

Title: Screening for Zika virus infection in 1057 potentially exposed pregnant women, Catalonia (Northeastern Spain)

Running title: Zika virus screening in 1057 pregnant women

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Dear editor:

A recent editorial in *Travel Medicine and Infectious Diseases* highlighted the lack of studies about Zika virus (ZIKV) in pregnancy and its implications in many countries [1]. Zika virus infection can induce congenital defects in the newborn such as microcephaly and miscarriage when mothers are infected during pregnancy [2]. However, relevant questions remain to be completely understood, such as the risk of infection for pregnant women and of subsequent congenital defects, and the ratio between symptomatic and asymptomatic ZIKV infections in the general population and in pregnant women. Here, we describe the results of a ZIKV screening of pregnant women in Catalonia, northeastern Spain. Testing for ZIKV was recommended for all pregnant women with history of travel to ZIKV endemic areas during pregnancy or in the 8 weeks before conception [3]. Symptomatic patients were screened by serological methods from day four after the onset of symptoms and by molecular methods within the first week (serum) and two weeks (urine) after illness. Asymptomatic patients were tested by serological methods. Seroneutralization assay for ZIKV was performed in samples positive for antibodies. Commercial diagnostic assays were used (RT-PCR, Altona Diagnostics and IIFT, Euroimmun). Neutralization titers $\geq 1/32$ were considered indicative of the presence of ZIKV neutralizing antibodies. Follow up at two designated reference obstetrical departments for early detection of microcephaly or other malformations was offered to pregnant women with laboratory evidence of ZIKV infection. When available, amniotic fluid and placental tissue samples were tested for ZIKV by RT-PCR in cases of microcephaly or miscarriage, respectively.

A ZIKV confirmed case was defined as i) a pregnant woman with a positive RT-PCR test for ZIKV in serum and/or urine samples and/or ii) a pregnant woman with a positive IgM against ZIKV, a negative IgM against dengue virus and a ZIKV neutralization titer $\geq 1/32$. A ZIKV probable case was defined as a pregnant woman with an isolated positive IgG test against ZIKV and a ZIKV neutralization titer $\geq 1/32$. All cases with no laboratory evidence of ZIKV infection and cases

with an isolated positive IgG test and a ZIKV neutralization titer $<1/32$ were considered negative cases.

A total of 1057 pregnant women with history of potential exposure to ZIKV were tested between January and December 2016. The results of the screening, clinical presentation and pregnancy outcomes are summarized in Figure 1.

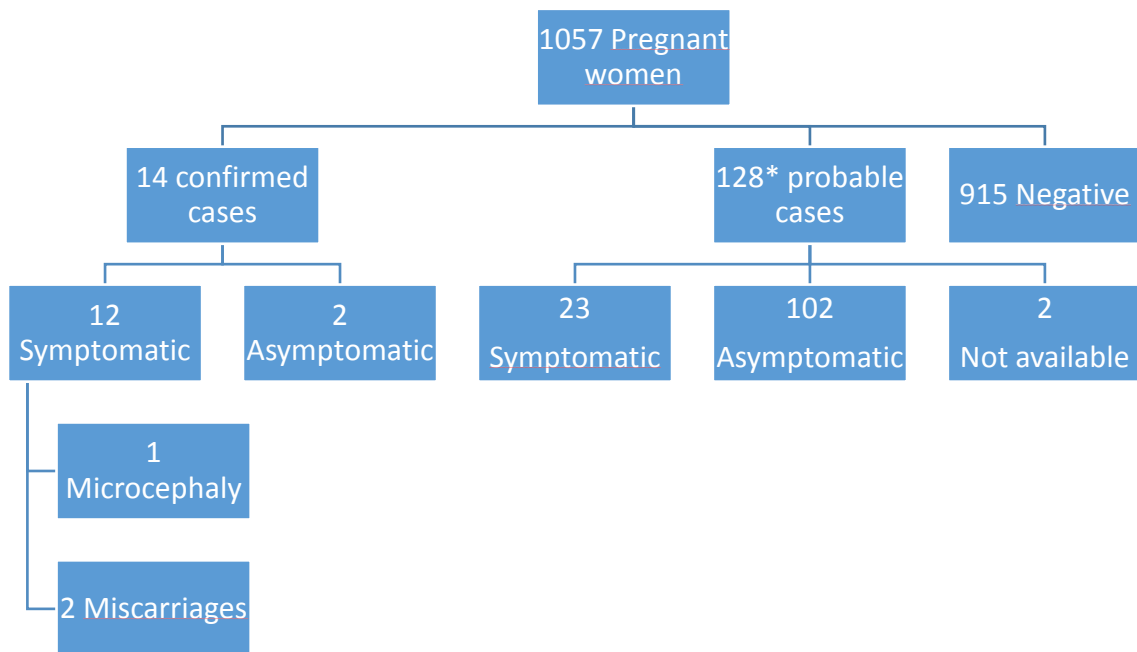


Figure 1. Screening results, clinical presentation and adverse pregnancy outcomes of 1057 pregnant women screened for ZIKV infection.

*Information on the final pregnancy outcomes was not available for 46 probable cases.

The median age of the pregnant women undergoing ZIKV screening was 31 years (range 15-49). The country most frequently visited was the Dominican Republic (113 women; 14.2%) followed by Bolivia (97, 12.2%), Ecuador (90; 11.3%) and Colombia (88; 11.0%). Information about the gestational age in which the women were potentially exposed to ZIKV was available in 683/1057 women (62.8%). Thirty-five (5.1%) stayed in ZIKV endemic areas within one month before conception, 482 (70.6%) during the first trimester, 117 (17.1%) during the second trimester and 49 (7.2%) during the third trimester. Clinical information was available in 946 patients (89.5%) and most of them (846; 89.4%) had not presented ZIKV compatible symptoms. Overall, ZIKV infection was confirmed in 14 out of the 1057 potentially exposed pregnant women (1.3%; 95%CI: 0.7-2.2%) and this figure rose to 142/1057 (13.4%; 95%CI: 11.4%-15.6%) considering women with any laboratory evidence of ZIKV infection (confirmed and probable cases). Of the 14 confirmed ZIKV cases, 12 were diagnosed by RT-PCR and two by serological methods.

We detected three adverse fetal outcomes associated with ZIKV infection: one newborn with microcephaly and two miscarriages. In the case with microcephaly, ZIKV RNA was detected in amniotic fluid [4]. In both cases of miscarriage, ZIKV RNA was detected in placental tissues and in one case ZIKV was, in addition, isolated from embryonic cells [5]. In forty-six probable cases, the information on the final pregnancy outcome was not available, but no fetal abnormalities were found at the time of screening. Our data show a prevalence of serious adverse effects (microcephaly or miscarriage) associated with ZIKV infection of 3/96 (3.1%; 95% CI:0.6%-8.9%) among pregnant women with laboratory evidence of ZIKV infection. Importantly, in the three cases, ZIKV infection occurred in the first trimester of pregnancy. Moreover, the three cases were symptomatic and laboratory confirmed.

ZIKV infection in pregnancy, particularly during the first trimester in which the central nervous system of the fetus is being formed, is associated with adverse fetal outcomes such as

miscarriage and microcephaly among others [2]. There is a lack of published data from cohorts of pregnant women residing in Europe and who have been potentially exposed to ZIKV during pregnancy. A recent systematic review and metaanalysis based on series from the Americas estimated the prevalence of microcephaly of 2.3% [95%CI: 1.0%–5.3%] among babies born to ZIKV-infected mothers [6]. Our results from pregnant women with any laboratory evidence of ZIKV infection are in concordance with these figures. Among eight published studies, the proportion of asymptomatic ZIKV infections in pregnant women ranged from 17.2 to 56% [6]. Our study revealed that among women with any laboratory evidence of ZIKV infection only 25% presented symptoms. However, 85.7% (12 out of 14) laboratory confirmed ZIKV cases were symptomatic. These differences may be explained considering that ZIKV symptomatic patients may be more likely to seek medical attention and therefore might be more likely to be laboratory confirmed by RT-PCR in the acute phase of the illness. In contrast, asymptomatic cases are mainly detected by serology and therefore are more likely to be classified as probable cases.

Our study has several limitations. Firstly, the high cross reactivity between dengue and ZIKV induced antibodies and the absence of available neutralization tests against DENV in our setting hampered a more precise classification of the group of probable ZIKV infections. Secondly, other relevant risk factors such as precise duration of the trip or stay in the endemic areas, magnitude of the ZIKV circulation in each country at the time of visit and use of repellents or other measures against mosquito bites, were not systematically assessed. Despite these limitations, some of them being certainly difficult to address, we present the screening results from more than 1000 pregnant women who were screened for ZIKV infection. Our results contribute to better understand the impact of ZIKV infection in pregnancy and might be helpful to estimate the risk of poor pregnancy outcomes for pregnant women living in Europe who travel to ZIKV affected areas, as well as to evaluate the performance of screening and surveillance programs.

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Conflicts of interest: All authors, no conflict.

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References

- [1] Alvarado-Arnez LE, Escalera-Antezana JP. Zika infection in pregnancy: Follow up and outcomes, where are all the data? *Travel Med Infect Dis* 2018;25:1–2. doi:10.1016/j.tmaid.2018.06.014.
- [2] Rice ME, Galand RR, Roth NM, Ellington SR, Moore CA, Prado MV, et al. Vital Signs: Zika-Associated Birth Defects and Neurodevelopmental Abnormalities Possibly Associated with Congenital Zika Virus Infection — U.S. Territories and Freely Associated States, 2018. *Morb Mortal Wkly Rep* 2018;67:858–67. doi:10.15585/mmwr.mm6731e1.
- [3] Protocol d’actuació davant de la infecció pel virus de Zika en l’àmbit obstètric i pediàtric de Catalunya Protocol d’actuació davant de la infecció pel virus de Zika en l’àmbit obstètric i pediàtric de Catalunya Subdirecció General de Vigilància i Resposta a Emergències de Salut Pública Protocol d’actuació davant de la infecció pel virus de Zika en l’àmbit obstètric i pediàtric de Catalunya 2017.

- [4] Suy A, Sulleiro E, Rodó C, Vázquez É, Bocanegra C, Molina I, et al. Prolonged Zika Virus Viremia during Pregnancy. *N Engl J Med* 2016;375:2611–3.
doi:10.1056/NEJMc1607580.
- [5] Goncé A, Martínez MJ, Marbán-Castro E, Saco A, Soler A, Alvarez-Mora MI, et al. Spontaneous Abortion Associated with Zika Virus Infection and Persistent Viremia. *Emerg Infect Dis* 2018;24:933–5. doi:10.3201/eid2405.171479.
- [6] Campos Coelho AV, Crovella S. Microcephaly prevalence in infants born to zika virus-infected women: A systematic review and meta-analysis. *Int J Mol Sci* 2017;18:1–10.
doi:10.3390/ijms18081714.