This is the published version of a paper published in *The Lancet Global Health*.

Citation for the original published paper (version of record):


https://doi.org/10.1016/S2214-109X(16)30176-0

Access to the published version may require subscription.

N.B. When citing this work, cite the original published paper.

Permanent link to this version:

http://urn.kb.se/resolve?urn=urn:nbn:se:umu:diva-126472
Association of Rift Valley fever virus infection with miscarriage in Sudanese women: a cross-sectional study

Maria Baudin, Ammar M Jumaa, Huda J E Jomma, Mubarak S Karsany, Göran Bucht, Jonas Näslund, Clas Ahlm, Magnus Evander*, Nahla Mohamed*

Summary

Background Rift Valley fever virus is an emerging mosquito-borne virus that causes infections in animals and human beings in Africa and the Arabian Peninsula. Outbreaks of Rift Valley fever lead to mass abortions in livestock, but such abortions have not been identified in human beings. Our aim was to investigate the cause of miscarriages in febrile pregnant women in an area endemic for Rift Valley fever.

Methods Pregnant women with fever of unknown origin who attended the governmental hospital of Port Sudan, Sudan, between June 30, 2011, and Nov 17, 2012, were sampled at admission and included in this cross-sectional study. Medical records were retrieved and haematological tests were done on patient samples. Presence of viral RNA as well as antibodies against a variety of viruses were analysed. Any association of viral infections, symptoms, and laboratory parameters to pregnancy outcome was investigated using Pearson’s χ² test.

Findings Of 130 pregnant women with febrile disease, 28 were infected with Rift Valley fever virus and 31 with chikungunya virus, with typical clinical and laboratory findings for the infection in question. 15 (54%) of 28 women with an acute Rift Valley fever virus infection had miscarriages compared with 12 (12%) of 102 women negative for Rift Valley fever virus (p=0.0001). In a multiple logistic regression analysis, adjusting for age, haemorrhagic disease, and chikungunya virus infection, an acute Rift Valley fever virus infection was an independent predictor of having a miscarriage (odds ratio 7.4, 95% CI 2.7–20.1; p=0.0001).

Interpretation This study is the first to show an association between infection with Rift Valley fever virus and miscarriage in pregnant women. Further studies are warranted to investigate the possible mechanisms. Our findings have implications for implementation of preventive measures, and evidence-based information to the public in endemic countries should be strongly recommended during Rift Valley fever outbreaks.

Funding Schlumberger Faculty for the Future, CRDF Global (31141), the Swedish International Development Cooperation Agency, the County Council of Västerbotten, and the Faculty of Medicine, Umeå University.

Introduction Rift Valley fever is an emerging mosquito-borne infection caused by Rift Valley fever virus (genus Phlebovirus, family Bunyaviridae), with outbreaks in Africa and more recently also in the Arabian Peninsula. However, the virus has the potential to spread to other continents. The infection is characterised by a high case-fatality rate in young animals and causes mass abortions in cattle, goats, and sheep. In human beings, Rift Valley fever presents in most cases as a mild illness with influenza-like symptoms. However, in 1–3% of cases it progresses to a more severe haemorrhagic disease involving liver necrosis, ocular disease, internal and external haemorrhaging, and encephalitis, which could be lethal.1

Infections during pregnancy pose a considerable threat to the mother and fetus. Emerging vector-borne infections by agents such as Zika virus, West Nile virus, Japanese encephalitis virus, Venezuelan equine encephalitis virus, malaria, brucellosis, and dengue can induce fetal malformation, miscarriage, or premature birth in human beings.2,5

Rift Valley fever virus has not previously been linked to miscarriage in pregnant women, although only a few studies have investigated this association. In one study from Mozambique, pregnant women seropositive for Rift Valley fever virus (IgG) showed a slightly higher frequency of stillbirth than seronegative women, but the difference was not statistically significant.4 Case reports have described vertical transmission of Rift Valley fever virus in one pregnant woman in Sudan5 and in one case in Saudi Arabia, where transplacental transmission led to the death of the infant.6 Sudan has had several Rift Valley fever outbreaks,2,5 but the possible association with miscarriage in pregnant women is not known.

Our objective was to determine which infectious agents were the cause of miscarriage in a cross-sectional study of febrile pregnant women who attended a hospital in Port Sudan, Sudan.

Materials and methods

Study design and participants

In June, 2011, we noted an increase in patients with fever, many with haemorrhagic symptoms, arriving at the
Research in context

Evidence before this study
We searched the PubMed database for studies published before May 8, 2016, using the MeSH terms “Rift Valley Fever” AND “Abortion, Spontaneous” AND “humans”. We found two relevant studies. By combining the MeSH terms “Rift Valley Fever” AND “Pregnancy” AND “humans” we found two relevant studies. We used no language restrictions. No additional articles or reports on the effect of Rift Valley fever in pregnant women were found using other databases or resources (WHO, Google scholar, Web of Science). Very few studies have attempted to investigate Rift Valley fever and pregnancy loss in human beings. Two retrospective studies, based on seroepidemiology, have investigated the relationship between Rift Valley fever and miscarriage or stillbirth in human beings. Although they did not detect an association between Rift Valley fever virus infection and miscarriage or stillbirth in human beings, the absence of reporting mechanisms in these countries (Egypt in 1978, Mozambique in 1983) would underestimate the actual miscarriage or stillbirth rate. However, two case reports found evidence of vertical transmission of Rift Valley fever virus in human beings. In animals, especially livestock, Rift Valley fever virus infection is almost 100% abortogenic at all stages of pregnancy. Rift Valley fever virus has been found in both placental and fetal tissue in aborted goats, deer, and sheep.

Added value of this study
The authors of the articles described above stated that there is a need for further studies into a possible link between Rift Valley fever virus and miscarriage in pregnant women, preferably during an outbreak in order to observe the effect of an acute infection. Our study has investigated for the first time an association between acute Rift Valley fever virus infection and miscarriage in a relatively large number of pregnant women with fever attending the hospital in Port Sudan. We found a significant association between acute Rift Valley fever virus infection and miscarriage. Rift Valley fever is in several ways a neglected disease, and the role of Rift Valley fever virus infection in miscarriage or stillbirth in human beings has not been recognised. This could be due to the fact that Rift Valley fever outbreaks mostly occur in rural areas in low-income countries that generally lack proper abortion records, have limited access to health care and diagnosis, especially during outbreaks.

Implications of the available evidence
Our findings have implications for implementation of preventive measures, and evidence-based information for the public in endemic countries is crucial during Rift Valley fever outbreaks. Preventive measures should be introduced to avoid Rift Valley fever virus infection; for example, women at a fertile age could be given information on the effect of Rift Valley fever during pregnancy and how to avoid being infected.
The amplified fragments were sequenced (Eurofins Genomics, Ebersberg, Germany) to confirm the Rift Valley fever virus qRT-PCR results.

Dengue IgM was detected by enzyme-linked immunosorbent assay using a PanBio Dengue Early ELISA kit (PanBio, Brisbane, Australia). Acute hepatitis B virus infection (HBsAg and anti-HBe), and acute hepatitis A virus infection (IgM) were analysed with an Abbot Architect i4000SR (Abbott Laboratories, Chicago, IL, USA). Antibodies to Rift Valley fever virus were detected by IgM analysis using an attenuated Rift Valley fever virus strain MP12 in Dulbecco’s minimal essential medium (Gibco; Thermo Fisher Scientific) in a final volume of 200 μL. The samples were added to nearly confluent baby hamster kidney (BHK)-21 cells in 12-well plates for virus attachment, and incubated for 1 h. After removing the samples and washing once with phosphate-buffered saline, 2 mL of a 1:5% aquacide (Calbiochem; Merck Millipore, Darmstadt, Germany) overlay in Dulbecco’s minimal essential medium supplemented with 2·5% fetal bovine serum and 0·5% penicillin and streptomycin (10000 U/mL) was added to the wells. The plates were incubated at 37°C in an atmosphere of 5% CO2 for 6 days. The cells were fixed in 10% paraformaldehyde in phosphate-buffered saline for 2 h before rinsing in tap water and counterstained using crystal violet. After destaining with water, samples with more than 70% plaque reduction were considered positive.

**Definitions**

Pregnancy outcome was defined as normal pregnancy (delivery of a healthy baby at full term), preterm delivery (delivery of a healthy baby at 8 months of gestation or earlier), and miscarriage (pregnancy loss at any stage of pregnancy). Early miscarriage was defined as occurring at 3 months’ gestation or earlier (first trimester), whereas a pregnancy loss at or after 4 months’ gestation was classified as a late miscarriage (second and third trimester). To be positive for acute Rift Valley fever virus infection, patients had to be positive for Rift Valley fever virus RNA in qRT-PCR or positive for Rift Valley fever virus IgM antibodies using ELISA confirmed by a plaque reduction neutralisation test. Patients who were IgM positive but negative by PCR and plaque reduction neutralisation test were classified as Rift Valley fever virus negative. For classification of severe disease, we constructed a category named haemorrhagic disease in which we included patients with any bleeding symptoms or moderate-to-severe thrombocytopenia (<100×10⁹ platelets per L).

**Table 1: Primers and probes used in the qRT-PCR analyses**

<table>
<thead>
<tr>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVFV PCR forward primer 5’-AAGGCCAAAGCAACTGAGTCAG-3’</td>
</tr>
<tr>
<td>RVFV PCR reverse primer 5’-CAGGGGCTCTGAGCCTGAG-3’</td>
</tr>
<tr>
<td>RVFV PCR probe 5’-GATGATGTGACCTTATCACGAGTTGC-3’</td>
</tr>
<tr>
<td>CHIKV PCR forward primer 5’-CAGCGAACAGAAATG-3’</td>
</tr>
<tr>
<td>CHIKV PCR reverse primer 5’-TGGGCTATTACGATGATG-3’</td>
</tr>
<tr>
<td>CHIKV PCR probe 5’-CTCATACCCGATCTGCAATCA-3’</td>
</tr>
<tr>
<td>HEV PCR forward primer 5’-GGGGTCTTCGAGTGAC-3’</td>
</tr>
<tr>
<td>HEV PCR reverse primer 5’-AGGGGTGATGGAATGAA-3’</td>
</tr>
<tr>
<td>HEV PCR probe 5’-TGGATTCTGCCCTGTCGC-3’</td>
</tr>
<tr>
<td>DENV PCR forward primer 5’-TCATATGCTGAAACGCGAGAGAAACCG-3’</td>
</tr>
<tr>
<td>DENV PCR reverse primer 5’-TTGCAACACGTCAGTTTTCGGTTC-3’</td>
</tr>
</tbody>
</table>

**Statistical analysis**

SPSS Statistics 23.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Miscarriage was the chief outcome variable. Categorical data such as pregnancy outcome, clinical symptoms in Rift Valley fever virus or chikungunya virus positive compared with negative patients, and if haemorrhagic disease was correlated with miscarriage, were analysed using Pearson’s χ² test. Fisher’s exact test was used in analysing the timing of miscarriages (early vs late) between patients positive and negative for Rift Valley fever virus. Analysis of the relation of scale variables (age, total white blood cell counts, platelets, haemoglobin, and haematocrit) to Rift Valley fever virus or chikungunya virus infection was done with independent-samples t tests. Multiple logistic regression was used to calculate the odds ratio (OR) for risk of miscarriage depending on Rift Valley fever virus infection with adjustment for age, haemorrhagic disease, and chikungunya virus infection. To test if chikungunya virus infection affected the association between Rift Valley fever virus infection and risk of miscarriage, the chikungunya virus by Rift Valley fever virus interaction was added to the model. CIs were set at 95% and statistical significance was 0·05.

**Role of the funding source**

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.
Results

Of 162 patients whose samples were stored for analysis, 130 pregnant women were included in the study. 32 patients were excluded from the analysis: one deceased pregnant woman, seven non-pregnant women (of which one succumbed to disease), and 24 men, although some analyses were done on their samples. Of the 130 surviving pregnant women, four had premature deliveries and 27 had miscarriages. Two women miscarried in the first trimester (early miscarriage), 17 in the second trimester, and four in the third trimester (late miscarriage); gestational age was unknown in four miscarriages. Four women had preterm deliveries; two at 7 months of gestation and two at 8 months (table 2). The mean age of the participants was 27 years (range 17–40).

According to the medical records, all women in the study had nausea or vomiting, jaundice, and joint pain in addition to other influenza-like symptoms such as fever, headache, and generalized pain. Malaise, diarrhoea, and rash were experienced by 52 (40%), 46 (35%), and 40 (31%) women, respectively, whereas 21 (16%) women had bleeding symptoms (subconjunctival bleeding, epistaxis, vaginal bleeding; table 2).

Before our analysis, the patient samples were negative for malaria, bacterial infections, and yellow fever. Viral RNA in blood is a sign of acute infection, and by analysing the samples for several virus infections by qRT-PCR we found that many patients were positive for Rift Valley fever virus and chikungunya virus RNA, whereas none were positive for hepatitis E virus or dengue virus RNA. Of the 130 pregnant women, 28 (22%) tested positive for an acute Rift Valley fever virus infection by qRT-PCR or IgM detection, whereas 31 (24%) were PCR-positive for chikungunya virus RNA (table 2). Eight patients were co-infected with both viruses. No patients had acute hepatitis A virus or hepatitis B virus infection, but nine patients had IgM antibodies to dengue virus (table 2). A selection of Rift Valley fever virus qRT-PCR amplification products were confirmed positive by sequencing (appendix).

The Rift Valley fever virus IgM-positive samples were further tested with a plaque reduction neutralisation test to confirm the presence of Rift Valley fever virus antibodies; 12 (71%) of 17 were positive in this assay (data not shown). Two of the samples negative by the plaque reduction neutralisation test were positive for Rift Valley fever virus RNA. Of the 130 pregnant women, 28 (22%) tested positive for an acute Rift Valley fever virus infection by qRT-PCR or IgM detection, whereas 31 (24%) were PCR-positive for chikungunya virus RNA (table 2). Eight patients were co-infected with both viruses. No patients had acute hepatitis A virus or hepatitis B virus infection, but nine patients had IgM antibodies to dengue virus (table 2). A selection of Rift Valley fever virus qRT-PCR amplification products were confirmed positive by sequencing (appendix).

The temporal distribution showed more pregnant women positive for Rift Valley fever virus and chikungunya virus from July to September in both 2011 and 2012 (figure). To assess if there were more cases of Rift Valley fever during the study period we also analysed samples from the excluded patients. We found that three (13%) of 24 men but none of the non-pregnant women tested positive for Rift Valley fever virus RNA by qRT-PCR. Two of the men were also positive for IgM but negative by PCR for dengue virus. Neither of the deceased women were positive for any infection tested for (data not shown).

When we analysed the association between virus infection and clinical and laboratory parameters, an acute Rift Valley fever virus infection was found to be significantly associated with bleeding, lower platelet counts, low haemoglobin concentrations, rash, and malaise (table 3). Chikungunya virus infection was associated with malaise, rash, high white blood cell count, low haemoglobin, and low haematocrit concentrations (table 4). Positivity for dengue virus IgM was not associated with adverse pregnancy outcome (data not shown).
Of all virus infections studied here, only Rift Valley fever virus was associated with miscarriage. 15 (54%) of 28 patients positive for Rift Valley fever virus had miscarriage compared with only 12 (12%) of 102 patients negative for Rift Valley fever virus (p<0·0001; table 3). There was no association between age and pregnancy outcome or to Rift Valley fever virus infection. In a multiple logistic regression analysis, adjusting for age, haemorrhagic disease, and chikungunya virus infection, an acute Rift Valley fever virus infection was an independent predictor of having a miscarriage (OR 7·4, 95% CI 2·7–20·1; p<0·0001). Patients positive for Rift Valley fever virus with miscarriage (15 women) had bleeding (p=0·005), joint pain (p<0·0001), and malaise (p<0·0001) to a higher degree than patients positive for Rift Valley fever virus with normal pregnancy (12 women). Otherwise, these two patient groups were not significantly different regarding age and other clinical and laboratory parameters (table 3).

Eight (26%) of 31 patients infected with chikungunya virus had miscarriage compared with 19 (19%) of 99 patients negative for Chikungunya virus (p=0·296; table 4). Five of the eight patients who had a Rift Valley fever virus and chikungunya virus co-infection had a miscarriage, but this interaction was not significant (p=0·573).

Gestational age of the pregnancy was known in 23 of the 27 women with miscarriage, and 12 of these were positive for Rift Valley fever virus. All 12 women with acute Rift Valley fever virus infection had a late miscarriage (second or third trimester), whereas four (36%) of 11 women who were negative for Rift Valley fever virus had an early miscarriage (first trimester). An acute Rift Valley fever virus infection was significantly associated with late miscarriage (p=0·037).

Of the four women with preterm delivery, one was co-infected with Rift Valley fever virus and chikungunya virus, whereas two others were positive for chikungunya virus only. Preterm delivery was associated with chikungunya virus infection (p=0·034; table 4), but not with Rift Valley fever virus (table 3) or co-infection (data not shown).

Discussion
In this study we found that infection of pregnant women with Rift Valley fever virus was significantly associated with miscarriage. The results were conclusive and they have not been described before. Acute infection was detected using complementary methods; the presence of Rift Valley fever virus RNA was detected by qRT-PCR and anti-Rift Valley fever virus IgM antibodies with neutralising capacity. Moreover, the pregnant women who were positive for Rift Valley fever virus had characteristic clinical symptoms, as reported from several previous outbreaks. The women positive for Rift Valley fever virus who had miscarriage had more severe clinical symptoms than positive women with normal pregnancy, but laboratory parameters did not differ. Many other factors could affect the severity of a Rift Valley fever virus infection, but those were not studied here.
Unfortunately, no placental or aborted fetal tissue was obtained for further analysis, which was a limitation of the present study. Further studies should be done to investigate the pathology and the possible presence of virus in such samples. Other limitations were a lack of records regarding miscarriages, stillbirths, and acute or historical Rift Valley fever cases in Port Sudan in human beings and livestock. Also, we could not follow up patients and newborns to study potential complications of the infection in those who did not miscarry.

In livestock, death of almost all newborns and abortions at all stages of pregnancy are early signs of an emerging Rift Valley fever outbreak. Fetuses often show marks of hepatic discolouration, haemorrhage, and necrosis. Virus has been recovered from both aborted fetal material and placental tissue and it appears that placental transmission of Rift Valley fever virus has only been severe haemorrhagic symptoms, which led to his death. Direct infection of the fetus through the placental barrier or severe febrile disease could explain miscarriages. However, the mechanism behind the observed association between Rift Valley fever virus infection and miscarriage is not known and warrants further study.

There are many other possible causes of miscarriage in developing countries, which makes it probable that the importance of Rift Valley fever virus infection in this respect has not been properly recognised. When these infections emerge in new regions, they might as for other mosquito-borne viral infections (eg, Zika virus) reveal their true range of clinical disease. Acquired immunity in the endemic regions might restrict infections in females by the time they reach childbearing age and in these circumstances, the potential for a virus to cause intrauterine infections might not be detected until it is introduced into an immunologically naive population. In a study of the Rift Valley fever outbreak in Egypt in 1977, no increased rate of miscarriages was reported. The authors commented that most abortions occur in the home and that medical assistance from the clinic is usually sought only if complications develop.

In Sudan the frequency of stillbirths (miscarriage after 28 weeks) is estimated to be 2·4% of all pregnancies, 14 times higher than in developed regions (as defined for the Millennium Development Goals). Sudan has had several Rift Valley fever outbreaks, most recently in 2010 in El Gezira state, but little is known about the incidence of disease between the outbreaks has not been well studied, the virus is endemic in this area. Many other infectious diseases are also present in this region, and a fatal outbreak of hepatitis E virus in pregnant women occurred in Port Sudan before the start of our study. We detected Rift Valley fever virus infection in pregnant women and in men from the region, but no Rift Valley fever outbreak was reported by authorities in the Port Sudan area during the study time. The latest described Rift Valley fever outbreak in Sudan was in 2010 in El Gezira state, but little is known about the cases and consequences. There is a dearth of information about the epidemiology and disease potential of Rift Valley fever in domestic livestock of Sudan. In a recent study, 9·4% of camels in Killour State, Sudan, sampled during 2014–15 had had a Rift Valley fever virus infection at any stage of pregnancy could be deleterious. Direct infection of the fetus through the placental barrier or severe febrile disease could explain miscarriages. However, the mechanism behind the observed association between Rift Valley fever virus infection and miscarriage is not known and warrants further study.

**Table 4: Association between chikungunya virus positivity and pregnancy outcome, clinical symptoms, and laboratory findings**

<table>
<thead>
<tr>
<th>Clinical symptoms</th>
<th>Chikungunya virus positive (n=31)</th>
<th>Chikungunya virus negative (n=99)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal pregnancy</td>
<td>24 (77%)</td>
<td>28 (28%)</td>
<td>0·0001</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>9 (29%)</td>
<td>19 (19%)</td>
<td>0·297</td>
</tr>
<tr>
<td>Preterm delivery</td>
<td>3 (10%)</td>
<td>1 (1%)</td>
<td>0·034</td>
</tr>
<tr>
<td>Malaise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleeding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemorrhagic disease*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total white blood cell count (&lt;10^10/L)</td>
<td>9 (5)</td>
<td>7 (3)</td>
<td>0·006</td>
</tr>
<tr>
<td>Platelet count (&lt;10^10/L)</td>
<td>224 (97)</td>
<td>193 (126)</td>
<td>0·219</td>
</tr>
<tr>
<td>Haemoglobin concentration (%)</td>
<td>9 (1)</td>
<td>10 (18)</td>
<td>0·007</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td></td>
<td></td>
<td>0·038</td>
</tr>
</tbody>
</table>

Data are n (%) or mean (SD), unless otherwise stated. *Defined as having any bleeding symptoms or moderate-to-severe thrombocytopenia (<100 × 10⁹ platelets per L). †Four of these women had a chikungunya virus and Rift Valley fever virus co-infection.
infection has been suspected to occur in Sudan, but was not associated with miscarriage. Dual infections with hepatitis E virus nor dengue virus was associated with miscarriage, but the high incidence in pregnant women indicates that chikungunya virus infection and treatments for infectious diseases. Switzerland: Springer International Publishing, 2015.


