Seroprevalence of Zika virus among asymptomatic pregnant mothers and their newborns in the Najran region of southwest Saudi Arabia

Mohammed S. Alayed,1 Mohamed A. Qureshi,2 Saif Ahmed,2 Ali S. Alqahtani,2 Awad M. Alqahtani,1 Khalid Alshaybari,1 Majed Alshahrani,1 Ahmed M. Asaad1

From the 1Department of Pediatrics, 2Department of Microbiology, College of Medicine, Najran University, Najran, Saudi Arabia; 3Department of Obstetrics and Gynecology, College of Medicine, Najran University, Najran, Saudi Arabia; 4Department of Family and Community Medicine, 5Department of Medical Laboratory Sciences, College of Applied Medical Sciences, College of Medicine, Zagazig University, Zagazig, Egypt

BACKGROUND: Zika virus (ZIKV) is a teratogenic flavivirus that can cause microcephaly. Its main vector, Aedes aegypti, has been previously identified in Saudi Arabia, but no ZIKV infection has yet been reported. Nevertheless, the country is at risk from ZIKV because it receives many travelers throughout the year, including pilgrims from ZIKV-endemic countries.

OBJECTIVES: Screen asymptomatic pregnant mothers and their newborns attending a major hospital in the Najran region for subclinical or past infections with ZIKV, using ELISA and RT-PCR.

DESIGN: Cross-sectional.

SETTING: Najran Maternity and Children Hospital (NMCH).

SUBJECTS AND METHODS: All pregnant women admitted to NMCH in labor between November 2016 and July 2017 were included in the study. Clinical and demographic data were collected by pre-validated physician-administered questionnaires. Paired umbilical and maternal serum samples were collected and frozen at -60°C, using ELISA to measure anti-ZIKA IgG and IgM antibodies and RT-PCR to further investigate positive samples.

MAIN OUTCOME MEASURES: Maternal and newborn serum anti-ZIKV IgM and IgG and ZIKV RT-PCR.

SAMPLE SIZE: 410 mother-newborn pairs.

RESULTS: The median gestational age was 38.5 weeks (range 33-42). Most (n=342, 83.41%) of the women were from Najran city. All of the newborns had normal growth parameters with no congenital malformations. None of the mothers had symptoms suggestive of ZIKV infection; 3 (0.7%) exhibited a low-grade fever (38°C), but did not test positive for anti-ZIKV antibodies. Thirty-five (8.53%) of mothers had travelled inside Saudi Arabia, but none outside the country. Twenty-four (5.85%) mothers tested positive for anti-ZIKV IgM and 52 (12.68%) tested positive for anti-ZIKV IgG, but all infant samples were negative. All seropositive ZIKV IgM were also ZIKV IgG positive, but RT-PCR testing of all seropositive samples was negative.

CONCLUSION: Although previous (resolved) ZIKV infection and cross-reactivity of the ELISA method with other flaviviruses cannot be excluded, the study found no confirmed cases of acute ZIKV infection. However, given the presence of the vector in Saudi Arabia, the presence of presumptive positive serology and the ongoing risk of ZIKV...
The World Health Organization (WHO) has reported an estimated 3 to 4 million cases of ZIKV infection worldwide. The causal agent is an arboviral flavivirus that is transmitted to humans primarily by *Aedes aegypti*, which has a teratogenic effect on growing fetal neurological tissues, causing microcephaly.1 Guillain-Barré syndrome, meningoencephalitis and acute myelitis in adults have also been reported with ZIKV infection.2 Infection with ZIKV can cause acute febrile illness or hemorrhagic manifestations. Most infected subjects display a fever, rash, arthralgia, headache, and/or conjunctivitis, but many are asymptomatic, constituting a potential source of future outbreaks. The rash caused by ZIKV infection often disappears within a week or so.3

The *A. aegypti* vector has been found previously in Saudi Arabia, including the Najran region, where other viruses that are transmitted by this vector, mainly dengue virus, have also been reported.4 The presence of the vector and the huge influx of travelers that occurs throughout the year, in particular the millions of tourists and pilgrims that visit the two holy cities during the Hajj and Umrah, including people from ZIKV-endemic areas, put Saudi Arabia at risk for ZIKV transmission and infections.5

In this study, we collected and tested the sera of pregnant women and their newborns cord blood for anti-ZIKV IgM and IgG antibodies. We further investigated the positive cases using the ZIKV real-time polymerase chain reaction (RT-PCR).

**SUBJECTS AND METHODS**

The cross-sectional study was carried out at Najran Maternity and Children Hospital, the main pediatric and gynecology and obstetrics center in Najran, which is located in southwestern Saudi Arabia and serves a population of approximately 550,000 according to the 2016 report of the Ministry of Interior. All pregnant women presenting at the hospital in labor between November 2016 and July 2017, who agreed to participate, were enrolled. The study received ethical approval from the Research Committee of the College of Medicine, Najran University. A pre-validated questionnaire (available upon request from the authors), was administered to the patient by a physician. The first part of the questionnaire elicited patient demographic information, including age, place of residency and any symptoms or signs suggestive of illness. The results obtained from laboratory analysis of patient serum samples were entered into the second part of the questionnaire.

Paired mother and infant samples of 3-5 mL of clotted maternal blood and umbilical cord blood were taken from the mothers and their infants. All blood samples were collected in plain tubes and then centrifuged at 3000 rpm for 10 minutes to obtain serum. Serum samples were frozen at -60°C at the research laboratory in the College of Medicine at Najran University before being tested for ZIKV by enzyme-linked immunosorbent (ELISA) and RT-PCR. A commercial sandwich ELISA kit was used to qualitatively analyze ZIKV IgG and IgM in the collected serum samples. Assays were performed in accordance with the instructions provided by the kit manufacturer (MyBioSource Inc., San Diego, USA). TRIzol reagent (supplied by Thermo Fisher Scientific, USA) was used to isolate high quality RNA from 200 μL serum samples according to the user’s manual. An internal Zika positive control extraction was performed for each sample by adding 2 μL of the internal control reagent directly to the lysis buffer and a negative control sample was run alongside the experimental and positive samples (Fast Track Diagnostics, Luxembourg) provided the internal control. Ten μL of each extracted RNA sample along with the negative and positive controls were mixed with 15 μL of Fast Track Mastermix (Fast Track Diagnostics, Luxembourg). Subsequently, the Agilent Mx3005P quantitative polymerase chain reaction was run with the samples under the following conditions: at 50°C for 15 minutes; at 94°C for 1 minute; 40 cycles at

**LIMITATIONS:** Small sample size, unavailability of plaque reduction neutralization tests to confirm serology results, and RT-PCR was only conducted on ELISA-positive serum samples, due to resource constraints.

**CONFLICT OF INTEREST:** None.
94°C for 8 seconds and 60°C for 1 minute. Only samples with cycle threshold values <33 were considered positive according to manufacturer’s instructions.

Data were analyzed using IBM SPSS software version 23 (IBM, Armonk, NY). Differences between groups were identified using the Pearson correlation coefficient and the chi-square test, with the threshold for statistical significance set at \( P < .05 \).

RESULTS

Four hundred ten mothers and newborns were recruited (Table 1). Thirty-five (8.54%) of the mothers had travelled inside Saudi Arabia before delivery, including the two holy cities during Hajj or Umrah, but none of them or their family members had been abroad. Three hundred and forty-two (83.41%) of the mothers were from Najran city. All of the mothers were asymptomatic except 3 (0.7%) had a low-grade fever (<38°C). The median gestation age was 38.5 weeks (range 33-42). All of the newborn babies had normal growth parameters as per gestational age norms. None of them or their siblings have congenital malformations.

Table 1. Demographic and clinical variables for 410 mothers and infants.

| Variable               | Number (%) | \( P \) value |
|------------------------|------------|--------------|
| **Residency**          |            |              |
| Najran city            | 342 (83.4) | .065         |
| Other                  | 68 (16.6)  |              |
| **Temperature**        |            |              |
| 37°C                   | 407 (99.3) | .509         |
| >38°C                  | 3 (0.7)    |              |
| **Conjunctivitis**     |            |              |
| No                     | 410 (100)  | --           |
| Yes                    | 0          |              |
| **History of travel**  |            |              |
| Travel                 | 35 (8.5)   | .020         |
| No travel              | 375 (91.5) |              |
| **Arthralgia**         |            |              |
| No                     | 410 (100)  | --           |
| Yes                    | 0          |              |
| **Skin rash**          |            |              |
| Yes                    | 0          | --           |
| No                     | 410 (100)  |              |

Of the 410 maternal serum samples, 24 (5.85%) and 52 (12.68%) tested positive for anti-ZIKV IgM and anti-ZIKV IgG, respectively, by ELISA. The higher positivity for anti-ZIKV IgG than for anti-ZIKV IgM among the study population was statistically significant (\( P < .001 \)). Among the positive maternal serum samples, anti-ZIKV IgG levels were positively correlated with anti-ZIKV IgM levels (\( P = .01 \)). Positive correlations between residency (Najran city) and both maternal anti-ZIKV IgG and anti-ZIKV IgM were identified, with non-significant \( P \) values (\( P = .09 \) and \( P = .06 \), respectively). There was a significant negative correlation between a maternal history of travel and anti-ZIKV IgG (\( P = .02 \)), but no significant correlation with the maternal anti-ZIKV IgM. None of the febrile mothers tested positive for anti-ZIKV antibodies. In addition, all newborn infant serum samples were negative for anti-ZIKV IgM and IgG. RT-PCR revealed that all the sera identified as anti-ZIKV IgM and IgG positive by ELISA were negative (Table 2).

DISCUSSION

ZIKV is an emerging flavivirus that is transmitted to hu-
mans primarily by the *Aedes aegypti* mosquito.\(^4\) It was discovered in 1947 and was thought at that time to lead to a relatively mild disease. However, it attracted worldwide attention when it was linked to human birth defects, mainly the fetal nervous system, following the 2015 ZIKV outbreak in Latin America.\(^7,8\) The incidence of central nervous system (CNS) anomalies among newborns in the Najran region has previously been reported as 3.79 per 1000 live births, almost 4% of which are classified as microcephaly with unknown underlying causes.\(^9\) However, in the central region of Saudi Arabia, CNS anomalies were reported in 29% of 855 babies based on radiological imaging.\(^10\)

After the announcement of a ZIKV outbreak in Brazil in May 2015, a public health alert was initiated internationally. There have been no reported cases in the Saudi Arabia, probably because there had been no active surveillance for ZIKV at the time. The presence of the vector in the country, mainly in the western and southwestern regions, represents a source of risk. People from the Najran region are at risk for ZIKV infection due to frequent travel to Mecca for the Hajj and Umrah, or to other countries where they may come into contact with pilgrims from ZIKV endemic areas. The present study was performed on asymptomatic mothers and their newborn babies, who were screened for any possibility of subclinical ZIKV cases or evidence of past infection (anti-ZIKV IgG) that might have otherwise passed unnoticed. We used ELISA to identify anti-ZIKV antibodies and positive samples were further investigated by RT-PCR, in accordance with US CDC guidelines for asymptomatic pregnant women.

It has been reported that ZIKV RNA may not be detected until as late as 107 days after onset of illness.\(^11\) Thus, there is a chance that asymptomatic mothers may have tested positive for ZIKV even though they may have had the infection. Although anti-ZIKV IgM antibodies are generally detectable approximately 4 days after symptom onset, and usually persist for up to 12 weeks after disease onset or exposure, they may persist for longer at variable levels.\(^12\) The findings of this study are presumptively positive though no confirmed cases of acute ZIKV infection were identified. Although the presence of anti-IgG and anti-IgM within the study population of mothers did not seem to relate to symptoms and there was no evidence of anti-ZIKV maternal antibodies being passed on to their infants, these positive anti-ZIKV antibodies among some individuals of the study population could be indicative of ZIKV circulation in the country. However, they could also be the result of cross-reactivity with other flaviviruses. However, the possibility of cross-reactivity of the ELISA results with other flaviviruses, such as dengue virus, which is endemic in Saudi Arabia, cannot be ruled out.\(^13\) Furthermore, both current and past infections of flaviviruses as well as their vaccines, such as the yellow fever virus vaccine, have previously been reported to be capable of generating false positive serological results for ZIKV.\(^14-16\) This could have affected the results in the present study.

Unfortunately, due to equipment constraints, we were unable to perform plaque reduction neutralization tests (PRNT) as a confirmatory test for the serological assay to rule out cross-reactivity with other flaviviruses among the collected serum samples. Nevertheless, ZIKV RT-PCR was performed on the serum samples of mothers and their paired newborn infants that had been identified by ELISA as IgG or IgM positive, in order to validate acute ZIKV infection in those cases, in accordance with CDC guidelines. All of the PCR tests were negative. As such, these findings are consistent with those of an earlier retrospective analysis of samples from patients with unexplained febrile illness between 2010 and 2015 in western Saudi Arabia, in which it was concluded that ZIKV had not yet appeared in Saudi Arabia.\(^17\)

Despite the relatively small sample size and the impossibility of excluding cross-reactivity with other flaviviruses from influencing the ELISA serological testing results, this study has provided insight into the possibility of subclinical or previous infection with ZIKV occurring among asymptomatic mothers and their newborn infants in the Najran region. Such data are considered important in providing clues as to the possible presence (or absence) of the virus in the region. No confirmed cases of acute ZIKV infection among the tested population were identified. However, given the presence of the vector organism in the country, the presence of presumptive positive serology and the ongoing risk of ZIKV entry via a regular influx of travelers from endemic areas, we propose that a program of continuous surveillance be conducted for ZIKV and other flaviviruses. It is also strongly recommended that larger-scale nationwide studies be performed in order to gain a broader picture of the potential threat from ZIKV in Saudi Arabia.

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