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Heat stability of the Rift Valley Fever Virus Clone 13 live vaccines

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ABSTRACT

Rift Valley Fever (RVF) is an emerging zoonotic disease present in sub-Saharan Africa and the Arabian Peninsula. Vaccination of cattle against RVF with a RVF virus clone 13 (CL13) strain has proven to be efficacious, and avoids the side effects caused by other available live vaccines. In order to determine the temperature stability of the CL13 vaccine, lyophilized and liquid forms were tested and titrated for the presence of live virus after storage for various time periods at various temperatures. Results showed that the virus could be stored lyophilized at 4 °C for more than 12 months, with no reduction of infectivity. However, the vaccine was shown to be unstable at room temperature and at 37 °C in both lyophilized and liquid forms. This data shows that the CL13 vaccine is highly reliant on a cold chain, emphasizing the need for the vaccine to be made thermostable in order to allow for efficient vaccine storage and delivery in endemic tropical countries.

Introduction

Rift Valley Fever Virus (RVFV) belongs to the family of *Bunyaviridae*, genus *Phlebobirus* [1]. It was first identified in 1931 in Kenya after isolation from a sheep in the Rift Valley [2]. More than 40 species of mosquitoes (primarily the genus *Aedes*, *Culex*, *Anopheles*) are likely to transmit the virus [3].

RVF infection is usually unapparent in humans, but can be associated with a moderate to severe non-fatal influenza-like illness [4,5]. In some cases the virus can however be lethal for humans and it results in major losses in the livestock industry. RVFV causes disease in camels [6], sheep, cattle and goats [2]. The disease in these species is characterized by high rates of abortion, high levels of mortality in neonates and hepatic necrosis. Mortality in adult cattle and sheep is 10% and 20% respectively [7]. However, the mortality in neonatal sheep and spontaneous abortion rates in pregnant ewes are close to 100% [8].

The first vaccine for RVF was developed in South Africa by attenuation of a field isolate (Smithburn) by serial passages in mouse brains [9]. This live vaccine has the advantage of inducing early and long-term immunity after a single injection [10]. Its use is however not recommended in the early stages of pregnancy in ewes due to residual virulence [11,12], as it is reported to induce a low percentage of abortions and stillbirth [13]. There is also a commercial inactivated vaccine available which is favored for use in non-endemic areas and during disease outbreak situations; however this vaccine is expensive and requires an initial course of two vaccines and then annual revaccination for optimal protection [14,15].

RVFV Clone 13 (CL13) is a natural live attenuated RVFV mutant, which was isolated from a non fatal human case of RVF [16]. The CL13 has a large deletion in the non-structural protein coded by the S segment (NSs), which has been identified as a virulence factor [12]. An evaluation of efficacy and safety of the CL13 vaccine in ewes at different stages of pregnancy indicated that the vaccine did not induce clinical manifestation of RVF such as abortion in pregnant ewes, teratogeny in their offspring, or pyrexia in vaccinated animals. Vaccination with CL13 vaccine also prevented clinical RVF following virulent challenge [17,18].

The objectives of this study were therefore to measure the stability of the CL13 vaccine strain, in both a lyophilized and liquid form, at various temperatures. Knowing this information is

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essential in order to allow for efficient vaccine storage and delivery in endemic tropical countries.

Material and methods

Virus growth and titration

The CL13 was passaged on baby hamster kidney cells (BHK) grown in DMEM (Dulbecco's modified Eagle's) medium with 10% calf serum. The virus was inoculated with a multiplicity of infection (MOI) of 0.01 in 25 cm^2 flasks containing confluent layer cells and incubated at 37 °C, after a 45 min of adsorption, the medium was added and flasks incubated 6 days at 37 °C. Every 24 h, a sample of supernatant (representing extracellular virus) was removed from the flasks and titrated for virus infectivity. Every 24 h one flask was frozen at −80 °C and titrated for virus after thawing (representing intracellular and extracellular virus).

Titration for virus was performed on BHK cells by making tenfold dilutions of the virus in medium. Each dilution (100 µl) was transferred to 6 wells of a micro-titer plate, and 150 µl of cell suspension was added to each well. Plates were incubated for 4 days at 37 °C in 5% CO_2. The highest dilution causing cytopathic effect in inoculated cells in 50% of the wells was calculated and expressed as TCID 50/ml following the method described by Reed and Muench temperature stability studies:

The virus was grown to a known titer. One aliquot was lyophilized and a second aliquot was stored in the 'wet' form.

Aliquots of the 'wet' virus were stored and titrated as follows:

1. At −80 °C, with aliquots of virus titrated at monthly intervals for 18 months.
2. At +4 °C, with aliquots of virus titrated every week for 9 weeks.
3. At room temperature (22–25 °C), with aliquots of virus titrated every 2 days for 10 days.
4. At 37 °C, with aliquots of virus titrated every 12 h for 96 h.
5. At 45 °C, with aliquots of virus titrated every 30 min for 3 h.
6. At 56 °C, with aliquots of virus titrated every 10 min for 2 h.

Aliquots of the lyophilized virus were stored and titrated as follows:

1. At +4 °C, with aliquots of virus titrated every month for 12 months.
2. At room temperature (22–25 °C), with aliquots of virus titrated every day for 7 days.
3. At 37 °C, with aliquots of virus titrated every day for 7 days.

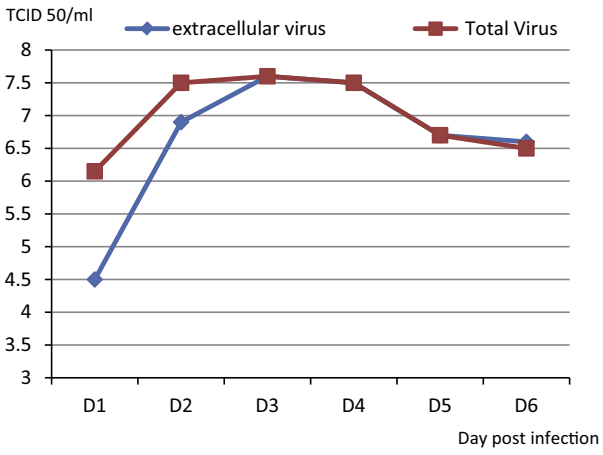

Fig. 1. Kinetics of viral multiplication (RVFV CL13) in BHK cells.

Results and discussion

Kinetics of CL13 growth

The maximum titer of virus ($10^{7.6}$/ml) was obtained at 3 dpi (Fig. 1). The titer of virus then decreased progressively up to 6 dpi, when it had a titer of $10^{6.5}$/ml. A difference was observed between the titer of the extracellular and the total virus during the first and second day of infection, with the total viral titer being higher than the titer of the extracellular virus (Fig. 1).

Temperature stability studies

The temperature stability studies carried out on both lyophilized and 'wet' forms of the CL13 virus revealed that:

- At −80 °C, the virus remained stable for at least 18 months. The titer dropped by 0.4 log (from $10^{6.5}$–$10^{6.1}$) in the first 6 months and then stabilized at $10^{6.1}$ /ml for more than 12 months.
- At +4 °C, the liquid form of the virus lost all its infectivity within 2 months, however the lyophilized form remained stable, with no significant reduction in viral titer being observed, for more than 12 months (Fig. 2).
- At room temperature (22–25 °C), the liquid form of the virus lost all its infectivity by day 11 of storage and the titer of the lyophilized form of the virus dropped by 1.2 logs (from $10^{5.5}$ to $10^{4.3}$) within 7 days of storage (Fig. 3). At 37 °C, the liquid and lyophilized form of the virus lost all infectivity by 2 and 7 dpi respectively (Fig. 3).
- The CL13 lost all its infectivity in 80 min at 56 °C and the titer of virus dropped by 1 log in 3 h at 45 °C (Fig. 4).

Infection of mammalian cells with RVFV generally leads to the production of virus followed by cell death. Billecocq and all reported that, when confluent cell cultures were infected with CL13, the peak of virus production occurred after 2 or 3 dpi, depending on the multiplicity of infection (MOI). Viral replication was associated with cells rounding up, detaching from the plate and dying after 5–6 days [16]. The same result was shown in our study, confirming that the maximum titer of the virus was obtained at 3 dpi in BHK cells and complete lyses of the infected cells occurred by 6 dpi.

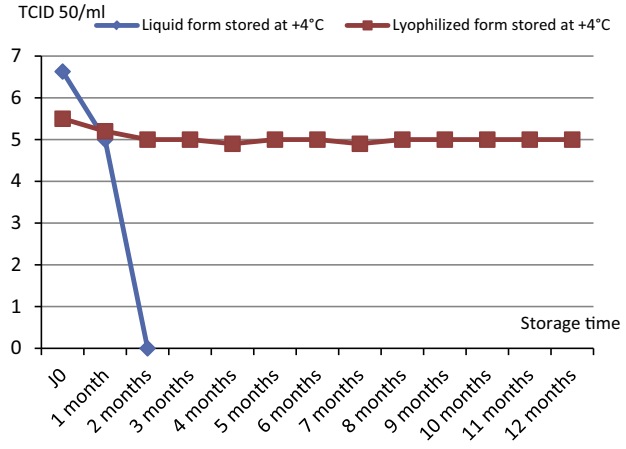

Fig. 2. Temperature stability of RVFV CL13 at 4 °C in the liquid and lyophilized form.