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Development of a Rift Valley fever virus viremia challenge model in sheep and goats

Hana M. Weingartl[a,b,*], Myrna Miller[c], Charles Nfon[a], William C. Wilson[d]

[a] National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, Manitoba, Canada
[b] Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada
[c] University of Wyoming, Laramie, WY, USA
[d] Arthropod-Borne Animal Diseases Research Unit, ARS, USDA, Manhattan, KS, USA

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ABSTRACT

Rift Valley fever virus (RVFV), a member of the family *Bunyaviridae*, causes severe to fatal disease in newborn ruminants, as well as abortions in pregnant animals; both preventable by vaccination. Availability of a challenge model is a pre-requisite for vaccine efficacy trials. Several modes of inoculation with RVFV ZH501 were tested on goats and sheep. Differences in development of infectious viremia were observed between animals inoculated with RVFV produced in mosquito C6/36 cells compared to Vero E6 cell-produced inoculum. Only C6/36-RVFV inoculation led to development of viremia in all inoculated sheep and goats. The C6/36 cell-produced RVFV appeared to be more infectious with earlier onset of viremia, especially in sheep, and may also more closely represent a field situation. Goats were somewhat more resistant to the disease development with lower and shorter infectious virus viremia, and with only some animals developing transient increase in rectal temperature in contrast to sheep. In conclusion, a challenge protocol suitable for goat and sheep vaccine efficacy studies was developed using subcutaneous inoculation of 10^7 PFU per animal with RVFV ZH501 produced in C6/36 cells.

1. Introduction

Rift Valley fever virus (RVFV) is a member of the family *Bunyaviridae*, genus *Phlebovirus*. This zoonotic arbovirus, endemic to Africa and Arabian Peninsula, causes acute disease in newborn ruminants with up to 100% fatality rate, as well as acute disease in pregnant animals resulting in abortion storms. Naturally infected animals develop high viremia sufficient to infect the arthropod vector, even if the infection is inapparent. The economically important affected species include sheep, goat, cattle and camel, with the primary route of infection being mosquito bites. Humans can be infected by mosquito bites, and importantly also by exposure to blood and tissues of infected ruminants during slaughter, necropsy or while assisting aborting animals [1,2].

Although the disease and development of viremia in ruminants is preventable by vaccination, and ruminant vaccination is recommended to protect human population from RVFV infections, the number of RVFV vaccines in use is limited [3,4]. Availability of a reliable challenge model is a pre-requisite for future vaccine development, registration and licensing. The clinical outcome of experimental infections of ruminants is dependent on RVFV strain used for inoculation, animal breed and age, as well as individual animal variations. The dramatically different clinical outcome of experimental infections makes vaccine evaluation difficult. There are currently two challenge models employed for vaccine efficacy trials in ruminants, both possessing inherent problems [5–8]. The abortion model is cumbersome with synchronization of the pregnancy and scheduling of high biosecurity facilities. The drawback of a viremia model can be a lack of consistency, as not all experimentally inoculated animals may develop detectable viremia [5,9–11], although sensitivity of detection may had been also an issue. For example Yedloutschnig et al. [12,13] titrated the virus inoculum for sheep and cattle inoculations in Vero cells, but used more sensitive intraperitoneal inoculation of 4–6 days old mice to detect viremia in the infected ruminants. Currently, RNA detection is used

* Corresponding author at: National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, 1015 Arlington Street, Winnipeg, MB R3E 3M4, Canada. Tel.: +1 204 789 2027; fax: +1 204 789 2038.
E-mail address: hana.weingartl@inspection.gc.ca (H.M. Weingartl).

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to compensate for the lower sensitivity of virus isolation in cell culture.

Different age animals were used in previous studies, ranging from one-day-old lambs to several years old adults. Our experimental target age was 3–4 months, when sheep and goats are usually vaccinated on farms.

Virus doses used in the inocula in the reviewed reports were of a wide range, titrated on different substrates, and therefore difficult to directly compare. Often, viremia outcome was not in correlation with the dose. This may be possibly related to individual and breed variations, and to a low number of animals used in most studies (two to four animals for the same route and dose). Overall it appears that lower doses lead to somewhat later development of viremia, delaying its detection from day one to 2–3 days post inoculation. An intraperitoneal route of inoculation was often used in the early experiments, while more recently subcutaneous route is used in majority of studies. Additional or alternative routes have been also tested, such as mucosal, intravenous, or intradermal inoculation [5–13,15,18,19].

There are very few, older publications on the experimental inoculations of goats, suggesting that the duration of viremia may be shorter than in sheep: between 1 and 3 dpi, both days inclusive [16,17]. There is one report currently published on vaccine safety in goats [20], but there are no reports on vaccine efficacy studies in goats; the second most susceptible ruminant species to Rift Valley fever virus. Recently, our group started to work on the experimental infections of goats [21], as vaccine immunogenicity, safety and efficacy testing in this target species may be also required.

The aim of this study was to develop a viremia model in goats and sheep of vaccine age (3–4 months) suitable for vaccine efficacy studies. Up to this point, the RVFV inocula were prepared using a substrate of mammalian origin, e.g. sheep and mouse serum, tissues from infected sheep and mice, or mammalian-origin cell cultures, most frequently Vero and BHK cells, regardless of the origin of the virus isolate [10–18]. To improve the infection model, virus propagated in *Aedes albopictus* cells (C6/36) was compared to virus propagated in mammalian cell line Vero E6. The outcomes of the experimental infections resulting in a proposed RVFV challenge model for vaccine evaluation are discussed.

2. Materials and methods

2.1. Cells and viruses

Vero E6 and C6/36 cells were obtained from American Tissue Culture Collection. Vero E6 cells were maintained in DMEM/10% fetal bovine serum (Wisent) at 37 °C in 5% CO_2 incubator. The C6/36 cells were maintained in 47% ESF-921 (Expression Systems)/47% EMEM/2.5% fetal bovine serum (Wisent)/2.5% HEPES (25 mM final)/1% sodium pyruvate (1 mM final)(Sigma–Aldrich) at 28 °C in sealed flasks (Corning).

RVFV, strain ZH501 [22], was kindly provided by Dr. Heinz Feldmann (National Microbiology Laboratory, Winnipeg). Passage no. 2 was transferred from National Microbiology Laboratory to National Centre for Foreign Animal Disease (NCFAD). The virus was then expanded in Vero E6 cells once, and NCFAD passage two was used in inoculations with RVFV-Vero E6. NCFAD passage two was used to prepare the RVFV-C6/36 stock for animal inoculations. The virus was sequenced at passage two in Vero E6 cells, and then at passage four (used for animal infections), and also at passage two in C6/36 cells (used in animal infections). All three genomic sequences were considered identical, also with the sequence published in GenBank for RVFV-ZH501. Both virus stocks were characterized on genomic and on protein level [21,23].

Single virus stock prepared either in Vero E6 cells or C6/36 cells was used for all respective animal inoculation experiments.

2.2. Virus plaque titration

The virus stocks, inocula and sera were plaque-titrated as follows: 400 μl/well of ten-fold serially diluted samples in DMEM were incubated on confluent monolayers of Vero E6 cells in 12 well plates in triplicates at 37 °C in 5% CO_2 for 1 h. The inoculum was replaced by 1.75% carboxymethyl cellulose (Sigma–Aldrich) in DMEM/0.3% (Wisent) supplemented with 25 mM HEPES (Sigma–Aldrich)/100 μg/ml of Streptomycin/100 IU/ml of Penicillin (Wisent), and incubated for 4 days at 37 °C, 5% CO_2. Formalin (10%) fixed plates were stained with crystal violet (0.5% (w/v) in 80% methanol in PBS), and virus titer determined in PFU/ml.

2.3. Virus detection

Serum samples were simultaneously analyzed by virus isolation using plaque titration as described above to determine viremia, and by real time RT-PCR to determine virus RNA load.

2.4. One-step real-time RT-PCR

RNA isolation from serum using TriPure (Roche Diagnostics) according to manufacturer's instructions was followed by one-step real time RT-PCR targeting the L gene [9].

2.5. Antibody detection

Virus neutralizing antibodies were determined by plaque reduction neutralization assay as described previously [21] on Vero E6 cells using virus produced in Vero E6 cells.

2.6. Animals

All animals in this study were 4 months old at the time of inoculation. Sheep (Suffolk cross, Rideau Arcott cross, Ile-de-France cross with Rideau Arcott) and goats (Alpine-Boer cross) were obtained from breeders in Manitoba. All animal manipulations were approved by the Animal Care Committee of the Canadian Science Centre for Human and Animal Health in compliance with the Canadian Council on Animal Care guidelines (Animal Use Documents #C-08-007, #C-09-004, #C-10-001, #C-11-011). The work with infected animals was performed under containment level 3 conditions (zoonotic BSL-3 Ag).

2.7. Experimental design

Animals were acclimatized for two weeks prior to inoculation and inoculated subcutaneously (SC) with 1 ml of RVFV (ZH501) into the right side of the neck, and if applicable re-inoculated SC or intravenously (IV) depending on the inoculation group. Two doses were compared: "low" dose of 10^5 PFU per animal and "high" dose of 10^7 PFU per animal. Rectal temperatures were taken for three days following arrival of the animal to the facility and for minimum of five days prior to inoculation, and daily during the first week post inoculation. Except for the first group (sheep group A; see below), blood was collected daily for up to 6 or 7 days post inoculation (dpi). At this time point animals were either euthanized to determine virus presence in liver and spleen, or were kept up to 35 dpi for serum production, and bled weekly to follow antibody development (not reported in this manuscript). Overview of the inoculation groups is provided in Table 1. Where it was possible to group animals to compare two experimental approaches, Student's

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