doi:10.20944/preprints201811.0022.v1

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

Full title: RVFV infection in goats by different routes of inoculation

Short title: RVFV infection in goats

Andrea L Kroeker[1], Valerie Smid[1], Carissa Embury-Hyatt[1], Estella Moffat[1], Brad Collignon[1], Oliver Lung[1,2], Robbin Lindsay[3,4], Hana Weingartl[1,5]

1 – Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada
2 – University of Manitoba, Biological Sciences, Winnipeg, Manitoba, Canada
3 – Public Health Agency of Canada, Winnipeg, Manitoba, Canada
4 – University of Manitoba, Entomology, Winnipeg, Manitoba, Canada
5 – University of Manitoba, Medical Microbiology, Winnipeg, Manitoba, Canada

Corresponding author:

Andrea.Kroeker@canada.ca

Hana.Weingartl@canada.ca

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

Abstract

Rift Valley Fever virus (RVFV) is a zoonotic arbovirus of the *Phenuiviridae* family. Infection causes abortions in pregnant animals, high mortality in neonate animals and mild to severe symptoms in both people and animals. There is currently an ongoing effort to produce safe and efficacious veterinary vaccines against RVFV in livestock to protect against both primary infection in animals and zoonotic infections in people. To test the efficacy of these vaccines it is essential to have a reliable challenge model in relevant target species, including ruminants. We evaluated two goats breeds (Nubian and LaMancha), three routes of inoculation (intranasal, mosquito-primed subcutaneous and subcutaneous) using an infectious dose of 10^7 pfu/ml, a virus strain from the 2006-07 Kenyan/Sudan outbreak and compared the effect of using virus stocks produced in either mammalian or mosquito cells. Our results demonstrated that Nubian goats achieved the highest levels and longer duration of viremia. In the Nubian goats, all three routes of inoculation were equally efficient at producing clinical signs, consistent viremia (peak viremia: $1.2x10^3$ - $1.0x10^5$ pfu/ml serum), nasal and oral shedding of viral RNA ($1.5x10^1$ – $8x10^6$ genome copies/swab), a systemic infection of tissues, and robust antibody responses. The Nubian goat breed and a needle-free intranasal inoculation technique could both be utilized in future vaccine and challenge studies.

Keywords: Rift Valley Fever virus; arbovirus; caprine; challenge model; animal vaccine; zoonosis;

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

Introduction

Rift Valley Fever virus (RVFV) is a zoonotic mosquito-borne virus that causes acute infections in ruminants such as goats, cattle, sheep and camels. Large outbreaks of RVFV have mainly occurred in Sub-Saharan Africa. However, an outbreak outside of the African continent, such as in the Arabian Peninsula in 2001, an imported case in China (Liu 2017), serological evidence in Turkey (Gür et al., 2017), and climate changes (Bett et al., 2017) have raised concerns about the potential spread of the virus to Europe, Asia and the Americas (Balkhy and Memish 2003; Bird 2009; Chevalier 2010; Pepin and Tordo 2010).

RVFV outbreaks in livestock are thought to be primarily transmitted by infected mosquitos. Studies have shown that RVFV epidemics typically follow periods of heavy rainfall conducive to large mosquito populations (Davies, Linthicum, & James, 1985; Hassan, Ahlm, Sang, & Evander, 2011; Leedale, Jones, Caminade, & Morse, 2016; Redding, Tiedt, Lo Iacono, Bett, & Jones, 2017) as well as the trade and importation of infected animals into susceptible regions (Napp et al., 2018). In Africa, RVFV is mainly transmitted by the *Aedes aegypti* mosquito, however, a growing number of studies have made it clear that other mosquito species present in Asia, Europe and North/South America are experimentally competent as RVFV vectors (Brustolin et al., 2017; Ndiaye et al., 2016; Turell et al., 2015). Therefore, if RVFV were to be introduced into other continents and their endemic mosquito populations, it could cause widespread epidemics and could seriously impact the health of human populations and economically important livestock herds.

RVFV infections in livestock are characterized by abortion storms in pregnant ruminants and high rates of mortality in young sheep, goats and cattle (Cotzer 1977 and 1982; Pepin and Tordo 2010). In contrast, human infections are thought to occur either through mosquito bites or via zoonotic aerosol transmission (Swanepoel and Coetzer,1994). For example, the 1977-78 outbreak in Egypt was identified as a mosquito-transmitted outbreak while the 2009-2011 outbreak in South Africa was

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

primarily an aerosol-transmitted epidemic (Archer et al., 2013; Métras et al., 2012; Monaco et al., 2013) in which veterinarians and livestock workers who were in close contact with infected animals were most at risk (Nicholas, Jacobsen, & Waters, 2014). Human infection can result in subclinical to severe illness that in some cases can progress to retinal vasculitis, resulting in blindness, encephalitis and fatal hepatitis with hemorrhagic fever (Ikegami & Makino, 2011). Although reported human case fatality rates are generally low, higher fatality rates (20%–40%) were observed in the Kenyan outbreak of 2007-08 (Nguku et al., 2010) and in Mauritania in 2012 (Sow et al., 2014). Therefore, vaccination of livestock against RVFV is an important consideration for both livestock and their associated workers.

There is currently an ongoing effort to produce safe and efficacious vaccines against RVFV in livestock (Dungu et al., 2010; Indran & Ikegami, 2012; Kortekaas et al., 2012; Mansfield et al., 2015; Morrill et al., 2013; Pittman et al., 2016; Soi et al., 2010; Warimwe et al., 2013) as well as reliable challenge models for testing these vaccines. Several groups have developed challenge models for RVFV in cattle (Wilson et al., 2016) and sheep (Faburay et al., 2016; Weingartl, Miller, Nfon, & Wilson, 2014), and we recently published a challenge model in 4-month old goats (Nfon, Marszal, Zhang, & Weingartl, 2012; Weingartl et al., 2014). These initial studies have identified important factors of pathogenesis such as infectious dose and the use of insect-derived virus compared to mammalian-derived virus (Weingartl et al., 2014). However, several interesting studies have also demonstrated that mosquito saliva can modulate RVFV infection in mice (Le Coupanec et al., 2013) and that aerosol exposure to RVFV led to different disease kinetics and outcomes in mice (Reed et al., 2013) and NHPs (Hartman et al., 2014; Smith et al., 2012). Furthermore, RVFV has been shown to be highly dependent on the viral strain, animal species, breed and age. Therefore, our goal in this study was to explore how some these factors affected the pathogenicity of our goat model and whether they could be utilized in future vaccine efficacy trials.

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

Materials & Methods

Ethics statement

All animal experiments were carried out in the enhanced biosafety level 3 (BSL3) facility at the National Centre for Foreign Animal Disease (NCFAD) in Winnipeg, Manitoba. All protocols for animal use were approved under the animal document use number C-17-002 at the Canadian Science Centre for Human and Animal Health (CSCHAH) in Winnipeg, Manitoba by the Animal Care Committee. Care was taken to minimise animal suffering and to follow the Canadian Council on Animal Care guidelines for animal manipulations.

Cells

Mosquito C6/36 cells (ATCC, USA) were grown and infected in 1:1 EMEM and ESF-921 (Expression Systems, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone) and 1% L-glutamine and maintained at 28°C without CO_2. Mammalian Vero E6 (VE6) cells were grown and infected in DMEM (Gibco) supplemented with 10% FBS and maintained at 37°C with 95% relative humidity and 5% CO_2.

Virus production and titration

VE6 cells were infected with a virus isolate from the 2006-2007 Kenyan outbreak (Genbank #MH175203, MH175204, MH175205) at an MOI 0.1 and maintained in DMEM with 10% FBS. Thereafter, virus was alternatively passaged between VE6 and C636 cells twice (Moutailler et al., 2011). All passages were titrated on VE6 cells with a plaque assay to determine virus concentration. The goats were then infected using passage 7 C6/36-derived virus or passage 8 VE6-derived virus.

Genome sequencing

RNA was converted to cDNA using the Superscript IV First Strand Synthesis Module (Invitrogen) according to the manufacturer’s specifications except for the following modifications: eight gene specific primers (10 μM; 0.25 μL each) were used to selectively enrich for RVFV RNA, and a total of 10 µL of RNA was added to the reaction. Second strand synthesis was carried out using the NEBNext mRNA Second Strand Synthesis Module (New England Biolabs) according to the manufacturer's specifications. The double-stranded cDNA was purified using the QiAquick PCR Purification Kit (QiAgen) according to the manufacturer's specifications and eluted in 20 µL of nuclease-free water. A volume of 2 µL of purified double-stranded cDNA from each sample was quantified on the Qubit 3.0 fluorometer (ThermoFisher Scientific) using the dsDNA High Sensitivity Kit (ThermoFisher Scientific). The samples were diluted to 0.2 ng/µL in nuclease-free water and a total of 5 µL of diluted material was used as input to generate sequencing libraries using the Nextera XT Library Preparation Kit (Illumina), then pooled with other libraries before sequencing on an Illumina MiSeq platform using a V2 300-cycle (2 x 150 bp reads) cartridge (Illumina) and Micro flow cell (Illumina).

Sanger sequencing

Sanger sequencing was used to sequence a short GC-rich area located in the intergenic region of the S segment that had no MiSeq reads mapping to the reference for all samples tested. Briefly, samples were amplified by singleplex PCR using the SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Invitrogen) and in-house designed primers (Forward: 5'-CTAGAGGACTCCTTTGTTGG-3', Reverse: 5'-CTTGAAAGCCTTTGGACTTG-3') to generate a 505 bp amplicon spanning the intergenic region of the S segment. Thermal cycling was performed for 30 minutes at 47°C for reverse transcription, 3 minutes at 94°C for initial denaturation, followed by 40 cycles of 15 seconds at 94°C, 30 seconds 47°C and 1 minute at 68°C and a final extension for 5 minutes at 68°C. Amplicons were sequenced using BigDye Terminator v3.1 technology on an ABI 3130xl Genetic Analyzer system (Applied Biosystems).

Bioinformatic sequencing analysis

Read quality of data from the MiSeq was first visualized in FastQC (v0.11.5), followed by quality filtering and trimming using Trimmomatic (v0.36 with headcrop 20 and sliding

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

window 4:20) and de novo assembly using SPAdes assembler (v3.11.1 in metagenomic mode with default settings). The resulting assembled contigs were then characterized with blastn to determine their closest match within the nr/nt database. The closest full length sequence match for each of the three viral gene segments was then used to perform a reference assembly with the raw data (Geneious v9.1.5 on Low Sensitivity/Fastest setting). Finally, for each sample MiSeq and Sanger sequencing data were combined to generate a consensus sequence for each segment (Geneious v9.1.5).

Goat inoculation

Twenty-nine healthy 4 month old Nubian or LaMancha goats were obtained from breeders in Manitoba, Canada and allowed 7 days to acclimatize to BSL3+ containment at NCFAD, during which they were monitored daily for any signs of disease. After acclimatization, the goats were divided into groups of 2-4 and housed in separate cubicles.

Subcutaneous (Nubian and LaMancha)

A group of Nubian and LaMancha goats were inoculated subcutaneously with 10^7 pfu insect-derived RVFV. A group of controls were also inoculated subcutaneously with PBS.

Mosquito-primed subcutaneous (Nubian goats only)

Naïve *Aedes aegyti* mosquitos were allowed to feed at a small surface area on the neck for 20 minutes. This same surface area was then inoculated subcutaneously with 10^7 pfu insect-derived RVFV.

Intranasal (Nubian and LaMancha goats)

One group of Nubian and one group of LaMancha goats were each inoculated intranasally with 10^7 pfu insect-derived RVFV. A second group of LaMancha goats was inoculated intranasally with 10^7 pfu mammalian-derived RVFV.

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

Goat sampling

All goats were monitored for clinical symptoms and rectal temperatures daily. Blood for serum isolation and oral and nasal swabs were collected prior to infection and daily for the first 7 days post infection. Blood, oral and nasal swabs were also collected on days 14, 21, and 28 post infection. Serum samples and swabs were stored at -70^{o}C.

Virus isolation by plaque assay

Serial dilutions of serum, nasal swabs and oral swabs were used to infect confluent monolayers of VE6 cells for 1 hour. The virus was then removed and the cells were overlayed with 1.75% carboxymethylcellulose (CMC). After 3 days the cells were formalin-fixed and stained with 0.5% crystal violet (Sigma) to visualize and count plaques.

RNA isolation and RT-PCR

RVFV RNA was extracted from serum using the TriPure Isolation Reagent (Roche) according to the manufacturer's instructions. Purified RNA was stored at -70^{0}C. We detected viral RNA with a one-tube real-time polymerase chain reaction (RT-PCR) mix (Rotor-Gene Dual Probe kit, Qiagen) (Espach, Romito, Nel, & Viljoen, 2002) as per manufacturer's instructions and ran the samples on the Rotor Gene PCR machine with the following conditions: 30 minutes at 50ºC, 2 minutes at 95ºC and 45 cycles of 15s at 95ºC and 30s at 60ºC. Primers (Invitrogen) and probe (Biosearch) targeted nucleotides 2912 to 3001 for the RVFV L gene segment (Bird, Bawiec, Ksiazek, Shoemaker, & Nichol, 2007). All Ct values were plotted on a standard curve using a DNA plasmid containing the targeted RVFV L gene segment (GenScript) and quantified.

In situ hybridization of RVFV-probe in tissues

Five-micron paraffin-embedded formalin fixed tissue sections were cut, air-dried, and melted onto charged slides in a 60^{0}C oven. The slides were then cleared and hydrated in xylene and 100% ethanol, and then air-dried. The sections were quenched for 10 minutes in aqueous H_2O_2, boiled in target retrieval solution for 15 minutes, rinsed in 100% ethanol and air-dried again. A final treatment of protease plus enzyme for 15

Peer-reviewed version available at Viruses 2018, 10, 709; doi:10.3390/v10120709

minutes at 40^{0}C was applied. Next, the probe (V-RVFV-ZH501-NP, from Advanced Cell Diagnostics) was applied and incubated at 40^{0}C for 2 hours. The hybridization amplification steps (AMP 1-6) were applied to the slides for the recommended times and temperatures as per the manual for the RNAscope® 2.5HD Detection Reagent – Red kit (ACD). The signal was then visualized with Fast Red after which the slides were counterstained with Gill's hematoxylin, dried, cleared and cover-slipped.

Neutralizing antibody detection (PRNT)

The presence of neutralizing antibodies to RVFV was determined by a plaque reduction neutralization test (PRNT). Serial 2-fold dilutions of serum were prepared in PBS and incubated with an equal volume of insect-derived RVFV for 1 hour at room temperature. Thereafter, 75µl of the sera-virus mixture was adsorbed to confluent monolayers of VeroE6 cells in 48-well plates in triplicate for 1 hour at 37ºC, 5% CO_2 and 95% relative humidity. A carboxymethylcellulose (CMC) overlay was then added to all wells and plates were further incubated at 37ºC, 5% CO2 and 95% relative humidity. At 4 days post infection the cells were fixed with 10% formalin, stained with 0.5% crystal violet and plaques were counted. The reciprocal of the highest serum dilution that reduced plaques by 80% CPE was read as the antibody-$PRNT_{80}$ titre for that sample.

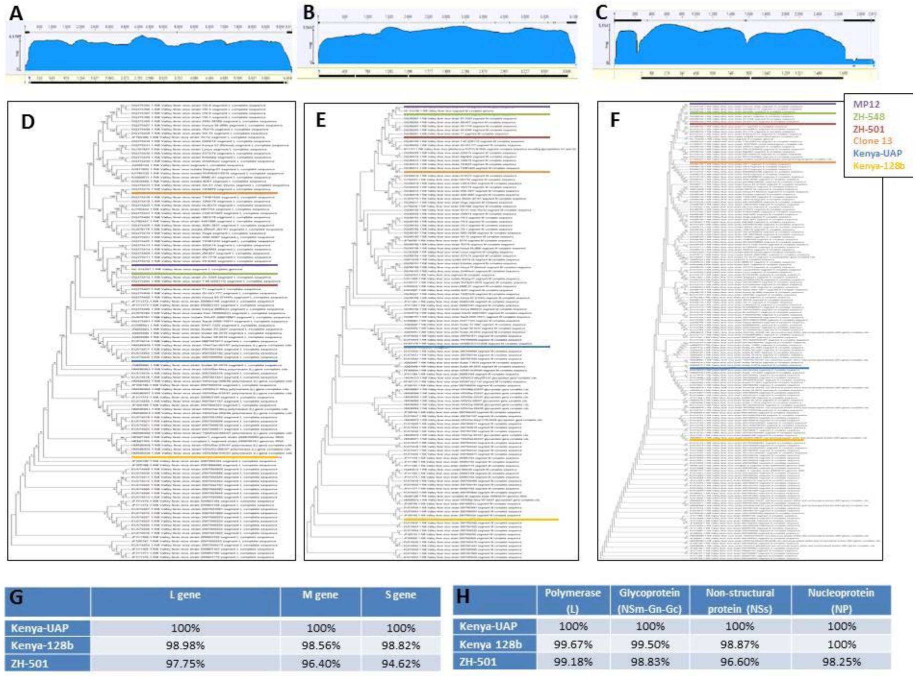
Results

Phylogenetic analysis of a 2006-07 strain of the CFIA-Kenya-UAP RVFV strain

The RVFV strain used in this study was sequenced and compared to other published RVFV sequences. Greater than 99% coverage of the reference ZH-501 strain and an average read depth of 400-2000 reads per nucleotide was achieved (Figure 1 A-C). A phylogenetic comparison demonstrated that the L, M and S segments all clustered with other strains isolated during the 2006-07 outbreak from Sudan or Kenya (Figure 1 D-F, respectively). We also directly compared sequences of our isolate to the Ken128b strain used in another study (Faburay et al., 2015; Wilson et al., 2016) as well as the commonly used ZH-501 strain. Our strain matched these with 96.6%-99.18% percent

Peer-reviewed version available at Viruses 2018, 10, 709; doi:10.3390/v10120709

identity at the amino acid level (Figure 1H) and with 94.6%-97.75% at the nucleotide level (Figure 1G).



G	L gene	M gene	S gene
Kenya-UAP	100%	100%	100%
Kenya-128b	98.98%	98.56%	98.82%
ZH-501	97.75%	96.40%	94.62%

H	Polymerase (L)	Glycoprotein (NSm-Gn-Gc)	Non-structural protein (NSs)	Nucleoprotein (NP)
Kenya-UAP	100%	100%	100%	100%
Kenya 128b	99.67%	99.50%	98.87%	100%
ZH-501	99.18%	98.83%	96.60%	98.25%

Figure 1

Experimental Design

We compared three different routes of RVFV infection in the Nubian and LaMancha goat breeds (Table 1). Five groups were infected with virus derived from the C6/36 (C6) mosquito cell line and one group was infected with virus grown in the Vero E6 (VE6) mammalian cell line. The subcutaneous infection method is widely used in RVFV infection models (Nfon et al., 2014; Weingartl et al., 2014; Faburay et al., 2015; Wilson et al., 2016; Smith et al., 2012) and mimics a natural infection via a mosquito bite. This group allowed our 2007 RVFV strain to be compared to many other published studies. Our second route of inoculation consisted of a mosquito-primed subcutaneous infection. Several interesting studies in mice have demonstrated that mosquito saliva can modulate RVFV infection (Le Coupanec et al., 2013) as well as other arbovirus infections (Agarwal et al., 2016; Styer et al., 2011; Wichit, Ferraris, Choumet, & Missé, 2016) and we sought to evaluate this effect in a large animal model. Thirdly, several groups were infected intranasally. A few studies have shown that aerosol exposure to RVFV could lead to different disease kinetics and outcomes in mice (Reed et al., 2013) and NHPs (Hartman et al., 2014; Smith et al., 2012).

Clinical signs and Gross Pathology

Inoculation of both the Nubian and LaMancha goats with RVFV resulted in mild clinical signs in all of the groups during the first week of infection. In the Nubian goats the subcutaneous group reached the highest clinical score (2-5 out of 10), the mosquito-primed group was intermediate (1-3 out of 10) and the intranasal was the lowest (1 out of 10). The LaMancha goats had higher clinical scores overall with the intranasal groups reaching the highest scores (2-7 out of 10) and the subcutaneous exhibiting the lowest score (2-5 out of 10) (Figure 2C-D). The observed clinical signs included a mild fever (Figure 2A-B), clumped stool, diminished eating and mild depression.

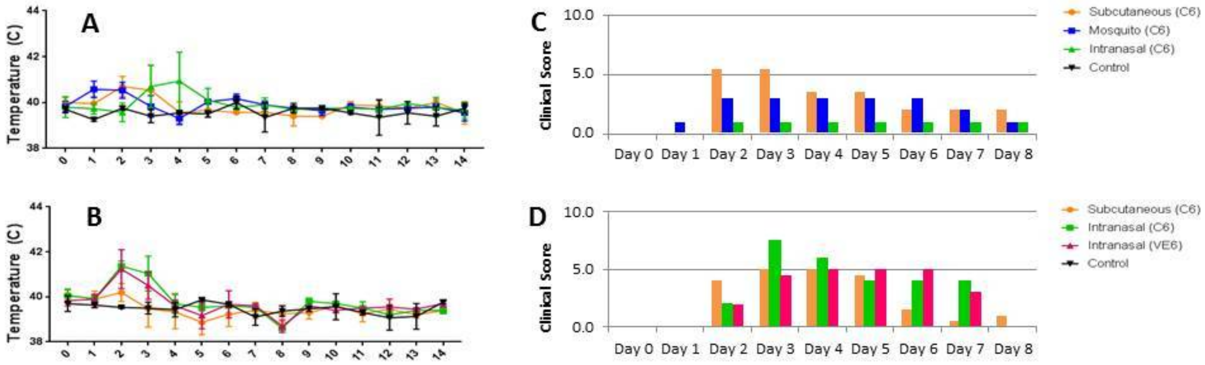


Figure 2

In the Nubian goats all signs generally resolved by 8-10 dpi. In the LaMancha goats most clinical signs improved at 8-10 dpi but did not resolve completely. However, ringworm was detected in all LaMancha goats at 14 dpi and likely contributed to the continued presence of mild clinical signs, primarily clumped stool. We postulated that the LaMancha goats had arrived with a latent ringworm infection which may have become apparent during the experiment. Finally, we performed autopsies and collected tissues at 1, 7, and 28 dpi. We did not detect any gross pathological changes at these time points.

Viremia, shedding and tissue viral load

All routes of inoculation in both Nubian and LaMancha goats led to infection and consistent viremia. In the Nubian goats, SQ and mosquito-primed-SQ inoculations produced peak titers of 10^3 pfu/ml while intranasally infected animals reached peak titers of 10^5 pfu/ml. All three inoculation routes produced viremia of similar duration with the virus detectable for 2-4 days by RT-PCR (Figure 3A-F, blue bars) and for 2 days by virus isolation (Figure 3A-F, orange bars). Interestingly, while subcutaneous and mosquito-primed-SQ injection led to viremia already on day 1 post infection, viremia in the intranasal group was delayed until day 2 or 3 post infection. In the LaMancha goats, a similar trend was seen with the mosquito-derived virus groups; however, virus duration tended to be a day shorter than in the Nubian goats (Figure 3D-F).

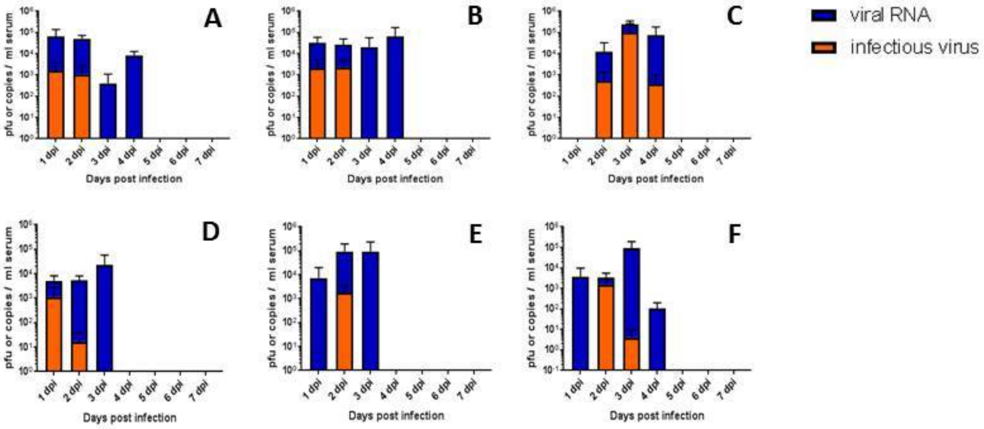


Figure 3

All of the Nubian goats had nasal (Figure 4A-C) and oral (Figure 4D-F) shedding of viral RNA (10^3–10^7 copies/swab) at 2-6 dpi; however no infectious virus was detected. In contrast, neither viral RNA nor infectious virus was found in nasal or oral swabs from any of the LaMancha goats.

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

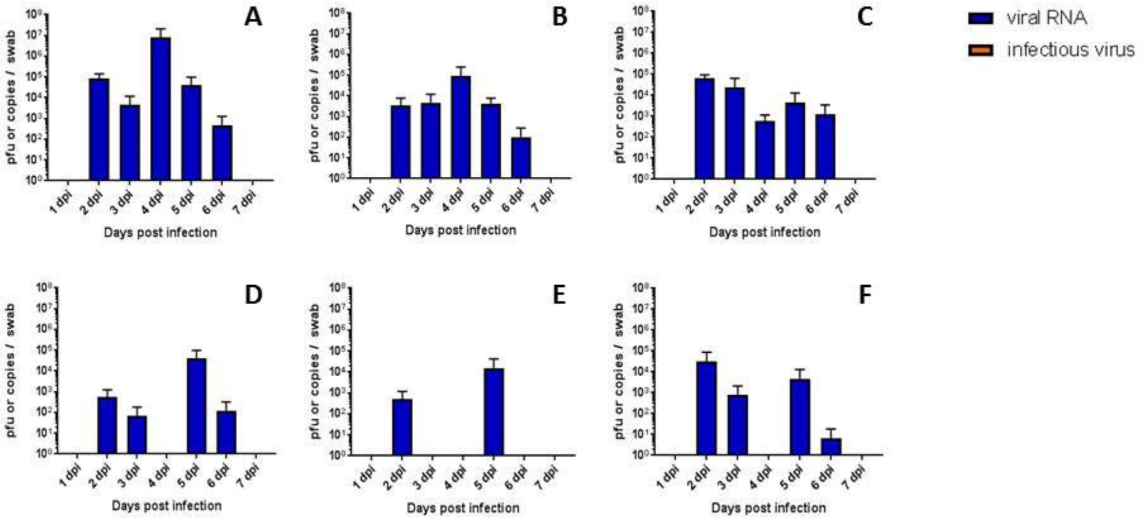


Figure 4

In the Nubian goats, all routes of inoculation also led to a systemic spread of the virus into the tissues. Although no infectious virus was isolated at our timepoints, viral RNA was detectable in the spleen, liver, lymph nodes and a variety of brain tissues. Our first timepoint consisted of 1 dpi to catch early localization of the virus *in vivo*. Indeed, we detected viral RNA in the trigeminal nerve in the intranasally infected group and in the spleen in the mosquito-primed-SQ group (Figure 5A). By 7 dpi, the mesenteric, prescapular and retropharyngeal lymph nodes were generally positive for viral RNA in all groups (Figure 5A) (10^2-10^6 copies/g tissue) and a few remained positive at 28 dpi (Figure 5A). The spleen was positive for the duration of the experiment in the mosquito-primed group (10^2-10^4 copies/g tissue), but only appeared by day 28 in the intranasal group (Figure 5A) and not at all in the subcutaneous group. In contrast, viral RNA was not detected in the liver except at day 28 for the mosquito-primed group (Figure 5A). Virus had begun to invade brain tissue in the subcutaneous and mosquito-primed groups by 7 dpi (Figure 5A), and in the intranasal group by 28 dpi. Notably, virus was particularly widespread throughout all the brain tissues tested in the mosquito-primed-SQ group at 28 dpi (Figure 5A). In contrast, neither viral RNA nor infectious virus was found in any tissues in the LaMancha goats at 1, 7 or 28 dpi (data not shown).

In-situ staining for RVFV

We also confirmed the presence of virus in some tissues using *in-situ hybridization* against the RVFV nucleoprotein (NP) sequence. For example, the spleen had positive staining at day 28 in all groups (Figure 5C-E) and at day 7 in the mosquito group (data not shown but similar to Figures 5C-D). In contrast, the SQ and IN groups had no staining at day 7 and none of the groups had staining at day 1 (Figure 5B is a representative image of negative staining in the spleen). Any positive staining was scattered only throughout the spleen follicles in the white pulp and ranged from weak (Figure 5C-D) to strong staining patterns (Figure 5E). Liver samples from all groups at day 28 and the trigeminal nerve from the intranasal group on day 1 were negative for virus staining.

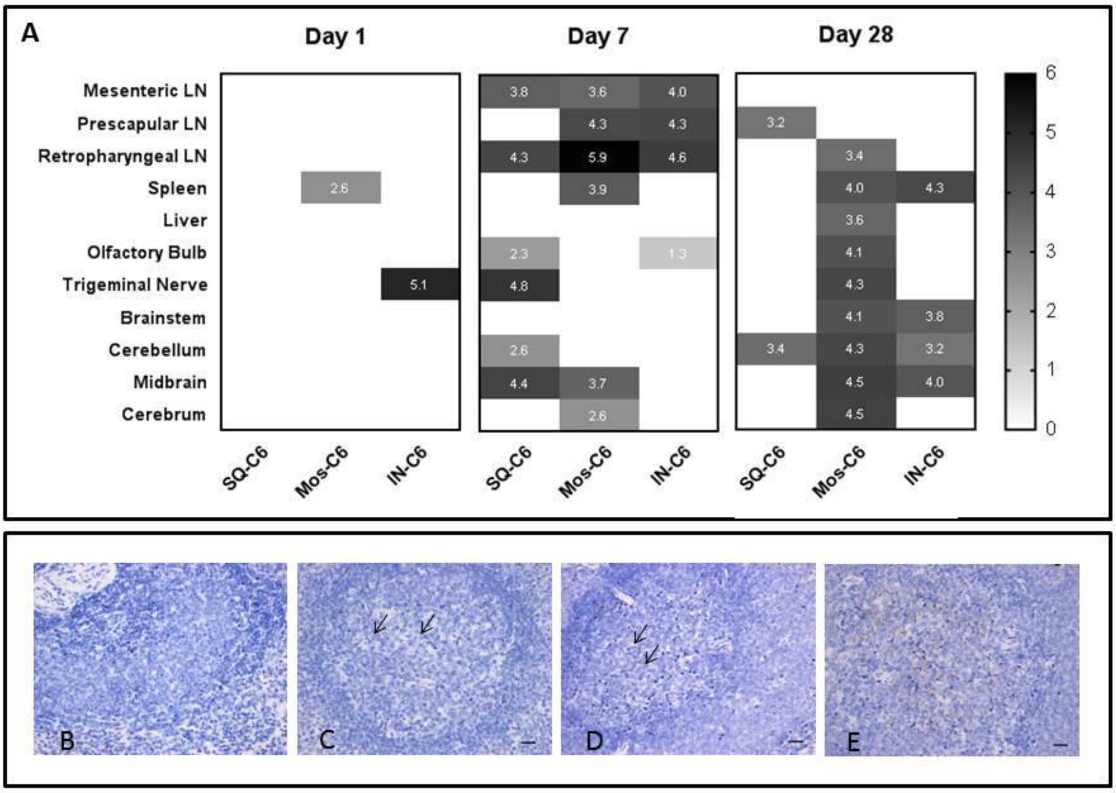
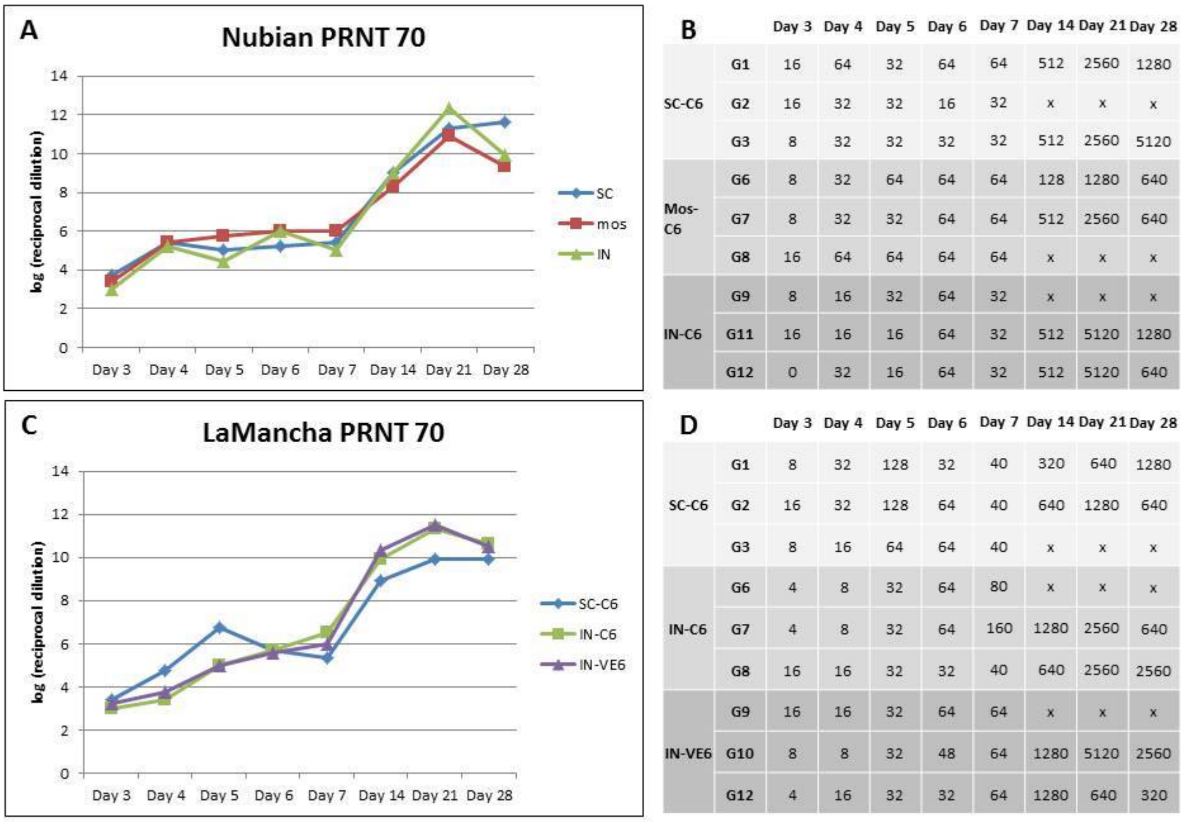


Figure 5

Peer-reviewed version available at Viruses 2018, 10, 709; doi:10.3390/v10120709

Neutralizing antibodies

We detected neutralizing antibodies against RVFV in both Nubian (Figure 6A-B) and LaMancha (Figure 6C-D) goats starting at 3 days post infection. In both goat breeds, all groups displayed similar titers and kinetics with a peak at 21 days post infection and peak titers of 1/1280 to 1/5120.



B		Day 3	Day 4	Day 5	Day 6	Day 7	Day 14	Day 21	Day 28
SC-C6	G1	16	64	32	64	64	512	2560	1280
	G2	16	32	32	16	32	x	x	x
	G3	8	32	32	32	32	512	2560	5120
Mos-C6	G6	8	32	64	64	64	128	1280	640
	G7	8	32	32	64	64	512	2560	640
	G8	16	64	64	64	64	x	x	x
IN-C6	G9	8	16	32	64	32	x	x	x
	G11	16	16	16	64	32	512	5120	1280
	G12	0	32	16	64	32	512	5120	640

D		Day 3	Day 4	Day 5	Day 6	Day 7	Day 14	Day 21	Day 28
SC-C6	G1	8	32	128	32	40	320	640	1280
	G2	16	32	128	64	40	640	1280	640
	G3	8	16	64	64	40	x	x	x
IN-C6	G6	4	8	32	64	80	x	x	x
	G7	4	8	32	64	160	1280	2560	640
	G8	16	16	32	32	40	640	2560	2560
IN-VE6	G9	16	16	32	64	64	x	x	x
	G10	8	8	32	48	64	1280	5120	2560
	G12	4	16	32	32	64	1280	640	320

Figure 6

Discussion

Robust challenge models for RVFV are an important prerequisite for testing novel therapeutics and vaccines and need further development for goats. We have previously established the necessary dose required for infection (Weingartl et al., 2014) and have demonstrated that mosquito-derived virus leads to more consistent viremia compared to mammalian-derived virus (Weingartl et al., 2014). In this follow-up study we investigated

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

whether other parameters affect the pathogenicity of RVFV in goats including different breeds of goats, routes of infection and a new strain of RVFV.

Sequencing and phylogenetic analysis of the virus isolate CFIA-Kenya-UAP determined that the virus clusters with other strains primarily isolated from Sudan, and that its origin is distinct from the Kenyan-128b strain utilized by Faburay and Wilson *et. al.* as well as other commonly used strains such as the Egyptian ZH-501 and ZH-548 strains.

Our previous experiments utilized Alpine-Boer goats infected with the RVFV ZH-501 strain. While infection produced consistent viremia and fever, it did not result in observable clinical disease or significant gross pathology (Weingartl et al., 2014). In contrast, we found that the Kenyan strain used in this study induced mild to moderate clinical symptoms in both the Nubian and LaMancha goats that lasted throughout the acute infection phase (days 2-5 post infection). Notably, this was not likely due to increased infectious virus production as peak viremia and duration in the LaMancha goats were similar to what we saw in the Alpine-Boer goats. Of the three goat breeds tested, Nubians infected intranasally with the Kenyan strain reached the highest peak viremia titers (10^5 pfu/ml).

Viral spread to the tissues differed greatly between the goat breeds, with the LaMancha completely lacking virus in any tissue tested at any time point, whereas many organs tested positive for viral RNA in the Nubians at both 7 and 28 dpi. We first looked at a variety of lymphoid tissues including the spleen which is well known to become infected in sheep and cattle (Faburay et al., 2016; Wilson et al., 2016). We then investigated other immune tissues including the mesenteric, prescapular and retropharyngeal lymph nodes. Dendritic cells are thought to be the primary cells to be infected by RVFV and once activated they travel to the lymph nodes. We hypothesized that lymph nodes close to the site of infection might be preferentially infected or contain higher amounts of virus. The prescapular lymph node was chosen for its proximity to the subcutaneous injection site behind the shoulder blade, the retropharyngeal lymph node for its proximity to the nasal and oral cavity, and the

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

mesenchymal lymph node as a site distant from either inoculation site. As viremia is present already at 1 or 2 dpi, we chose an early time point to look at the lymph nodes. While we did not find detectable virus in the lymph nodes at 1 dpi and could not distinguish any kinetics, all of the lymph nodes were generally infected in all groups by 7 dpi indicating systemic circulation of the virus.

Previous studies in calves, sheep, and non-human primates have found extensive involvement of the liver in RVFV pathology including lesions and changes in blood liver enzymes on days 3, 4, and 5 (Faburay et al., 2016; Rippy, Topper, Mebus, & Morrill, 1992; Smith et al., 2012; Wilson et al., 2016). In contrast, we did not detect significant viral loads in the liver in the Nubian or LaMancha goats at any time and this was supported by a lack of *in-situ* staining in liver tissues. It is possible that tissue tropism is different in the goats compared to sheep and cattle. Alternatively, we propose that perhaps the liver is only infected during the acute phase of infection, and any liver lesions or impairment may have been resolved in the goats by 7 dpi.

Interestingly, we detected substantial viral burden in different brain tissues in the Nubian goats. For example, at 1 day post infection the trigeminal nerve was positive for viral RNA in the intranasally-infected group. This time point is particularly notable in that it detected neural infection prior to the presence of viremia. This would suggest that neurons could be directly targeted during an intranasal exposure, rather than occurring through viremia and a breakdown of the blood brain barrier. Unfortunately though, we were unable to confirm the presence of virus in the trigeminal nerve with *in-situ* staining. We also detected viral RNA in various brain tissues at 7 dpi in the subcutaneous and mosquito-primed Nubian groups and in all groups by 28 dpi. The most consistently infected brain tissues included the cerebellum, midbrain and brainstem. RVFV has been detected in the brain in a few other studies. For example, in a mouse model of aerosolized RVFV the authors found virus in the neuroepithelium of the olfactory bulb at 7 days post infection (Reed et al., 2013). In addition, the neurons of 21-day old calves at 9 days after subcutaneous infection were positive for RVFV (Rippy et al.,1992). *In vitro* cultures of brain tissues from a variety of different ruminants have also been shown to

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

support robust RVFV replication (Gaudrealt et al., 2015). Notably, our study is the first indication of infected brain tissue in goats, the first to show positivity at such an early timepoint post infection and the only study to have investigated different regions of the brain.

In ruminants RVFV transmission appears to be primarily mosquito driven although results from transmission experiments have been mixed. For example, a few studies in sheep have demonstrated transmission through oral and respiratory routes (Busquets et al., 2010; Easterday et al., 1962; Harrington et al., 1980; Yedloutschnig et al., 1981). We hypothesized that the intranasal infection group might shed virus and so we measured viral shedding in both nasal and oral swabs. Surprisingly, all three routes of inoculation induced high and similar levels of shedding of viral RNA throughout acute infection in the Nubian goats, while shedding was completely absent in the LaMancha goats. This highlights a potential utility for nasal swabs in diagnostic testing, however, shedding may be breed specific and requires further investigations for reliability. We did not detect any infectious virus, suggesting that under our laboratory conditions the risk of transmission from shedding is very low. However, a few studies have demonstrated horizontal transmission from infectious virus isolated from nasal and oral swabs in sheep (Busquets et al., 2010; Harrington et al., 1980). Hence, further studies in the field with different breeds, species or viral strains could be useful.

Both the Nubian and LaMancha goats produced low levels of neutralizing antibodies by 3 dpi, a sharp increase by 14 dpi and strong peak titers at 21 dpi. These kinetics are similar to what we have seen with the Alpine-Boer goats infected with ZH-501 (Nfon et al., 2014), and are similar to what we have seen in sheep challenged with ZH-501 (Weingartl et al., 2014). Peak levels of neutralizing antibodies were generally similar between the LaMancha and Nubian goats, but were somewhat higher than similar groups in the ZH-501 infected Boer goats (Nfon et al., 2012). In contrast to what we have seen previously (Weingartl et al., 2014), no difference was seen between the mammalian- and mosquito-derived virus groups in the LaMancha. Similarly, no

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

difference was seen between the subcutaneous and mosquito-primed groups in the Nubian goats.

Conclusion

Overall, we have identified a novel goat breed that is useful for RVFV vaccine efficacy testing and have demonstrated that a needle-free intranasal inoculation method produces robust viremia in goats. In addition we have demonstrated the presence of viral RNA in goat brain tissue up to 28 days post infection and a high degree of viral RNA shedding after infection using both subcutaneous and intranasal routes. Importantly, our data also suggests that airborne virus may cause direct invasion of RVFV into the central nervous system. These findings highlight the impact that a variety of different parameters have on RVFV infection in goats and could be utilized in future vaccine and surveillance studies.

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

Acknowledgments

We graciously acknowledge the help of our dedicated animal care staff at NCFAD and our lab manager Peter Marszal for his training and support through this project. We would also like to thank the Canadian Safety and Security Program for funding this research through the Biosafety Level 4 Zoonotic Disease Laboratory Network (BSL4ZNET).

Author Contributions

Conceptualization, H.W.

Methodology, E.M., R.L.

Formal Analysis, A.K., C.E., B.C., O.L.

Investigation, A.K., V.S., O.L.

Resources, R.L.

Data Curation, A.K.

Writing – Original Draft Preparation, A.K.

Writing – Review & Editing, A.K., H.W.

Visualization, A.K.

Supervision, A.K. H.W.

Project Administration, A.K.

Funding Acquisition, H.W.

Conflicts of Interest

None

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

References

Agarwal, A., Joshi, G., Nagar, D. P., Sharma, A. K., Sukumaran, D., Pant, S. C., … Dash, P. K. (2016). Mosquito saliva induced cutaneous events augment Chikungunya virus replication and disease progression. *Infection, Genetics and Evolution*, *40*, 126–135. http://doi.org/10.1016/j.meegid.2016.02.033

Archer, B. N., Thomas, J., Weyer, J., Cengimbo, A., Landoh, D. E., Jacobs, C., … Blumberg, L. (2013). Epidemiologic investigations into outbreaks of Rift Valley fever in humans, South Africa, 2008-2011. *Emerging Infectious Diseases*, *19*(12), 1918–1925. http://doi.org/10.3201/eid1912.121527

Bett, B., Kiunga, P., Gachohi, J., Sindato, C., Mbotha, D., Robinson, T., … Grace, D. (2017). Effects of climate change on the occurrence and distribution of livestock diseases. *Preventive Veterinary Medicine*, *137*, 119–129. http://doi.org/10.1016/j.prevetmed.2016.11.019

Bird, B. H., Bawiec, D. A., Ksiazek, T. G., Shoemaker, T. R., & Nichol, S. T. (2007). Highly sensitive and broadly reactive quantitative reverse transcription-PCR assay for high-throughput detection of Rift Valley fever virus. *Journal of Clinical Microbiology*, *45*(11), 3506–3513. http://doi.org/10.1128/JCM.00936-07

Brustolin, M., Talavera, S., NuÑez, A., SantamarÍa, C., Rivas, R., Pujol, N., … Busquets, N. (2017). Rift Valley fever virus and European mosquitoes: vector competence of Culex pipiens and Stegomyia albopicta (= Aedes albopictus). *Medical and Veterinary Entomology*, *31*(4), 365–372. http://doi.org/10.1111/mve.12254

Busquets, N., Xavier, F., Martín-Folgar, R., Lorenzo, G., Galindo-Cardiel, I., del Val, B. P., … Brun, A. (2010). Experimental infection of young adult European breed sheep with Rift Valley fever virus field isolates. *Vector Borne and Zoonotic Diseases (Larchmont, N.Y.)*, *10*(7), 689–96. http://doi.org/10.1089/vbz.2009.0205

Davies, F. G., Linthicum, K. J., & James, A. D. (1985). Rainfall and epizootic Rift Valley fever. *Bulletin of the World Health Organization*, *63*(5), 941–943.

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

Dungu, B., Louw, I., Lubisi, A., Hunter, P., von Teichman, B. F., & Bouloy, M. (2010). Evaluation of the efficacy and safety of the Rift Valley Fever Clone 13 vaccine in sheep. *Vaccine*, *28*(29), 4581–4587. http://doi.org/10.1016/j.vaccine.2010.04.085

EASTERDAY, B. C., McGAVRAN, M. H., ROONEY, J. R., & MURPHY, L. C. (1962). The pathogenesis of Rift Valley fever in lambs. *American Journal of Veterinary Research*, *23*, 470–479.

Espach, A., Romito, M., Nel, L. H., & Viljoen, G. J. (2002). Development of a diagnostic one-tube RT-PCR for the detection of Rift Valley fever virus. *Onderstepoort J Vet Res*, *69*(3), 247–252.

Faburay, B., Gaudreault, N. N., Liu, Q., Davis, A. S., Shivanna, V., Sunwoo, S. Y., … Richt, J. A. (2016). Development of a sheep challenge model for Rift Valley fever. *Virology*, *489*, 128–140. http://doi.org/10.1016/j.virol.2015.12.003

Gaudreault, N. N., Indran, S.V., Bryant, P.K., Richt, J.A., Wilson, W.C. (2015). Comparison of Rift Valley fever virus replication in NOrth American livestock and wildlife cell lines. *Frontiers in Microbiology, 6:664.* doi: 10.3389/fmicb.2015.00664

Gür, S., Kale, M., Erol, N., Yapici, O., Mamak, N., & Yavru, S. (2017). The first serological evidence for Rift Valley fever infection in the camel, goitered gazelle and Anatolian water buffaloes in Turkey. *Tropical Animal Health and Production*, *49*(7), 1531–1535. http://doi.org/10.1007/s11250-017-1359-8

Harrington, D. G., Lupton, H. W., Crabbs, C. L., Peters, C. J., Reynolds, J. A., & Slone, T. W. (1980). Evaluation of a formalin-inactivated Rift Valley fever vaccine in sheep. *American Journal of Veterinary Research*, *41*(10), 1559–1564.

Hartman, A. L., Powell, D. S., Bethel, L. M., Caroline, A. L., Schmid, R. J., Oury, T., & Reed, D. S. (2014). Aerosolized Rift Valley Fever Virus Causes Fatal Encephalitis in African Green Monkeys and Common Marmosets. *Journal of Virology*, *88*(4), 2235–2245. http://doi.org/10.1128/JVI.02341-13

Hassan, O. A., Ahlm, C., Sang, R., & Evander, M. (2011). The 2007 Rift valley fever outbreak in Sudan. *PLoS Neglected Tropical Diseases*. http://doi.org/10.1371/journal.pntd.0001229

Ikegami, T., & Makino, S. (2011). The pathogenesis of rift valley fever. *Viruses*. http://doi.org/10.3390/v3050493

Indran, S. V., & Ikegami, T. (2012). Novel approaches to develop Rift Valley fever vaccines. *Frontiers in Cellular and Infection Microbiology*, *2*. http://doi.org/10.3389/fcimb.2012.00131

Kortekaas, J., Antonis, A. F. G., Kant, J., Vloet, R. P. M., Vogel, A., Oreshkova, N., … Moormann, R. J. M. (2012). Efficacy of three candidate Rift Valley fever vaccines in sheep. *Vaccine*, *30*(23), 3423–3429. http://doi.org/10.1016/j.vaccine.2012.03.027

Le Coupanec, A., Babin, D., Fiette, L., Jouvion, G., Ave, P., Misse, D., … Choumet, V. (2013). Aedes Mosquito Saliva Modulates Rift Valley Fever Virus Pathogenicity. *PLoS Neglected Tropical Diseases*, *7*(6). http://doi.org/10.1371/journal.pntd.0002237

Leedale, J., Jones, A. E., Caminade, C., & Morse, A. P. (2016). A dynamic, climate-driven model of rift valley fever. *Geospatial Health*, *11*(1S). http://doi.org/10.4081/gh.2016.394

Mansfield, K. L., Banyard, A. C., McElhinney, L., Johnson, N., Horton, D. L., Hernández-Triana, L. M., & Fooks, A. R. (2015). Rift Valley fever virus: A review of diagnosis and vaccination, and implications for emergence in Europe. *Vaccine*. http://doi.org/10.1016/j.vaccine.2015.08.020

Métras, R., Porphyre, T., Pfeiffer, D. U., Kemp, A., Thompson, P. N., Collins, L. M., & White, R. G. (2012). Exploratory Space-Time Analyses of Rift Valley Fever in South Africa in 2008-2011. *PLoS Neglected Tropical Diseases*, *6*(8). http://doi.org/10.1371/journal.pntd.0001808

Monaco, F., Pinoni, C., Cosseddu, G. M., Khaiseb, S., Calistri, P., Molini, U., … Lelli, R. (2013). Rift Valley fever in Namibia, 2010. *Emerging Infectious Diseases*, *19*(12),

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

2025–2027. http://doi.org/10.3201/eid1912.130593

Morrill, J. C., Laughlin, R. C., Lokugamage, N., Wu, J., Pugh, R., Kanani, P., … Peters, C. J. (2013). Immunogenicity of a recombinant Rift Valley fever MP-12-NSm deletion vaccine candidate in calves. *Vaccine*, *31*(43), 4988–4994. http://doi.org/10.1016/j.vaccine.2013.08.003

Moutailler, S., Roche, B., Thiberge, J. M., Caro, V., Rougeon, F., & Failloux, A. B. (2011). Host alternation is necessary to maintain the genome stability of Rift Valley fever virus. *PLoS Neglected Tropical Diseases*, *5*(5). http://doi.org/10.1371/journal.pntd.0001156

Napp, S., Chevalier, V., Busquets, N., Calistri, P., Casal, J., Attia, M., … Bayomy, S. (2018). Understanding the legal trade of cattle and camels and the derived risk of Rift Valley Fever introduction into and transmission within Egypt. *PLoS Neglected Tropical Diseases*, *12*(1). http://doi.org/10.1371/journal.pntd.0006143

Ndiaye, E. H., Fall, G., Gaye, A., Bob, N. S., Talla, C., Diagne, C. T., … Diallo, M. (2016). Vector competence of Aedes vexans (Meigen), Culex poicilipes (Theobald) and Cx. quinquefasciatus Say from Senegal for West and East African lineages of Rift Valley fever virus. *Parasites and Vectors*, *9*(1). http://doi.org/10.1186/s13071-016-1383-y

Nfon, C. K., Marszal, P., Zhang, S., & Weingartl, H. M. (2012). Innate immune response to rift valley fever virus in goats. *PLoS Neglected Tropical Diseases*, *6*(4). http://doi.org/10.1371/journal.pntd.0001623

Nguku, P. M., Sharif, S. K., Mutonga, D., Amwayi, S., Omolo, J., Mohammed, O., … Breiman, R. F. (2010). An investigation of a major outbreak of rift valley fever in Kenya: 2006-2007. *American Journal of Tropical Medicine and Hygiene*, *83*(2 SUPPL.), 5–13. http://doi.org/10.4269/ajtmh.2010.09-0288

Nicholas, D. E., Jacobsen, K. H., & Waters, N. M. (2014). Risk factors associated with human Rift Valley fever infection: systematic review and meta-analysis. *Tropical Medicine & International Health : TM & IH*, *19*(12).

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

http://doi.org/10.1111/tmi.12385

Pittman, P. R., McClain, D., Quinn, X., Coonan, K. M., Mangiafico, J., Makuch, R. S., … Peters, C. J. (2016). Safety and immunogenicity of a mutagenized, live attenuated Rift Valley fever vaccine, MP-12, in a Phase 1 dose escalation and route comparison study in humans. *Vaccine*, *34*(4), 424–429. http://doi.org/10.1016/j.vaccine.2015.12.030

Redding, D. W., Tiedt, S., Lo Iacono, G., Bett, B., & Jones, K. E. (2017). Spatial, seasonal and climatic predictive models of Rift Valley fever disease across Africa. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1725), 20160165. http://doi.org/10.1098/rstb.2016.0165

Reed, C., Lin, K., Wilhelmsen, C., Friedrich, B., Nalca, A., Keeney, A., … Smith, D. R. (2013). Aerosol Exposure to Rift Valley Fever Virus Causes Earlier and More Severe Neuropathology in the Murine Model, which Has Important Implications for Therapeutic Development. *PLoS Neglected Tropical Diseases*, *7*(4). http://doi.org/10.1371/journal.pntd.0002156

Rippy, M. K., Topper, M. J., Mebus, C. A., & Morrill, J. C. (1992). Rift Valley Fever Virus-induced Encephalomyelitis and Hepatitis in Calves. *Veterinary Pathology*, *29*(6), 495–502. http://doi.org/10.1177/030098589202900602

Smith, D. R., Bird, B. H., Lewis, B., Johnston, S. C., McCarthy, S., Keeney, A., … Hensley, L. E. (2012). Development of a novel nonhuman primate model for Rift Valley fever. *Journal of Virology*, *86*(4), 2109–20. http://doi.org/10.1128/JVI.06190-11

Soi, R. K., Rurangirwa, F. R., McGuire, T. C., Rwambo, P. M., DeMartini, J. C., & Crawford, T. B. (2010). Protection of sheep against Rift Valley fever virus and sheep poxvirus with a recombinant capripoxvirus vaccine. *Clinical and Vaccine Immunology*, *17*(12), 1842–1849. http://doi.org/10.1128/CVI.00220-10

Sow, A., Faye, O., Ba, Y., Ba, H., Diallo, D., Faye, O., … Sall, A. A. (2014). Rift valley fever outbreak, Southern Mauritania, 2012. *Emerging Infectious Diseases*, *20*(2),

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

296–299. http://doi.org/10.3201/eid2002.131000

Styer, L. M., Lim, P.-Y., Louie, K. L., Albright, R. G., Kramer, L. D., & Bernard, K. A. (2011). Mosquito Saliva Causes Enhancement of West Nile Virus Infection in Mice. *Journal of Virology*, *85*(4), 1517–1527. http://doi.org/10.1128/JVI.01112-10

Turell, M. J., Britch, S. C., Aldridge, R. L., Xue, R. De, Smith, M. L., Cohnstaedt, L. W., & Linthicum, K. J. (2015). Potential for Psorophora columbiae and Psorophora ciliata Mosquitoes (Diptera: Culicidae) to Transmit Rift Valley Fever Virus. *Journal of Medical Entomology*, *52*(5), 1111–1116. http://doi.org/10.1093/jme/tjv093

Warimwe, G. M., Lorenzo, G., Lopez-Gil, E., Reyes-Sandoval, A., Cottingham, M. G., Spencer, A. J., … Gilbert, S. C. (2013). Immunogenicity and efficacy of a chimpanzee adenovirus-vectored Rift Valley Fever vaccine in mice. *Virology Journal*, *10*. http://doi.org/10.1186/1743-422X-10-349

Weingartl, H. M., Miller, M., Nfon, C., & Wilson, W. C. (2014). Development of a rift valley fever virus viremia challenge model in sheep and goats. *Vaccine*, *32*(20), 2337–2344. http://doi.org/10.1016/j.vaccine.2014.02.066

Wichit, S., Ferraris, P., Choumet, V., & Missé, D. (2016). The effects of mosquito saliva on dengue virus infectivity in humans. *Current Opinion in Virology*. http://doi.org/10.1016/j.coviro.2016.10.001

Wilson, W. C., Davis, A. S., Gaudreault, N. N., Faburay, B., Trujillo, J. D., Shivanna, V., … Richt, J. A. (2016). Experimental infection of calves by two genetically-distinct strains of rift valley fever virus. *Viruses*, *8*(5). http://doi.org/10.3390/v8050145

Yedloutschnig RJ, Dardiri AH, W. J. (1981). Rift Valley Fever Infection in sheep by contact exposure. *Contr. Epidem. Biostatist*, *3*, 53–59.

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

Table 1 – Experimental groups

Group	**Route of infection**	**Cells used to grow virus stock**	**Dose**
	Nubian goats		
A	Subcutaneous	Mosquito cells	10^7 pfu/ml
B	Mosquito-primed subcutaneous	Mosquito cells	10^7 pfu/ml
C	Intranasal	Mosquito cells	10^7 pfu/ml
	LaMancha goats		
D	Subcutaneous	Mosquito cells	10^7 pfu/ml
E	Intranasal	Mosquito cells	10^7 pfu/ml
F	Intranasal	Mammalian cells	10^7 pfu/ml

Peer-reviewed version available at *Viruses* **2018**, *10*, 709; doi:10.3390/v10120709

Figure Legends

Figure 1 - Sequence of RVFV strain Kenya-AUP.

(A-C) The amount of the total genome that was sequenced, with the height of the curve indicated the number of reads achieved and the length representing the basepairs of the gene. (D-F) A phylogenetic analysis of the three RVFV genome segments L, M, and S, respectively. A nucleotide (G) and amino acid (H) alignment of the three RVFV genome segments, L, M and S, respectively.

Figure 2 – Clinical signs

(A-B) Rectal temperatures were taken and recorded daily. (C-D) Each goat was examined daily for signs of illness and given a clinical score between 1 and 10.

Figure 3 - Viremia

Quantification of RVFV viremia in the Nubian (A-C) and LaMancha (D-F) goats after infection with RVFV by different routes of infection. Blue bars indicate the presence of viral RNA as measured by RT-PCR. Orange bars indicate the presence of infectious virus as measured by plaque assay.

Figure 3 – Oral and nasal shedding

Quantification of RVFV in oral (A-C) and nasal (D-F) swabs in the Nubian goats. Blue bars indicate the presence of viral RNA as measured by RT-PCR. Orange bars indicate the presence of infectious virus as measured by plaque assay.

Figure 4 – Viral load in tissues

(A) Quantification of RVFV in tissues in the Nubian goats at 1 dpi, 7 dpi and 28 dpi. (B-E) In-situ hybridization staining of spleen tissues from day 7 SQ, day 28 SQ, day 28 mosquito and day 28 IN, respectively. Arrows indicate individual dots in the slides where weak staining is present.

Figure 5 – Neutralizing antibody response

Quantification of neutralizing antibodies against RVFV in serum. The titers are given as a reciprocal dilution for the Nubian (A-B) and LaMancha (C-D) goats.