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Association of Rift Valley fever virus infection with miscarriage in Sudanese women: a cross-sectional study

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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 χ^2 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.**

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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.[2–7]

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.[8] Case reports have described vertical transmission of Rift Valley fever virus in one pregnant woman in Sudan[9] and in one case in Saudi Arabia, where transplacental transmission led to the death of the infant.[10] Sudan has had several Rift Valley fever outbreaks,[11,12] 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.

governmental hospital of Port Sudan, Sudan. Simultaneously, the Department of Obstetrics and Gynaecology at the same hospital observed an increase in miscarriages among febrile women. Patients with these characteristics were examined and sampled between June 30, 2011, and Nov 17, 2012. Samples from patients whose fever could not be explained by bacterial infections, malaria, or yellow fever, and thus suspected to be of another viral origin, were stored for later analysis. We then retrieved clinical information and laboratory test results from the patients' medical records and stored blood samples were analysed.

Ethical clearance was granted from the Regional Ethics Review Board at the Red Sea University in Sudan. All participants gave their consent after being informed about the objectives of the study and the confidentiality of the information and results.

Procedures

Venous blood samples were collected 2–5 days after the onset of illness. Shortly after the initial sampling, haemoglobin, haematocrit, total white blood cell count, and platelet count were measured with a haematology analyser (Kx 21; Sysmex, Kobe, Japan). Plasma samples were prepared from the blood and stored at –20°C. Later, total RNA was extracted using the QIAamp viral RNA mini kit (Qiagen, Hilden, Germany), and recovered in 20 µL of nuclease-free water. The RNA obtained was either stored at –80°C or used directly for cDNA synthesis using the GoScript Reverse Transcription System (Promega, Madison, WI, USA). Sera from the venous blood samples were spotted on filter papers (FTA Cards; Whatman, GE Healthcare, Maidstone, UK), dried, and stored as described previously.[13] Elution of samples from filter papers was done at room temperature under constant rocking of filter cut-outs (1 cm^2 in area) in 1 mL of phosphate-buffered saline for 30 min. After this elution, which corresponded to a 1 in 25 dilution, the samples were stored at 4°C for analysis.

Eluted patient samples were prepared and analysed by qRT-PCR for Rift Valley fever virus RNA, chikungunya virus RNA, hepatitis E virus RNA, and dengue virus RNA using the primers and probes listed in table 1. For the probe-based PCR assays (Rift Valley fever virus, chikungunya virus, and hepatitis E virus), a qRT-PCR QuantiTect Probe PCR kit was used (Qiagen, Hilden, Germany). The assay was done in a 25-µL final reaction volume containing 2 µL of cDNA, each primer at 400 nM, each probe at 225 nmol/L, and RNase-free water (Ambion; Thermo Fisher Scientific, Waltham, MA, USA). The reaction was run for 2 min at 50°C and 15 min at 95°C, followed by 45 cycles of 94°C for 15 s and 60°C for 1 min. All samples were tested in duplicate (including positive and negative controls) in a 96-well reaction plate

using the ABI Prism 7900HT Sequence Detection System 2.4 (Applied Biosystems; Thermo Fisher Scientific). For detection of dengue virus, an SYBR green-based assay was done as previously described.[14] For sequencing, PCR products of Rift Valley fever virus positive cDNA samples were generated by using Rift Valley fever virus forward and reverse primers (table 1). 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-HBc), 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[15] in an assay we have previously described.[16] The presence of neutralising antibodies to Rift Valley fever virus in serum samples was detected using a plaque reduction neutralisation test as previously described.[17] In brief, eluted patient samples (at a final dilution of 1:100) were pre-incubated for 60 min with 50 plaque-forming units of 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 (10 000 U/mL) was added to the wells. The plates were incubated at 37°C in an atmosphere of 5% CO_2 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.

	Sequence
RVFV PCR forward primer	5′-AAGGCAAAGCAACTGTGGAG-3′
RVFV PCR reverse primer	5′-CAGTGACAGGAAGCCACTCA-3′
RVFV PCR probe	5′- GATGAGTTGACTCTATCACGAGTTGC-3′
CHIKV PCR forward primer	5′-CATGCAAAACAGAATTTGC-3′
CHIKV PCR reverse primer	5′-TAGGCAGTTACAGTGATG-3′
CHIKV PCR probe	5′-CTCATACCGCATCTGCATCA-3′
HEV PCR forward primer	5′-GGTGGTTTCTGGGGTGAC-3′
HEV PCR reverse primer	5′-AGGGGTTGGTTGGATGAA-3′
HEV PCR probe	5′-TGATTCTCAGCCCTTCGC-3′
DENV PCR forward primer	5′-TCAATATGCTGAAACGCGAGAGAAACCG-3′
DENV PCR forward primer	5′-TTGCACCAACAGTCAATGTCTTCAGGTTC-3′

RVFV=Rift Valley fever virus. CHIKV=chikungunya virus. HEV=hepatitis E virus. DENV=dengue virus.

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

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 ($<100\times10^9$ platelets per L).

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 χ^2 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.

See Online for appendix

	Febrile pregnant women (n=130)
Age (years; mean [range, SD])	27·0 (17–40, 5·5)
Pregnancy outcome	
Normal pregnancy	99 (76%)
Miscarriage	27 (21%)
Preterm delivery	4 (3%)
Viral infections	
RVFV infection (PCR or IgM ELISA or PRNT positive)	28 (22%)
CHIKV infection (PCR positive)	31 (24%)
RVFV/CHIKV coinfection	8 (6%)
DENV infection (IgM ELISA positive)	9 (7%)
DENV infection (PCR positive)	0
HEV infection (PCR positive)	0
HBV infection (HBsAg, anti-HBc positive)	0
HAV infection (HAV anti-IgM positive)	0
Clinical symptoms	
Fever	130 (100%)
Headache	130 (100%)
Generalised pain	130 (100%)
Nausea or vomiting	130 (100%)
Jaundice	130 (100%)
Joint pain	130 (100%)
Malaise	52 (40%)
Diarrhoea	46 (35%)
Rash	40 (31%)
Bleeding	21 (16%)
Laboratory findings	
Total white blood cell count (×10^9/L)*	7·5 (3·9)
Platelet count (×10^9/L)†	201 (102)
Haemoglobin concentration (%)‡	9·8 (1·8)
Haematocrit (%)§	31·7 (5·3)

Data are n (%) or mean (SD), unless otherwise stated. RVFV=Rift Valley fever virus. PRNT=plaque reduction neutralisation test. CHIKV=chikungunya virus. DENV=dengue virus. HEV=hepatitis E virus. HBsAg=hepatitis B surface antigen. HBV=hepatitis B virus. HAV=hepatitis A virus. Normal range for pregnant women:[18] *5·6–16·9. †146–429. ‡9·5–15·0. §28·0–41·0.

Table 2: **Pregnancy outcome, clinical symptoms, and laboratory findings in febrile pregnant women in Port Sudan, Sudan**

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 generalised 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 and classified as Rift Valley fever virus positive. The remaining three were regarded as Rift Valley fever virus negative.

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.[19] 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.

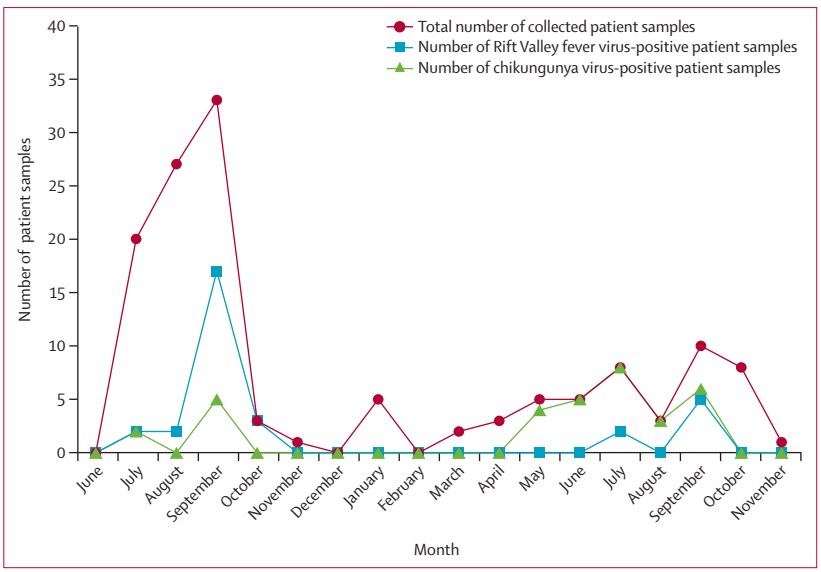

Figure: **Temporal distribution of patient samples during the study period**
Temporal distribution of samples positive for Rift Valley fever virus and chikungunya virus in relation to all samples collected during the study period (June 30, 2011, to Nov 17, 2012).

	Rift Valley fever virus positive (n=28)	Rift Valley fever virus negative (n=102)	p value
Age (years; mean [range, SD])	27·8 (17–37, 5·0)	26·8 (17–40, 5·7)	0·382
Pregnancy outcome			
Normal pregnancy	12 (43%)	87 (85%)	<0·0001
Miscarriage	15 (54%)	12 (12%)	<0·0001
Preterm delivery	1 (3%)	3 (3%)	0·422
Clinical symptoms			
Malaise	19 (68%)	33 (32%)	0·001
Diarrhoea	8 (29%)	38 (37%)	0·395
Rash	10 (36%)	30 (29%)	0·522
Bleeding	11 (39%)	10 (10%)	<0·0001
Haemorrhagic disease*	17 (61%)	31 (30%)	0·003
Laboratory findings			
Total white blood cell count (×10⁹/L)	7·0 (3·5)	7·6 (4·0)	0·469
Platelet count (×10⁹/L)	161 (89)	211 (126)	0·050
Haemoglobin concentration (%)	9·1 (1·8)	10·0 (1·7)	0·024
Haematocrit (%)	30·2 (5·1)	32·1 (5·3)	0·091

Data are mean (SD) or n (%), unless otherwise stated. *Defined as having any bleeding symptoms or moderate-to-severe thrombocytopenia (<100 × 10⁹ platelets per L).

Table 3: **Association between Rift Valley fever virus positivity and pregnancy outcome, clinical symptoms, and laboratory findings**

	Chikungunya virus positive (n=31)	Chikungunya virus negative (n=99)	p value
Age (years; mean [range, SD])	26·9 (18–36, 4·4)	27·0 (17–40, 5·9)	0·934
Pregnancy outcome			
Normal pregnancy	20 (64%)	79 (80%)	0·081
Miscarriage	8 (26%)	19 (19%)	0·296
Preterm delivery	3 (10%)	1 (1%)	0·034
Clinical symptoms			
Malaise	24 (77%)	28 (28%)	<0·0001
Diarrhoea	8 (26%)	38 (38%)	0·201
Rash	31 (100%)	9 (9%)	<0·0001
Bleeding	6 (19%)	15 (15%)	0·579
Haemorrhagic disease*	9 (29%)†	38 (38%)	0·297
Laboratory findings			
Total white blood cell count (×10⁹/L)	9·1 (5·1)	7·0 (3·3)	0·006
Platelet count (×10⁹/L)	224 (97)	193 (126)	0·219
Haemoglobin concentration (%)	9·0 (1·1)	10·0 (1·8)	0·007
Haematocrit (%)	30·0 (4·9)	32·3 (5·3)	0·038

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.

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

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 discoloration, haemorrhage, and necrosis.[20,21] Virus has been recovered from both aborted fetal material and placental tissue[19] and it appears that placental necrosis or infection of the fetal liver might be major causes of abortion.[20,21] In human beings, vertical transmission of Rift Valley fever virus has only been shown in two cases. One infected woman became ill a few days before an uneventful delivery. The infant was IgM-positive for Rift Valley fever virus and developed severe haemorrhagic symptoms, which led to his death 6 days after admission to hospital.[10] In another case, a 38-week-pregnant woman with Rift Valley fever virus-like symptoms delivered a child with enlarged liver and skin rash. 3 days after birth, the child developed jaundice and both he and the mother tested positive for Rift Valley fever virus IgM.[9] In the present study, all women with an acute Rift Valley fever virus infection had miscarriages that occurred in the second or third trimester of pregnancy. This finding might suggest that 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.

There are many other possible causes of miscarriage in developing countries,[22] 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.[23] 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.[3] 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.[24]

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).[25] Sudan has had several Rift Valley fever outbreaks,[11,26] most recently in 2010[12] in both human beings and livestock. Although 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.[27] 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.[12] 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 Khartoum State, Sudan, sampled during 2014–15 had had a Rift Valley fever virus infection as measured by IgG antibodies.[28] Furthermore, the main export market for Sudanese livestock is Saudi Arabia, just across the Red Sea from Port Sudan,[29] and imported goats and sheep in Makkah, Saudi Arabia, during November 2011, showed a high prevalence of anti-Rift Valley fever virus IgG

(18%).[30] This finding indicates that Rift Valley fever virus could have been circulating among livestock in the Port Sudan region during the study period.

Both Rift Valley fever virus and chikungunya virus are transmitted by mosquitoes, which increase in numbers during the warm periods that follow after heavy rainfall.[31] The rainy period in the Red Sea State is not as pronounced as in other Sudanese areas.[32] In general, the wettest period in Sudan is in July–September, whereas in Port Sudan a short rainy season also appears during December–January.[33] We noted an increase in the number of patients infected with Rift Valley fever virus and chikungunya virus for both 2011 and 2012 mainly during July–September when the weather is warm and conditions are conducive for mosquitoes. This pattern is similar to the temporal pattern of dengue virus transmission in Port Sudan, where several transmission peaks were preceded by peaks of mosquito densities.[33,34]

Dengue is present in the Red Sea State, and maternal and fetal deaths due to dengue haemorrhagic fever have been observed in Port Sudan.[4] However, neither hepatitis E virus nor dengue virus was associated with clinical disease in the present study. Chikungunya virus infection has been suspected to occur in Sudan,[35] but only a few cases of IgM positivity have been described.[36,37] We detected chikungunya virus RNA in 24% of the patients and could describe their clinical symptoms, which were consistent with what has previously been reported.[38,39] Chikungunya virus infection was not associated with miscarriage, but the high incidence in pregnant women indicates that chikungunya virus infections are relatively common in this coastal region. Although chikungunya virus has been shown to cross the placenta leading to fetal infection and miscarriage, this is a very rare occurrence.[40] Perinatal infections with chikungunya virus, which can cause severe neurocognitive outcomes in the newborn, are also very rare.[41] Interestingly, dual infection with Rift Valley fever virus and chikungunya virus was found in eight women but was not associated with miscarriage. Dual infections with mosquito-borne pathogens have been reported (eg, dengue virus and chikungunya virus[38] and malaria together with several different arboviruses[42]), but the importance of such combined infections for disease development is not clear.

In conclusion, this study of 130 febrile pregnant Sudanese women showed that infection with Rift Valley fever virus was significantly associated with miscarriage. For animals, this correlation is well known, but it has not previously been described in human beings. Further studies are needed to investigate the possible mechanisms. Meanwhile, preventive measures should be implemented to avoid Rift Valley fever virus infection during pregnancy.

Contributors

NM, MSK, AMJ, CA, and ME conceived and designed the study. AMJ, HJEJ, NM, MB, JN, and GB collected and analysed the data. MB, NM, CA, and ME interpreted the data. MB and NM drafted the report. GB, CA, and ME supervised the study, interpreted the results, and revised the report. All authors contributed to the writing of the report.

Declaration of interests

We declare no competing interests.

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