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GEM

Rift Valley Fever: a Threat to Pregnant Women Hiding in Plain Sight?

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ABSTRACT The potential for emerging mosquito-borne viruses to cause fetal infection in pregnant women was overlooked until the Zika fever outbreak several years ago. Rift Valley fever virus (RVFV) is an emerging arbovirus with a long history of fetal infection and death in pregnant livestock. The effect of RVFV infection on pregnant women is not well understood. This Gem examines the effects that this important emerging pathogen has during pregnancy, its potential impact on pregnant women, and the current research efforts designed to understand and mitigate adverse effects of RVFV infection during pregnancy.

KEYWORDS Rift Valley fever virus, bunyavirus, vertical transmission, fetal infection, pregnancy, *in utero*

The conventional TORCH pathogens (*Toxoplasma gondii*, rubella virus, cytomegalovirus, herpes simplex virus, plus others) are transmitted transplacentally from mother to fetus during either pregnancy or the delivery process. They are known to cause a range of adverse outcomes from developmental and cognitive defects to premature delivery and even fetal death (1). Viruses such as measles virus, influenza virus, varicella-zoster virus, smallpox virus, and poliovirus are not specifically transmitted to fetuses *in utero*, yet the resulting disease in pregnant women is often much more severe than in nonpregnant women (2, 3). For emerging viruses like Ebola and Lassa viruses, pregnant women are significantly more likely to suffer severe disease and death, especially when infected during the third trimester (4, 5). Complicating matters, pregnant women are often excluded from clinical trials for therapeutics or vaccines. In some cases, they are contraindicated without evidentiary basis (6).

The potential for mosquito-borne viruses to be transmitted to developing fetuses *in utero* was not appreciated until the Zika fever outbreak in 2015 to 2016 (7). Zika virus infection during pregnancy, in which the pregnant woman has inapparent or mild disease, resulted in fetal infection and subsequent physical deformity and/or developmental abnormalities in the fetuses, termed congenital Zika syndrome (8). The association of Zika virus infection with fetal deformities caught the medical and scientific communities off guard because there had never been a previous indication that this could occur. The mosquito-transmitted Rift Valley fever (RVF) virus (RVFV) is different because it has a long history of fetal infection and death in pregnant livestock (9, 10). This Gem examines the effects that this agriculturally important emerging pathogen has during pregnancy, its potential impact on pregnant women, and the current research efforts designed to understand and mitigate the adverse effects of RVFV infection in this important yet often overlooked population.

HISTORY OF RIFT VALLEY FEVER

RVFV is a negative-sense RNA virus with 3 genome segments. It belongs to the large and diverse *Phlebovirus* genus within the *Phenuiviridae* family of RNA viruses. RVFV is

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TABLE 1 Selected Rift Valley fever epizootics with reported fetal loss in livestock

Yr(s)	Location	Species, # or % of spontaneous fetal abortions[a]	Reference(s)
1930–1931	Kenya	Sheep, # not reported	11
1950–1951	South Africa	Sheep, 500,000	71
1977–1979	Egypt	Sheep, 80–100% Goats, # not reported Cattle, # not reported Camels, # not reported	9, 15
1978	Zimbabwe	Cows, 15%	72
1990	Madagascar	Cows, 16%	73
1997	Egypt	Sheep, 60–70% Cows, 30–40%	74 10
1998	Mauritania	Sheep, 10% Cows, 5% Goats, 47% Camels, 21% Donkeys, 0%	75, 76
2000–2001	Saudi Arabia	Sheep, 90% Goats, 90%	77 78
2006–2007	Kenya	Sheep, 70% Goats, 63% Cattle, 47%	79
2006–2007	Tanzania	Sheep, 13% Goats, 31% Cattle, 8%	79
2010	Mauritania	Sheep, 30% Goats, 26%	80 76
2016	Uganda	Sheep, 24%	81

[a]#, number.

classified as biosafety level 3 (BSL3), and its use is regulated by the Federal Select Agent Program (FSAP). The discovery of RVFV can be traced to a farm northwest of Nairobi, Kenya, in 1930, which reported unusual mortality in sheep, including abortions in ewes that were not visibly ill and death of newborn lambs between 3 and 7 days after birth (11). This initial illuminating outbreak revealed the age-dependent susceptibility of sheep to RVFV disease, with newborn lambs being the most susceptible to severe disease and death. Especially noteworthy were the large numbers of fetal abortions in pregnant animals (12).

Since that time, RVF disease in animals and humans has been documented periodically throughout much of Africa, including outbreaks in Saudi Arabia and Yemen in 2000 to 2001 ostensibly caused by livestock trade across the Red Sea (Table 1). Human disease became more apparent over time, with the first documented human deaths from RVFV noted during the South African outbreak of 1974 to 1975 (13). Major outbreaks in humans in Egypt (1977 to 1979), Mauritania (1987), and Saudi Arabia (2000 to 2001) have resulted in hundreds of human deaths and many more thousands of estimated human infections. The severity of human infection can vary from presumed asymptomatic to mild flu-like illness to the rarer occurrence of hemorrhagic fever or encephalitis (14, 15). During interepidemic periods, serosurveys suggest that 10 to 20% of people living within areas of endemicity have been exposed to RVFV, meaning that

TABLE 2 Reports of adverse effects of select live-attenuated RVFV vaccines in pregnant livestock[a]

Vaccine	Gestation period	Dose and/or delivery route	No. of pregnant animals with spontaneous abortions/total no. of animals vaccinated (%)	Effect(s) on fetuses	Description	Reference(s)
Smithburn or MLVV	Not reported	s.c.	Cattle, 28/100 (28)	Early fetal death and mummification	Animals received multiple doses of formalin-inactivated vaccine prior to vaccination with Smithburn	26
	Various (from both experimental and field vaccinations)	s.c., i.v., or i.d.	Sheep, 386/1,150 (44)	Stillbirths, teratogenesis, viremia	Hydranencephaly, hypoplasia (cerebrum, cerebellum, spinal), brachygnathia, arthrogryposis, scoliosis, hydrops amnii	27
Clone 13	Early gestation	1×10^5 PFU s.c.	Cattle, 0/17 (0) Sheep, 0/17 (0) Goats, 0/12 (0)	None reported		30
	Late gestation	1×10^5 PFU s.c.	Cattle, 0/8 (0) Sheep, 0/30 (0) Goats, 0/36 (0)	None reported		31
	Late gestation (120 days)	6.8 $\log_{10}$ $TCID_{50}$/ml s.c. or i.d.	Sheep, 0/12 (0)	0/28 teratogenicity 4/28 (14%) viremic		32
MP-12	Early gestation (28–56 days)	1×10^6 PFU s.c.	Sheep, 2/50 (4)	15–23% teratogenic effects in delivered lambs	Hydranencephaly, hypoplasia (cerebellum, spinal), prognathia inferior, brachygnathia, arthrogryposis, scoliosis, lordosis, kyphosis, domed head	41
	Late gestation (90–110 days)	5×10^3 PFU s.c.	Sheep (0)	None reported	NA	39, 82
	Early/midgestation (90 or 150 days)	1×10^5 PFU s.c. to pregnant sow or i.m. directly to fetus	Cattle (0)	None reported	NA	38, 40
ΔNSsΔNSm	Early (42 days)	1×10^4 PFU s.c.	0/29 (0)	None reported	No RVFV vRNA found in lamb tissues	83

[a]MLVV, modified live virus vaccine; s.c., subcutaneous; i.m., intramuscular; i.d., intradermal; i.v., intravenous; $TCID_{50}$, 50% tissue culture infective dose; vRNA, viral RNA; NA, not applicable.

the circulation of RVFV is much wider than often reported, and as a result, inapparent/mild illness may occur with regularity (16–19).

While the severe cases of RVF come to the attention of health care personnel, a legitimate unanswered question is what effect the wider circulation of RVFV has on people, in particular on pregnant women, especially in resource-limited nations. In an attempt to address this question, we first review what is known about RVFV infection of pregnant livestock, including live-attenuated vaccines (LAVs), followed by evidence of vertical transmission in women. Finally, we end with a discussion of important unanswered research and epidemiological questions.

WHAT IS KNOWN ABOUT THE ABORTOGENIC EFFECTS OF RVFV IN LIVESTOCK?

Spontaneous abortions in livestock are the trademark of RVF epizootics, and oftentimes, the first indication of RVFV circulation is an increase in fetal deaths among ruminant herds (20). Statistics on the number of fetal deaths and the rate of fetal deaths per species are difficult to pin down, as fetal deaths are often guesstimated,

overlooked, or not recorded at all. Sheep seem to be the species that is most susceptible to RVFV infection and fetal death. The reported spontaneous abortion rate for sheep can vary between 25 and 90%, with relatively less frequent occurrences in other livestock species like cows (10 to 40%), goats (10 to 60%), and camels (20%) (Table 1) (20). Aborted fetuses from livestock have severe liver necrosis similar to that in adult animals, and the nervous and musculoskeletal systems of fetuses are also affected, resulting in hydranencephaly and arthrogryposis (21, 22).

Given the devastating nature of RVF outbreaks in livestock, the widespread use of live-attenuated vaccines (LAVs) began in the 1950s with the development of the Smithburn neurotropic vaccine (23). Between 1952 and 1973, 28 million doses of this live-attenuated vaccine were administered throughout South Africa (24). A single injection protects sheep and cattle from subsequent natural infection with RVFV, and offspring from vaccinated animals retain protection from natural infection for several months (25). However, this vaccine causes abortion and fetal abnormalities in pregnant sheep and cattle (26, 27), and thus, the use of the vaccines is prohibited in pregnant animals.

Despite the known effects on pregnant animals, LAVs, including Smithburn, continue to be used to this day to curb the spread of RVFV in animals (24) (Table 2). Clone 13 is a naturally occurring isolate of RVFV that has a major deletion in the gene that encodes the viral interferon antagonist protein NSs (28). The removal of NSs renders RVFV apathogenic in most immunocompetent animals (29). While found to be generally safe in pregnant livestock (30, 31), one study administered a high dose of clone 13 to pregnant ewes and found transmission of the virus to fetuses, fetal deaths, and malformations (32). Pregnant ewes vaccinated earlier in gestation delivered stillborn and abnormal fetuses containing "malformations of the central nervous system such as hydranencephaly, hypoplasia of cerebrum, cerebellum and spinal cord and/or malformations of the musculoskeletal system such as brachygnathia, arthrogryposis, and scoliosis" (32). While no deformities were noted in offspring from ewes inoculated later in gestation, several lambs contained detectable levels of viral RNA, indicating that *in utero* transmission occurred.

Another LAV is the attenuated MP-12 strain that was generated in the 1980s by chemical mutagenesis (33). This strain contains point mutations throughout all 3 segments of the genome (29, 34, 35). While not currently used in livestock, it has been experimentally tested in livestock and is under development as a human vaccine (36, 37). Several studies demonstrated no fetal effects when MP-12 was administered to ewes and cows during pregnancy (38–40). In contrast, another study showed that administration of MP-12 to pregnant ewes resulted in fetal malformations in the nervous and musculoskeletal tissues (41). Interestingly, this study also identified a case of dizygous twins that were differentially affected. Ongoing studies are geared toward further attenuating MP-12 to reduce the potential for fetal infection (42–44), and more studies are warranted to confirm its safety.

Based on years of natural history and vaccination of livestock, it is apparent that (i) some live-attenuated vaccines can cause abortion or developmental defects when given to pregnant animals due to the high efficiency of RVFV for transplacental transmission (Table 2) and (ii) vaccinated animals can be protected from natural or experimental RVF disease, yet their fetuses may still become infected and suffer abnormalities or even death as a result of infection and/or vaccination.

WHAT IS KNOWN ABOUT THE EFFECTS OF RVFV INFECTION IN PREGNANT WOMEN?

Given the well-known occurrence of fetal infection in livestock, including after vaccination with LAVs, it is understandable to ask whether transplacental infection happens in pregnant women. Epidemiological data are limited regarding the rates of miscarriage or vertical transmission within women infected with or previously exposed to RVFV. The reasons for this may be due to the fact that spontaneous abortions or

stillbirths are, in general, drastically underreported, and this may be exacerbated in resource-poor countries. Epidemiological studies of RVFV typically focus on animal infections and individuals in close contact with animals, who tend to be male. Follow-up with exposed communities after RVFV outbreaks is limited, and as such, information on lost pregnancies is likely not gathered. Below, we review the available data regarding RVFV infection during human pregnancy.

Two early studies found little association between RVFV infection and miscarriages in women based on retrospective seropositivity (45, 46). A hemagglutination inhibition (HI) test was used to determine prior RVFV infection in women who suffered spontaneous abortions prior to or during the 1977 Egyptian epidemic, and both groups were compared to randomly selected individuals. No difference was found among the 3 groups, and no increase in fetal loss was noted (45). A similar study was performed during an outbreak in Mozambique in 1981, which used an IgG enzyme-linked immunosorbent assay (ELISA) to determine previous RVFV exposure (46). The rates of RVFV-specific antibody prevalence were not different between women who did and those who did not suffer late-term spontaneous fetal loss. However, 24% (5/21) of seropositive women had a history of fetal loss, compared to only 15% (143/969) of seronegative women. Both of these studies assumed that the included individuals had no prior exposure to RVFV because there had been no previous reports of RVFV-related disease within livestock in the respective areas. Similarly, neither the HI tests nor the IgG ELISAs detected RVFV-specific IgM, which was the primary means, at the time, of detecting recent or active infection. The authors of both studies emphasize the need for more epidemiological studies with a larger pool of participants. "Considering the extreme virulence of the virus and its tendency to induce abortions in a variety of animals, it is important that this subject be further investigated" (45).

Almost 20 years after the last serological study, the first case report of vertical transmission in humans was documented in Saudi Arabia (47). In 2000, during a massive animal and human outbreak of RVFV on the Arabian Peninsula, a 5-day-old infant was admitted to the hospital with respiratory distress. Blood tests detected RVFV-specific IgM antibodies and elevated white blood cells, platelets, and liver enzymes, indicative of organ damage; the infant died 2 days later. Family history revealed that the infant's grandfather recently died of confirmed RVF disease, and six other family members developed fever within 2 weeks of the infant's birth. The mother and other family members had all come into contact with sick or aborting animals during the outbreak. Four days prior to the infant's delivery, the mother developed fever, headache, dizziness, and muscle ache; her blood collected at 3 months postpartum contained RVFV-specific IgG antibodies. It is unclear in this case whether transmission occurred ante-, peri-, or postnatally.

Subsequently, in 2007, another pregnant woman was hospitalized with fever, headache, dizziness, and muscle aches (48). Blood tests confirmed the presence of RVFV-specific IgM. Labor commenced at 38 weeks, and a male baby was delivered with an enlarged spleen and liver (Apgar score of 5). Cord blood contained RVFV-IgM antibodies. On the third day, after the mother and the child were in stable condition, the parents insisted on discharge despite the child developing clinical jaundice; it is unknown whether the child recovered from infection, and the mother's history was not provided. These 2 case reports implicate RVFV as being able to infect human fetuses at or around the time of birth.

In 2011, patients with symptoms characteristic of RVF arrived at a hospital in Sudan, and many of them progressed to hemorrhagic disease (49). The maternity clinic of the same hospital reported an increased rate of miscarriages in febrile women. To determine if a virus was associated with the unusually high rate of miscarriages within this hospital, serological studies were performed to distinguish between RVFV, chikungunya virus (CHIKV), hepatitis E virus, and dengue virus infections. Out of the 130 pregnant women included in the study, 22% tested positive for RVFV, 24% were positive for CHIKV, and 8% were coinfected with both viruses. CHIKV-infected women had no

increase in miscarriages, while RVFV-infected women were 4 times more likely to have a miscarriage than seronegative controls (54% versus 12%, respectively; $P < 0.0001$). RVFV-infected pregnant women also had more severe bleeding, joint pain, and malaise than their uninfected counterparts. Acute RVFV infection during pregnancy was an independent predictor of miscarriage (odds ratio [OR], 7.4), and RVFV infection was significantly associated with late-term spontaneous abortion or stillbirth. This study did not analyze the placenta or aborted fetal tissue for the presence of virus or other pathological changes related to RVFV infection, nor were the infants monitored to determine whether maternal RVFV infection results in developmental abnormalities. This study became the first midsized, cross-sectional study to show a significant association between RVFV infection and late-term miscarriages. Additionally, of the four viruses examined, only RVFV infection was associated with adverse fetal outcomes.

HOW DOES RVFV INFECT THE PLACENTA?

In an experimental *ex vivo* setting, two studies have shown that RVFV can infect human placental explants from midgestation (50) and full-term (51) fetuses. The human placenta contains chorionic villi, which are tree-like structures surrounded by maternal blood (reviewed in reference 52). The outer cell layer of the villi is comprised of syncytiotrophoblasts, which are fused, multinucleated cells that form the cellular barrier between maternal and fetal blood. Cytotrophoblasts reside under the syncytiotrophoblast layer and have proliferative capacity. Extending off the branched villi are highly invasive extravillous trophoblasts that invade the maternal uterus and anchor the villi to the maternal endometrial tissue. Syncytiotrophoblasts are interesting from a pathogen standpoint because they are resistant to viral, bacterial, and fungal pathogens and thus provide a primary barrier of protection for the fetus. Pathogens that are known to cause human fetal infection (*Listeria*, *Toxoplasma*, cytomegalovirus, and Zika virus) infect extravillous trophoblasts and/or cytotrophoblasts but not syncytiotrophoblasts, which maintain high resistance to pathogens (53–55). RVFV, in contrast to these other fetal infections, appears capable of infecting both the syncytiotrophoblast and cytotrophoblast layers of the human placenta (50, 51), which may portend the potential for *in utero* infection of human fetuses.

In livestock animals, which have a placental structure very different from that of humans, RVFV demonstrates extraordinarily high tropism for placental tissue. In pregnant sheep, widespread viral infection occurs throughout the maternal epithelial cells and fetal trophoblasts comprising the placentomes (51). Fetal death in sheep can be due to severe placental pathology with or without direct fetal infection. In cases where the fetus is infected, viral antigen has been found throughout the liver, brain, and endothelial cells (51).

Ex vivo human placental tissue is difficult to obtain, and experimental studies in pregnant sheep are logistically challenging. As such, a rodent model would be useful for understanding potential mechanisms of vertical transmission of RVFV. A small study from 1956 infected pregnant rodents with a mouse-passaged strain of RVFV, and virus was detected in murine embryos taken from sick dams; however, no abortions or fetal deaths were reported (56). More recently, a rodent model of vertical infection was described in outbred Sprague-Dawley rats (50). Unlike mice, rats demonstrate a wider range of disease outcomes after infection with RVFV by different routes (57, 58). Rats are also a preferred rodent model for embryogenesis and toxicology studies (59, 60). In Sprague-Dawley rats, RVFV was highly tropic for placental tissue in rats infected during late gestation (day 14 of a 21-day gestation period). At lower infectious doses of RVFV, pregnant dams remained healthy and disease free yet delivered pups that were dead and full of virus (50). Some dams delivered a combination of live and dead pups, while others delivered only deceased offspring. Live-born pups still had detectable viral RNA, highlighting the efficiency of transplacental transmission of RVFV. The mechanisms of RVFV infection of placental tissue are not well understood in any species.

UNANSWERED RESEARCH QUESTIONS

Many epidemiological questions remain unaddressed regarding the impact of RVFV on pregnant women. Active surveillance of both early- and late-term pregnancy loss in areas where RVFV is endemic is needed, both during and in between outbreaks of disease in livestock and humans. Serological studies are performed on patients who present clinical symptoms of RVF; however, oftentimes, the pregnancy status of the patients is not documented, likely due to the limited understanding of the potential for miscarriages in women with RVF. Accurate clinical history, documentation of exposures, and adequate follow-up are essential. Diagnostic testing of pregnant women, including pathological and virological testing of their placenta and autopsy of fetuses in the event of a loss, will better inform the clinical picture of RVF during pregnancy. Equally important is the follow-up of infants for later developmental issues and the monitoring of women for future pregnancies and infertility. For example, irregular menstruation and adverse pregnancy outcomes were frequently reported in survivors of Ebola (61).

Important mechanistic questions can be addressed by laboratory studies involving a combination of primary tissue and animals, including both livestock and rodent models. For instance, why is the placenta a major target of RVFV infection in livestock, rodents, and potentially humans? Is there a difference in receptor or attachment factors? Does the virus have the ability to evade the innate antiviral response of syncytiotrophoblasts via the NSs protein or other mechanisms? Are human placentas as permissible to infection as those of livestock? What explains the apparent ranking of the abortogenic frequency of sheep $>$ cows $>$ humans? In the case of twins or multiple births, do all or some fetuses become infected? Are there genetic factors associated with varied susceptibility?

The fact that RVFV can be transmitted to fetuses via the placenta in diverse species ranging from livestock to rodents to humans is of considerable interest given that RVFV is designated by the World Health Organization (WHO) an important emerging viral infection (62). It also means that while rodent placentas have a structure distinct from that of humans, there may be crosscutting mechanisms that can be understood by studying rodents (63). Remarkably little work has been done on the vertical transmission of RVFV outside livestock, and most livestock studies that have been performed have centered around the safety of live-attenuated vaccines rather than a deeper understanding of the mechanism of transplacental infection. Thus, there is a major gap in the field in understanding how RVFV is efficiently transmitted from mother to fetus.

Finally, given the emerging nature of members of the *Bunyavirales* order, like RVFV, and the ability to undergo reassortment, do related emerging bunyaviruses have an unrecognized potential for vertical transmission? Jamestown Canyon virus (JCV) and La Crosse virus (LCV) are endemic in the United States and circulate in mosquitoes (64). Evidence of abortions has been reported for JCV-infected white-tailed deer, the predicted animal reservoir of JCV. Experimental infection of sheep, rabbits, and gerbils with LCV resulted in vertical transmission (65–67). Schmallenberg virus causes miscarriages and fetal malformations in sheep throughout western Europe; however, there appears to be no evidence of human infection (68). Severe fever with thrombocytopenia syndrome virus (SFTSV) and the related Heartland virus (HRTV) are found in Asia and North America, respectively. Both are tick-borne viruses that cause thrombocytopenia and RVFV-like symptoms in humans (69, 70). These examples and many other related bunyaviruses with shared pathogenesis and hosts should be evaluated for their potential to undergo vertical transmission.

CONCLUSION

The World Health Organization includes Rift Valley fever on its annual list of viral diseases with high potential to cause a public health emergency due to the lack of approved vaccines and therapeutic drugs (62). Recent experimental evidence reviewed

here highlights the fact that RVFV is a credible threat to pregnant women and their unborn babies in areas in Africa where the disease is endemic. Clearly, more work is warranted on both an epidemiological and a mechanistic front. The Zika fever epidemic of 2014 to 2015 emphasizes the cost of ignoring emerging viral threats that are hiding in plain sight.

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