZS remains a threat for childhood development. As noted in recent studies, adverse neurological outcomes are occurring in children exposed to ZIKV *in utero*, although not clear if this occurs even without congenital infection, and this requires further study and follow-up [75,76]. As transmission became endemic in certain areas, such as most of the Colombian territory, pregnant women living or visiting those zones should be assessed for ZIKV and other arboviruses, even if they are asymptomatic. It remains unclear as to how many pregnant women are asymptomatic and past infection is only discovered post-partum. Recently, others have demonstrated the utility of molecular tests in detecting asymptomatic cases of ZIKV in pregnant women during recent outbreak settings in northern Peru [77]. This has also implications for other potential emerging arboviral disease with possible compromise during pregnancy. Studies like this, contributing to the clinical description of pregnant women with ZIKV, are still needed in different endemic areas. Finally, long-term follow-up of exposed children is required to define comprehensively the outcomes of disease and potentially mitigate their impacts through targeted intervention.

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Conflicts of interest

AJRM served as member of the Committee of International Experts for the Development of a Zika Vaccine (Sanofi Pasteur, 2016–2017).

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References

[1] Rodriguez-Morales AJ, Villamil-Gomez WE, Franco-Paredes C. The arboviral burden of disease caused by co-circulation and co-infection of dengue, chikungunya and Zika in the Americas. Trav Med Infect Dis 2016;14:177–9.
[2] Musso D, Rodriguez-Morales AJ, Levi JE, Lormeau V-MC, Gubler DJ. Unexpected outbreaks of arbovirus infections: lessons learned from the Pacific and tropical America. Lancet Infect Dis 2018. http://dx.doi.org/10.1016/S1473-3099(18)30269-X.
[3] Villamil-Gomez WE, Mendoza-Guete A, Villalobos E, Gonzalez-Arismendy E, Uribe-Garcia AM, Castellanos JE, et al. Diagnosis, management and follow-up of pregnant women with Zika virus infection: a preliminary report of the ZIKERNCOL cohort study on Sincelejo, Colombia. Trav Med Infect Dis 2016;14:155–8.
[4] Kuehnert MJ, Basavaraju SV, Moseley RR, Pate LL, Galel SA, Williamson PC, et al. Screening of blood donations for zika virus infection - Puerto Rico. MMWR Morbidity and mortality weekly report 2016;65:627–8. April 3-June 11, 2016.
[5] Alvarado-Socarras JL, Idrovo AJ, Contreras-Garcia GA, Rodriguez-Morales AJ, Audcent TA, Mogollon-Mendoza AC, et al. Congenital microcephaly: a diagnostic challenge during Zika epidemics. Trav Med Infect Dis 2018. http://dx.doi.org/10.1016/j.tmaid.2018.02.002.
[6] Rodriguez-Morales AJ. Zika and microcephaly in Latin America: an emerging threat for pregnant travelers? Trav Med Infect Dis 2016;14:5–6.
[7] Villamil-Gomez WE, Guijarro E, Castellanos J, Rodriguez-Morales AJ. Congenital Zika syndrome with prolonged detection of Zika virus RNA. J Clin Virol 2017;95:52–4. the official publication of the Pan American Society for Clinical Virology.
[8] Blohm GM, Lednicky JA, Marquez M, White SK, Loeb JC, Pacheco CA, et al. Evidence for mother-to-child transmission of zika virus through breast milk. Clin Infect Dis 2018;66:1120–1. an official publication of the Infectious Diseases Society of America.
[9] Blohm GM, Lednicky JA, Marquez M, White SK, Loeb JC, Pacheco CA, et al. Complete genome sequences of identical zika virus isolates in a nursing mother and her infant. Genome Announc 2017:5.
[10] Pessoa A, van der Linden V, Yeargin-Allsopp M, Carvalho M, Ribeiro EM, Van Naarden Braun K, et al. Motor abnormalities and epilepsy in infants and children with evidence of congenital zika virus infection. Pediatrics 2018;141:S167–79.
[11] Saad T, PennaeCosta AA, de Goes FV, de Freitas M, de Almeida JV, de Santa Ignez LJ, et al. Neurological manifestations of congenital Zika virus infection. Child's Nerv Syst : CHNS (Child's Nerv Syst) 2018;34:73–8. official journal of the International Society for Pediatric Neurosurgery.
[12] Ventura CV, Maia M, Bravo-Filho V, Gois AL, Belfort Jr. R. Zika virus in Brazil and macular atrophy in a child with microcephaly. Lancet 2016;387:228.
[13] Ventura CV, Maia M, Dias N, Ventura LO, Belfort Jr. R. Zika: neurological and ocular findings in infant without microcephaly. Lancet 2016;387:2502.
[14] Cuevas EL, Tong VT, Rozo N, Valencia D, Pacheco O, Gilboa SM, et al. Preliminary report of microcephaly potentially associated with zika virus infection during pregnancy - Colombia. MMWR Morbidity and mortality weekly report 2016;65:1409–13. January-November 2016.
[15] Villamil-Gomez WE, Rodriguez-Morales AJ, Uribe-Garcia AM, Gonzalez-Arismendy E, Castellanos JE, Calvo EP, et al. Zika, dengue, and chikungunya co-infection in a pregnant woman from Colombia. Int J Infect Dis : Int J Infect Dis 2016;51:135–8. official publication of the International Society for Infectious Diseases.
[16] Mattar S, Ojeda C, Arboleda J, Arrieta G, Bosch I, Botia I, et al. Case report: microcephaly associated with Zika virus infection, Colombia. BMC Infect Dis 2017;17:423.
[17] Parra-Saavedra M, Reefhuis J, Piraquive JP, Gilboa SM, Badell ML, Moore CA, et al. Serial head and brain imaging of 17 fetuses with confirmed zika virus infection in Colombia, south America. Obstet Gynecol 2017;130:207–12.
[18] Sanz Cortes M, Rivera AM, Yepez M, Guimaraes CV, Diaz Yunes I, Zarutskie A, et al. Clinical assessment and brain findings in a cohort of mothers, fetuses and infants infected with zika virus. Am J Obstet Gynecol 2018;218:440.e1–440.e36.
[19] Butler D. First Zika-linked birth defects detected in Colombia. Nature 2016;531:153.
[20] Rodriguez-Morales AJ, Gil-Restrepo AF, Ramirez-Jaramillo V, Montoya-Arias CP, Acevedo-Mendoza WF, Bedoya-Arias JE, et al. Post-chikungunya chronic inflammatory rheumatism: results from a retrospective follow-up study of 283 adult and child cases in La Virginia, Risaralda, Colombia vol. 5. 2016. p. 360. F1000Research.
[21] Rodriguez-Morales AJ, Restrepo-Posada VM, Acevedo-Escalante N, Rodriguez-Munoz ED, Valencia-Marin M, Castrillon-Spitia JD, et al. Impaired quality of life after chikungunya virus infection: a 12-month follow-up study of its chronic inflammatory rheumatism in La Virginia, Risaralda, Colombia. Rheumatol Int 2017;37:1757–8.
[22] Rodriguez-Morales AJ, Bedoya-Arias JE, Ramirez-Jaramillo V, Montoya-Arias CP, Guerrero-Matituy EA, Cardenas-Giraldo EV. Using geographic information system (GIS) to mapping and assess changes in transmission patterns of chikungunya fever in municipalities of the Coffee-Triangle region of Colombia during 2014-2015 outbreak: implications for travel advice. Trav Med Infect Dis 2016;14:62–5.
[23] Quintero-Herrera LL, Ramirez-Jaramillo V, Bernal-Gutierrez S, Cardenas-Giraldo EV, Guerrero-Matituy EA, Molina-Delgado AH, et al. Potential impact of climatic variability on the epidemiology of dengue in Risaralda, Colombia, 2010-2011. Journal of infection and public health 2015;8:291–7.
[24] Rodriguez-Morales AJ, Ruiz P, Tabares J, Ossa CA, Yepes-Echeverry MC, Ramirez-Jaramillo V, et al. Mapping the ecoepidemiology of Zika virus infection in urban and rural areas of Pereira, Risaralda, Colombia, 2015-2016: implications for public health and travel medicine. Trav Med Infect Dis 2017;18:57–66.
[25] Harapan H, Alleta A, Anwar S, Setiawan AM, Maulana R, Wahyuniati N, et al. Attitudes towards Zika virus infection among medical doctors in Aceh province, Indonesia. Journal of infection and public health 2018;11:99–104.
[26] Honein MA, Dawson AL, Petersen EE, Jones AM, Lee EH, Yazdy MM, et al. Birth defects among fetuses and infants of US women with evidence of possible zika virus infection during pregnancy. Jama 2017;317:59–68.
[27] Johansson MA, Mier-y-Teran-Romero L, Reefhuis J, Gilboa SM, Hills SL. Zika and the risk of microcephaly. N Engl J Med 2016;375:1–4.
[28] Rodriguez-Morales AJ. Zika: the new arbovirus threat for Latin America. Journal of infection in developing countries 2015;9:684–5.
[29] Sabogal-Roman JA, Murillo-Garcia DR, Yepes-Echeverri MC, Restrepo-Mejia JD, Granados-Alvarez S, Paniz-Mondolfi AE, et al. Healthcare students and workers' knowledge about transmission, epidemiology and symptoms of Zika fever in four cities of Colombia. Trav Med Infect Dis 2016;14:52–4.
[30] Rodriguez-Morales AJ, Galindo-Marquez ML, Garcia-Loaiza CJ, Sabogal-Roman JA, Marin-Loaiza S, Ayala AF, et al. Mapping Zika virus disease incidence in Valle del Cauca. Infection 2017;45:93–102.
[31] Counotte M, Egli-Gany D, Riesen M, Abraha M, Porgo T, Wang J, et al. Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: from systematic review to living systematic review [version 1; referees: awaiting peer review]. 2018.
[32] Chibueze EC, Tirado V, Lopes KD, Balogun OO, Takemoto Y, Swa T, et al. Zika virus infection in pregnancy: a systematic review of disease course and complications. Reprod Health 2017;14:28.
[33] Coronell-Rodriguez W, Arteta-Acosta C, Suarez-Fuentes MA, Burgos-Rolon MC, Rubio-Sotomayor MT, Sarmiento-Gutierrez M, et al. [Zika virus infection in pregnancy, fetal and neonatal impact]. Revista chilena de infectologia : organo oficial de la Sociedad Chilena de Infectologia vol. 33. 2016. p. 665–73.
[34] de Carvalho NS, de Carvalho BF, Doris B, Silverio Biscaia E, Arias Fugaca C, de Noronha L. Zika virus and pregnancy: an overview. AJRI (Am J Reprod Immunol) 2017;77. (New York, NY : 1989).
[35] Hoen B, Schaub B, Funk AL, Ardillon V, Boullard M, Cabie A, et al. Pregnancy outcomes after ZIKV infection in French territories in the Americas. N Engl J Med 2018;378:985–94.
[36] Alvarado-Socarras JL, Rodriguez-Morales AJ. Etiological agents of microcephaly: implications for diagnosis during the current Zika virus epidemic. Ultrasound Obstet Gynecol 2016;47:525–6. the official journal of the International Society of Ultrasound in Obstetrics and Gynecology.
[37] Carvalho FHC. Response to "Associated ultrasonographic findings in fetuses with microcephaly because of suspected Zika virus (ZIKV) infection during pregnancy". Prenat Diagn 2017;37:207–8.
[38] Haque U, Ball JD, Zhang W, Khan MM, Trevino CJ. Clinical and spatial features of Zika virus in Mexico. Acta Trop 2016;162:5–10.
[39] Cerbino-Neto J, Mesquita EC, Souza TM, Parreira V, Wittlin BB, Durovni B, et al. Clinical Manifestations of Zika Virus Infection, Rio de Janeiro, Brazil. Emerg Infect Dis 2015;2016(22):1318–20.
[40] de Laval F, Leparc-Goffart I, Meynard JB, Daubigny H, Simon F, Briolant S. Zika virus infections. Medecine et sante tropicales 2016;26:145–50.
[41] de Paula Freitas B, de Oliveira Dias JR, Prazeres J, Sacramento GA, Ko AI, Maia M, et al. Ocular findings in infants with microcephaly associated with presumed zika virus congenital infection in salvador, Brazil. JAMA ophthalmology 2016;134:529–35.
[42] Brasil P, Pereira Jr. JP, Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, et al. Zika Virus Infection in Pregnant Women in Rio de Janeiro. N Engl J Med 2016;375:2321–34.
[43] Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia December 2013 and February 2014 Euro Surveill 2014;19. bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin.
[44] Flamand C, Fritzell C, Matheus S, Dueymes M, Carles G, Favre A, et al. The proportion of asymptomatic infections and spectrum of disease among pregnant women infected by Zika virus: systematic monitoring in French Guiana, 2016. Euro Surveill 2017;22. bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin.
[45] Hancock WT, Soeters HM, Hills SL, Link-Gelles R, Evans ME, Daley WR, et al. Establishing a timeline to discontinue routine testing of asymptomatic pregnant women for zika virus infection - American Samoa, 2016-2017. MMWR Morbidity and mortality weekly report 2017;66:299–301.
[46] Saade GR. Asymptomatic pregnant women returning to the United States from countries experiencing a Zika virus outbreak should be tested for Zika virus: AGAINST: not so fast. BJOG An Int J Obstet Gynaecol 2016;123:1272.
[47] Sheffield JS. Asymptomatic pregnant women returning to the United States from countries experiencing a Zika virus outbreak should be tested for Zika virus : FOR: importance of identification in ALL pregnant women. BJOG An Int J Obstet Gynaecol 2016;123:1272.

[48] Meaney-Delman D, Oduyebo T, Polen KN, White JL, Bingham AM, Slavinski SA, et al. Prolonged detection of zika virus RNA in pregnant women. Obstet Gynecol 2016;128:724–30.
[49] Froeschl G, Huber K, von Sonnenburg F, Nothdurft HD, Bretzel G, Hoelscher M, et al. Long-term kinetics of Zika virus RNA and antibodies in body fluids of a vasectomized traveller returning from Martinique: a case report. BMC Infect Dis 2017;17:55.
[50] Paz-Bailey G, Rosenberg ES, Doyle K, Munoz-Jordan J, Santiago GA, Klein L, et al. Persistence of zika virus in body fluids - preliminary report. N Engl J Med 2017. http://dx.doi.org/10.1056/NEJMoa1613108.
[51] Zammarchi L, Stella G, Mantella A, Bartolozzi D, Tappe D, Gunther S, et al. Zika virus infections imported to Italy: clinical, immunological and virological findings, and public health implications. J Clin Virol 2015;63:32–5. the official publication of the Pan American Society for Clinical Virology.
[52] Boyer Chammard T, Schepers K, Breurec S, Messiaen T, Destrem AL, Mahevas M, et al. Severe thrombocytopenia after zika virus infection, Guadeloupe, 2016. Emerg Infect Dis 2017;23:696–8.
[53] Karimi O, Goorhuis A, Schinkel J, Codrington J, Vreden SGS, Vermaat JS, et al. Thrombocytopenia and subcutaneous bleedings in a patient with Zika virus infection. Lancet 2016;387:939–40.
[54] Sarmiento-Ospina A, Vasquez-Serna H, Jimenez-Canizales CE, Villamil-Gomez WE, Rodriguez-Morales AJ. Zika virus associated deaths in Colombia. Lancet Infect Dis 2016;16:523–4.
[55] Sharp TM, Munoz-Jordan J, Perez-Padilla J, Bello-Pagan MI, Rivera A, Pastula DM, et al. Zika virus infection associated with severe thrombocytopenia. Clin Infect Dis 2016;63:1198–201. an official publication of the Infectious Diseases Society of America.
[56] Simeone RM, Shapiro-Mendoza CK, Meaney-Delman D, Petersen EE, Galang RR, Oduyebo T, et al. Possible zika virus infection among pregnant women - United States and territories, may 2016. MMWR Morbidity and mortality weekly report 2016;65:514–9.
[57] Suwanmanee S, Luplertlop N. Dengue and Zika viruses: lessons learned from the similarities between these Aedes mosquito-vectored arboviruses. J Microbiol 2017;55:81–9.
[58] Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika virus and birth defects — reviewing the evidence for causality. N Engl J Med 2016;374:1981–7.
[59] Duca LM, Beckham JD, Tyler KL, Pastula DM. Zika virus disease and associated neurologic complications. Curr Infect Dis Rep 2017;19:4.
[60] Noronha L, Zanluca C, Azevedo ML, Luz KG, Santos CN. Zika virus damages the human placental barrier and presents marked fetal neurotropism. Mem Inst Oswaldo Cruz 2016;111:287–93.
[61] Machado-Alba JE, Machado-Duque ME, Gaviria-Mendoza A, Orozco-Giraldo V. Diagnosis of neurological disorders and the Zika virus epidemic in Colombia 2014-2016. Int J Infect Dis : Int J Infect Dis 2016;51:133–4. official publication of the International Society for Infectious Diseases.
[62] Alvarado-Socarras JL, Meneses-Silvera K, Zarate-Vergara AC, Guerrero-Gomez C, Rodriguez-Morales AJ. Not everything is Zika: congenital toxoplasmosis, still prevalent in Colombia? Rev Peru Med Exp Salud Publica 2017;34:332–6.
[63] Villamil-Gomez WE, Gonzalez-Camargo O, Rodriguez-Ayubi J, Zapata-Serpa D, Rodriguez-Morales AJ. Dengue, chikungunya and Zika co-infection in a patient from Colombia. Journal of infection and public health 2016;9:684–6.
[64] Villamil-Gomez WE, Rodriguez-Morales AJ. Reply: dengue RT-PCR-positive, chikungunya IgM-positive and zika RT-PCR-positive co-infection in a patient from Colombia. Journal of infection and public health 2017;10:133–4.
[65] Carrillo-Hernandez MY, Ruiz-Saenz J, Villamizar LJ, Gomez-Rangel SY, Martinez-Gutierrez M. Co-circulation and simultaneous co-infection of dengue, chikungunya, and zika viruses in patients with febrile syndrome at the Colombian-Venezuelan border. BMC Infect Dis 2018;18:61.
[66] Rodriguez-Morales AJ, Paniz-Mondolfi AE. Venezuela's failure in malaria control. Lancet 2014;384:663–4.
[67] Daniels JP. Increasing malaria in Venezuela threatens regional progress. Lancet Infect Dis 2018;18:257.
[68] Grillet ME, Villegas L, Oletta JF, Tami A, Conn JE. Malaria in Venezuela requires response. Science 2018;359:528.
[69] Rodriguez-Morales AJ, Paniz-Mondolfi AE. Venezuela: far from the path to dengue and chikungunya control. J Clin Virol 2015;66:60–1. the official publication of the Pan American Society for Clinical Virology.
[70] Li J, Xiong Y, Wu W, Liu X, Qu J, Zhao X, et al. Zika virus in a traveler returning to China from caracas, Venezuela, February 2016. Emerg Infect Dis 2016;22:1133–6.
[71] Rodriguez-Morales AJ, Castaneda-Hernandez DM. Relationships between morbidity and mortality from tuberculosis and the human development index (HDI) in Venezuela, 1998-2008. Int J Infect Dis : Int J Infect Dis 2012;16:e704–5. official publication of the International Society for Infectious Diseases.
[72] Torres-Duque CA, Fuentes Alcala ZM, Rendon A, Migliori GB. Roadmap for tuberculosis elimination in Latin America and the caribbean. Arch Bronconeumol 2018;54:7–9.
[73] Sarmiento H, Cobo OB, Morice A, Zapata R, Benitez MV, Castillo-Solorzano C. Measles outbreak in Venezuela: a new challenge to postelimination surveillance and control? J Infect Dis 2011;204(Suppl 2):S675–82.
[74] Burki T. Re-emergence of neglected tropical diseases in Venezuela. Lancet Infect Dis 2015;15:641–2.
[75] Kanda PAM, Aguiar AAX, Miranda JL, Falcao AL, Andrade CS, Reis L, et al. Sleep EEG of microcephaly in zika outbreak. Neurodiagn J 2018;58:11–29.
[76] Medina MT, Medina-Montoya M. New spectrum of the neurologic consequences of Zika. J Neurol Sci 2017;383:214–5.
[77] Weilg C, Troyes L, Villegas Z, Silva-Caso W, Mazulis F, Febres A, et al. Detection of Zika virus infection among asymptomatic pregnant women in the North of Peru. BMC Res Notes 2018;11:311.