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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.

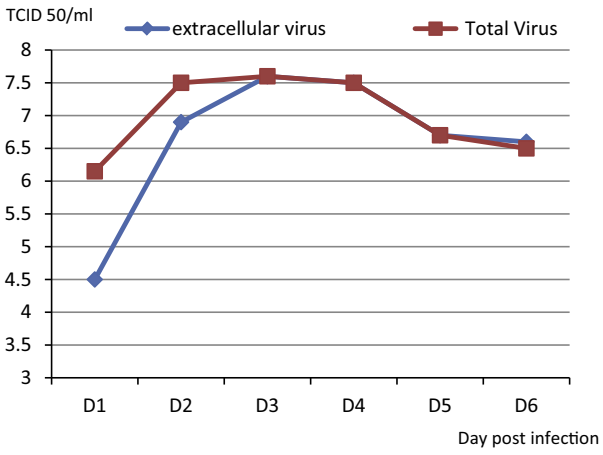

Fig. 1. Kinetics of viral multiplication (RVFV CL13) in BHK cells.

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.

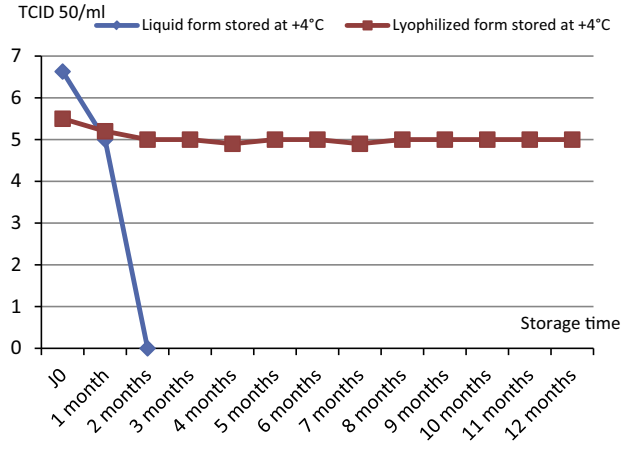

Fig. 2. Temperature stability of RVFV CL13 at 4 °C in the liquid and lyophilized form.

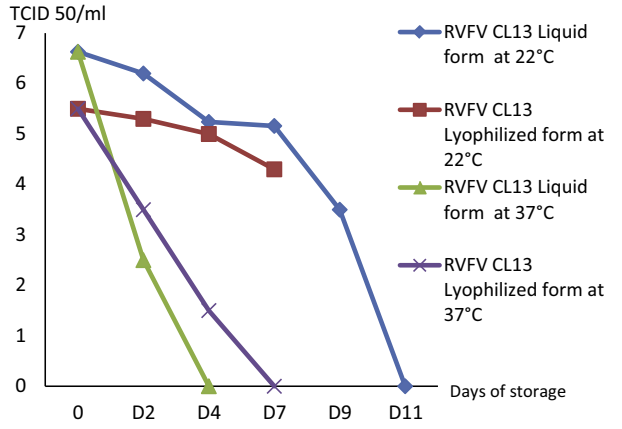

Fig. 3. Temperature stability of RVFV CL13 in a liquid and lyophilized form at room temperature (22–25 °C) and at 37 °C.

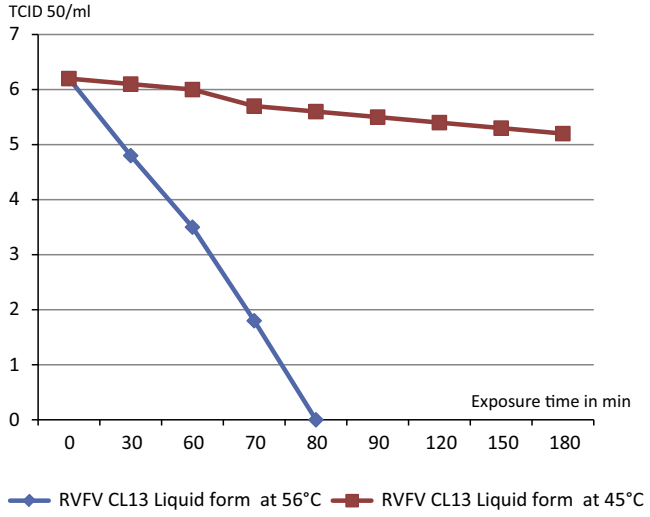

Fig. 4. Temperature stability of the RVFV CL13 in a liquid form at 45 °C and 56 °C.

Fundamental problems associated with the preservation of live vaccines relate to the potential drop of virus titer during lyophilization, improper storage conditions and/or an inability to maintain the cold chain during the process of vaccine delivery. RVF is an important zoonotic emerging disease and currently very few good quality vaccines have been developed to control and prevent its spread. CL13 is probably the safest and efficacious RVFV candidate vaccine strain currently available [12], however nothing is known about the temperature stability of this vaccine virus in both lyophilized and liquid forms. This is the first published study that analyzes the temperature stability of the CL13 vaccine when stored for various time periods at various temperatures in a liquid or lyophilized form.

Standard recommendations for the storage of similar live vaccines would be to store the liquid form at −80 °C and the lyophilized form at +4 °C. This study revealed that the CL13 virus maintained its initial viability when stored at −80 °C for at least 18 months. Another study also found that −70 °C was the temperature of choice for storage of the RVFV van Wyk strain [17].

This study also showed that there was no loss of infectivity of the lyophilized CL13 when stored at +4 °C for up to 12 months. The lyophilized Smithburn RVFV vaccine has an expiry date of 2 years when stored at −20 °C [19]. Another RVFV live-attenuated vaccine candidate (MP-12 strain) was developed in the 1980s by the US Army Medical Research Institute of infectious Diseases (USAMRIID) [20]. This vaccine however proved to be highly teratogenic, causing fetal pathology between day 35 and 52 of pregnancy, in ewes vaccinated at different stages of pregnancy [21]. However, the new recombinant RVFV MP-12 with NSm deletion was shown to be safe in pregnant sheep causing neither abortion nor fetal malformation [22]. It has also been shown that the lyophilized MP-12 strain is stable when maintained cold, and retains full potency following storage at −30 °C [23].

At room temperature (22–25 °C), in the liquid form, no live CL13 virus was detected after 10 days storage and when the liquid form of the virus was stored at 37 °C, its infectivity disappears completely by the fourth day of storage. Storage of the lyophilized form of the virus at 37 °C resulted in a complete loss of infectivity by 7 days of storage. The stability of the RVFV Smithburn strain was tested at 37.5 °C and after 5 days of storage at this temperature no viral infectivity remained. At 24 h there was a 10-fold decrease in the titer; by 48 h there was a 10,000-fold decrease in the titer and after 5 days there was no demonstrable infectious virus [24].

Complete inactivation of CL13 was achieved in 80 min at 56 °C and the titer of virus dropped one log after 3 h at 45 °C. When Vero cells were infected with RVF viruses, the virus was completely inactivated after heating for 1 h at 60 °C [25].

Conclusion

This study set out to measure the temperature stability of the CL13 vaccine strain when stored for various time periods at various temperatures in a liquid or lyophilized form. The CL13 maintained its viability for more than 18 months when stored at -80 °C in liquid form and for at least 12 months when stored at +4 °C in the lyophilized form. However, the CL13 virus was shown to be unstable at room temperature (22–25 °C), and at 37 °C, in both lyophilized and liquid forms. Complete inactivation of the virus was reached after only 80 min at 56 °C.

This data raises issues about the risks associated with using the CL13 vaccine in tropical (hot) countries without strict maintenance of the cold chain during vaccine storage and delivery. It is clear that the CL13 strain is a promising and effective vaccine when used to control for RVF, and that the vaccine has reduced side effects when compared to other available RVF live vaccines, such as the Smithburn vaccine. However, efforts to improve the thermostability of this vaccine strain are needed in order to optimize its efficacy when used in the tropical climates in which RVFV is currently circulating.

References

[1] F.A. Murphy, C.M. Fauquet, D.H.L. Bishop, S.A. Ghabrial, A.W. Jarvis, G.P. Martelli, M.A. Mayo, M.D. Summers, Family Bunyaviridae, in: The International Committee on Taxonomy of Viruses (Ed.), Sixth Report. Virus Taxonomy. Classification and Nomenclature of Viruses, Springer-Verlag, Wien, 1995, pp. 300–315.
[2] R. Daubney, J.R. Hudson, P.C. Garnham, Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep, cattle and man from East Africa, J. Pathol. Bacteriol. 34 (1931) 545–579.
[3] S. Moutailler, G. Krida, F. Schaffner, M. Vazeille, A.B. Failloux, Potential vectors of Rift Valley fever virus in the Mediterranean region, Vector Borne Zoonotic Dis. 8 (2008) 749–754.
[4] B.M. Macintosh, D. Russel, I. Dos Santos, J.H.S. Gear, Rift Valley fever in humans in South Africa, S. Afr. Med. J. 58 (1980) 803–806.
[5] G.F. Meadors, P.H. Gibbs, C.J. Peters, Evaluations of a new Rift Valley fever vaccine: safety and immunogenicity trials, Vaccine 4 (1986) 179–184.
[6] G.R. Scott, W. Coackley, R.W. Roach, N.R. Cowdy, Rift Valley fever in camels, J. Pathol. Bacteriol. 86 (1963) 229–231.
[7] B.C. Easterday, Rift Valley fever, Int. J. Adv. Vet. Sci. 10 (1965) 65–127.
[8] Coetzer, The pathology of Rift Valley fever. I. Lesions occurring in naturalcases in new-born lambs, Onderstepoort J. Vet. Res. 44 (4) (1977) 205–211.

[9] K.C. Smithburn, Rift Valley fever: the neurotropic adaptation of the virus and the experimental use of this modified virus as a vaccine, Brit. J. Exp. Pathol. 30 (1 (February)) (1949) 1–16.
[10] F. Assad, F.G. Davies, G.A. Eddy, The use of veterinary vaccines for prevention and control of Rift Valley fever, Bull. World Health Organ. 61 (1983) 261–268.
[11] B.J.H. Barnard, Rift Valley fever vaccine antibody and immune response in cattle to a live and an inactivated vaccine, J. S. Afr. Vet. Assoc. 50 (1979) 155–157.
[12] R. Muller, J.-F. Saluzzo, N. Lopez, T. Dreier, M. Turell, J. Smith, et al., Characterization of Clone 13, a naturally attenuated avirulent isolate of Rift Valley fever virus, which is altered in the small segment, Am. J. Trop. Med. Hyg. 53 (1995) 405–411.
[13] P. Hunter, M. Bouloy, Investigation of Clone 13 Rift Valley fever mutant as a vaccine strain, <http://www.up.ac.za/academic/lhpg/vi/hunter.htm> (accessed February 2013).
[14] B. Dungu, I. Louw, A. Lubisi, P. Hunter, B.F. von Teichman, M. Bouloy, Evaluation of the safety and efficacy of the Rift Valley fever Clone 13 vaccine in sheep, Vaccine 28 (29) (2010) 4581–4587.
[15] J.A.W. Coetzer, B.J.H. Barnard, Hydrops amnii in sheep associated with hydranencephaly and arthrogryposis with wesselsbron disease and rift valley fever viruses as aetiological agents, Onderstepoort J. Vet. Res. 44 (1977) 119–126.
[16] A. Billecocq, P. Vialat, M. Bouloy, Persistent infection of mammalian cells by Rift Valley fever virus, J. Gen. Virol. 77 (Pt 12 (December)) (1996) 3053–3062.
[17] V.R. Kaschula, The propagation and modification of strains of Rift Valley fever viruses in embryonated eggs and their use as immunizing agents for domestic ruminants, Thesis Doctor of Veterinary Science, University of Pretoria, South Africa, 1953.
[18] OIE, Rift Valley Fever. OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, fifth ed., vol. 1, Office International des Epizooties, 2008, pp. 323–333 (chapter 2.1.14) <http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.14_RVF.pdf>.
[19] VSVRI, Rift Valley Fever Attenuated Vaccine for Veterinary use only, 2011 Egyptian Veterinary Serum and Vaccine Research Institute, <http://www.vsvrieg.com/ Products.htm>.
[20] H. Caplen, C.J. Peters, D.H. Bishop, Mutagen-directed attenuation of Rift Valley fever virus as a method for vaccine development, J. Gen. Virol. 66 (Pt 10) (1985) 2271–2277.
[21] P. Hunter, B.J. Erasmus, J.H. Voster, Teratogenicity of a mutagenized Rift Valley fever virus (MVP12) in sheep, Onderstepoort J. Vet. Res. 69 (2001) 95–98.
[22] H.M. Weingartl, C.K. Nfon, S. Zhang, P. Marszal, W.C. Wilson, J.C. Morrill, G.E. Bettinger, C.J. Peters, Efficacy of a recombinant Rift Valley fever virus MP-12 with NSm deletion as a vaccine candidate in sheep, Vaccine (2014). pii: S0264-410X(13).
[23] FAO, Rift Valley Fever Vaccine Development, Progress and Constraints, Food and Agriculture Organization of the United Nations, Rome, 2011. <http://www.fao.org/docrep/014/i2310e/i2310e00.pdf>.
[24] C.A.C. Mims, Rift Valley fever virus in mice. II. Adsorption and multiplication of virus, Brit. J. Exp. Pathol. 37 (1956) 110–119.
[25] J.F. Saluzzo, B. Leguenno, G. Van der Groen, Use of heat inactivated viral haemorrhagic fever antigens in serological assays, J. Virol. Methods 22 (2–3 (December)) (1988) 165–172.