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A thermostable presentation of the live, attenuated *peste des petits ruminants* vaccine in use in Africa and Asia

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ABSTRACT

The research objective was to develop a thermostable vaccine against peste des petits ruminants (PPR), a morbilliviral disease of small ruminants targeted for eradication that is a major constraint on the livelihoods of the rural poor throughout much of Africa and Asia. Although existing PPR vaccines provide lifelong immunity, they require continuous refrigeration. This limits their utility in developing countries. Methods for the lyophilization of a related morbillivirus, rinderpest (RP), resulted in vaccine that could be used in the field for up to 30 days without refrigeration which was a major contribution to the global eradication of RP completed in 2011. The present research applied the rinderpest lyophilization method to the attenuated Nigeria 75/1 PPR vaccine strain, and measured thermostability in accelerated stability tests (AST) at 37 °C. The shelf-life of the vaccine was determined as the time a vial retained the minimum dose required as a 25-dose presentation at the specified temperature. A lactalbumin hydrolysate and sucrose (LS) stabilizer was compared to stabilizers based on trehalose. PPR vaccine produced using the Xerovac drying method was compared to vaccine produced using the rinderpest lyophilization method in AST. LS vaccine was evaluated in AST at 37, 45 and 56 °C and an Arrhenius plot was constructed for estimation of stability at temperatures not tested. Vaccines produced using LS and the rinderpest method of lyophilization were the most stable. The shelf-life of the Xerovac preparation was 22.2 days at 37 °C. The three LS vaccine batches had shelf-lives at 37 °C of 177.6, 105.0 and 148.9 days, respectively, at 37 °C. At 56 °C, the shelf-life was 13.7 days. The projected half-life at 25 °C was 1.3 years. This is sufficient thermostability for use without a cold chain for up to 30 days which will greatly facilitate the delivery of vaccination in the global eradication of PPR.

1. Introduction

Peste des petits ruminants (PPR) is a highly contagious, acute viral disease that primarily affects of domestic small ruminants [6] associated with high mortality and severe socio-economic impact. The disease is caused by the virus of the genus *Morbillivirus*, which includes rinderpest (RP), measles, and canine distemper and the phocid distemper viruses. The clinical symptoms associated with the disease in small ruminants are pyrexia, oculo-nasal discharge, stomatitis, pneumonia and diarrhoea. The apparent range of PPR has expanded in recent years to include parts of North Africa, sub-Saharan Africa as far south as Zambia, the Middle East, Central and South Asia [3]. In late 2013, the disease entered China for the second time with 244 outbreaks from across China reported to the World Animal Health Organization by June 2014 [18,29].

Small ruminants play an important role in the livelihoods of many livestock economies. They play a greater role in household food security than large ruminants and are more easily marketed to meet immediate cash needs. PPR is often ranked as one of the top two or three disease constraints to small ruminant production. International recognition of the pivotal role of PPR in the livelihoods of the poor has led to increasing recognition of the need for a globally coordinated eradication program [16]. Lessons from the global eradication of RP completed in 2011, a close relative of PPR, suggest that PPR eradication is an achievable and appropriate goal [2,13] The international animal health community launched the PPR Global Control and Eradication Program on April 1, 2015.

The principal method for the control of PPR is vaccination. Historically, the Plowright RP vaccine [20] was fully efficacious against PPR [27,14] and was widely used until the final stages of rinderpest eradication. The Plowright RP vaccine was fully protective against all strains of RP and PPR, was never noted to cause any adverse

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reactions and resulted in life-long immunity against either disease [19]. A single 50% tissue culture infective dose ($TCID_{50}$) was immunogenic. Delivery was constrained by the need for a strict cold chain, however this was overcome by the development of a thermostable production process [15] that greatly facilitated the eradication of rinderpest from the remote areas of Africa [22]. With the eradication of rinderpest, international norms now prohibit the production and use of RP vaccines.

The first homologous PPR vaccine was developed using an attenuated African strain of PPR virus designated Nigeria 75/1 [5]. Subsequently, two live attenuated vaccines were developed based on strains of Indian origin [23,26]. The performance of the Nigeria 75/1 vaccine is fully analogous to the Plowright RP vaccine: it protects against all lineages of PPR virus, has not been associated with an adverse reaction and has a duration of immunity of at least three years which is essentially life-long in small ruminants. The minimum dose recommended by the World Organization for Animal Health, 2.5 $\log_{10}$ $TCID_{50}$, is based on the results of a parallel titration in goats and cell culture [17] that found that a dose of approximately 1 $TCID_{50}$ is protective. This remarkable finding is equivalent to the results obtained for the Plowright RP vaccine. Vaccine based on the Nigeria 75/1 strain has been widely used throughout Africa, the Middle East and parts of Asia.

Given the considerable advantages provided by a thermostable vaccine in the RP campaign, the initial aim of this work was to adapt and validate the RP thermostabilization method to use with PPR. A second aim was to compare the thermostability of PPR vaccines produced using the method used in the manufacture of the thermostable RP vaccine with candidate PPR technologies described in the intervening years. The candidate methods were the 'Xerovac' anhydrobiotic approach to preserving PPR vaccine [28] and the use of trehalose as a stabilizer component in lyophilized vaccines. Representative lots of PPR vaccine were produced using the rinderpest method, trehalose stabilizers and the 'Xerovac' method and compared in accelerated stability tests [1].

2. Materials and methods

2.1. Viruses and cells

A working seed was produced from the first passage of PPR Nigeria 75/1, LK6 vero 75(14/02/1997) vaccine seed provided by CIRAD. The vaccine virus was propagated on Vero cells (ATCC CCL-81) between 1 and 38 passage levels and cultivated in Eagle's minimum essential medium (E-MEM) supplemented with 10% fetal bovine serum (Hyclone), gentamycin and L-glutamine.

2.2. Vaccine stabilizers

Three different vaccine stabilizers were used: lactalbumin hydrolysate (LAH) and sucrose (LS) [21] trehalose dehydrate alone (TD) [28] and LAH and trehalose dehydrate (LT) were compared in this study. The final concentrations achieved when the stabilizer and viral harvest were 2.5% LAH and 5% for the two sugars.

The LS stabilizer was prepared as 5% LAH and 10% sucrose in Hank's balanced salt solution (HBSS), pH 7.2; and TD was used at 10% in distilled water, LT was prepared as 5% LAH and 10% trehalose dehydrate. These were added 1:1 to the viral harvest. In Batches 4 and 5, a more concentrated LS stabilizer was prepared as 12.5% LAH and 25% sucrose mixed in the ratio of 1:4 with the virus supernatant.

2.3. Preparation of the PPR vaccine

Vero cells were seeded into T-150 culture flask (162 cm^2) at a concentration of 0.4×10^6 cells/ml in 30 ml E-MEM. The seeded cells were immediately infected with 100 μl of a reconstituted PPR N75/1 vaccine giving at a multiplicity of infection of approximately 0.0001. Infected cells were incubated at 37 °C incubator with 5% CO_2. Viral harvests were made when 90% cytopathic effect was evident at approximately 5 days post inoculation. The virus-cell suspension was produced by addition of sterile glass beads and agitation to detach the cell monolayer. The suspensions were pooled with stabilizer in sterile bottles and frozen at −80 °C until lyophilization.

2.4. Lyophilization

Lyophilization was carried out using Lyomax freeze-drier. Stoppers were sterilized by autoclaving followed by drying for 4 h in a hot air oven at 141C. Five ml tube glass vials with a 1 ml fill of stabilized vaccine mixture were used for all vaccine preparations.

The lyophilization protocol followed the method used in the manufacture of thermostable RP vaccine [15]. The vaccine vials were first chilled to −45 °C in a span of 1 h and maintained at that temperature for another 2 h. For Batch 1, the temperature was then brought to −30 °C in duration of one hour and a vacuum set point of 100 mT. Primary drying was conducted at that shelf temperature and pressure for an additional 16 h. The shelf temperature was then raised to 0 °C over 8 h. Upon completion of the ramp to 0 °C, maximum vacuum (~25 mT) was drawn and maintained for the remainder of the cycle. The shelf temperature was maintained at 0 °C and for another 18 h. This was followed by ramping the shelf temperature 25 °C over 8 h and maintaining 25 °C for another 18 h. The shelf temperature was then increased to a final temperature of 35 °C over 2 h and maintained at 35 °C for 4 h. Stoppering was done under dry nitrogen. Batches 2 through 5 were lyophilized in the same manner except the shelf temperature and vacuum level during the primary drying step were set to −34 °C and 80 mT, respectively.

For Batch 2, the LS and LT formulations were prepared from the same viral harvest and lyophilized in the same run. The LS and LD formulations in Batch 3 were prepared also used one harvest and were lyophilized simultaneously.

2.5. Xerovac procedure

Xerovac RP was produced in accordance with the published method [28] for primary and secondary drying with assistance of a Lyomax technician to ensure appropriate operation of the lyophilizer in compliance with the protocol. The liquid vaccine was placed on the shelf and a partial vacuum gradient was created from the shelf to the condenser. During primary drying, evaporation cooling chilled the liquid vaccine which was observed to foam resulting in a product with the typical Xerovac appearance of a foam matrix.

2.6. Accelerated stability tests

Accelerated stability tests [1] were conducted at 37 °C on all batches using the same protocol as was applied to RP vaccine [15]. Briefly, the vaccine was placed in a 37 °C incubator and sampled on days 0, 3, 7, 10, 14, 21, 28, 35, 42, 56, 70, 84, 98, 122, 140, 168, 196, 224, 252 and 280. In addition, Batch 5 was tested at 45 °C and 56 °C. The sampling points in the 45 °C test were days 0, 2, 4, 6, 8, 10, 12, 14, 17, 21, 24, 28, 35, 42, 49, 56, 63, 70, 77 and 84. The 56 °C test utilized a water bath rather than an incubator and the vaccine was sampled on days 0, 2, 4, 6, 8, 10, 12, 14, 17, 21, 24 and 28.

2.7. Virus titration

Virus titrations were conducted in Vero cells in a 96 well microtitre plates. Ten-fold serial dilutions of samples in E-MEM contain-

ing 10% fetal bovine serum were prepared. Six replicates per dilution were plated using 25 μl of virus suspension and 100 μl of cell suspension (10^6 cells/ml) per well. All the test plates were incubated at 37 °C in a humidified incubator with 5% CO_2. Titrations were read on day 7.

Two vials were titrated per time point and each vial was titrated twice to give four titrations values per time point. Titrations were done sequentially by time point until the end of the sample series was reached or the titer fell below 3 $\log_{10}$ $TCID_{50}$ per vial.

2.8. Analysis of results

The results were analyzed using the 2-component degradation model developed for measles by Allison [1]. This method divides the degradation into a period of rapid initial loss followed by a period of more gradual linear decay. The linear decay of the second component was analyzed by standard regression analysis in Excel to estimate the slope of the decay or degradation constant (k) and intercept. As a measure of the first component, the rapid initial loss was taken as the difference between the intercept of the second component and the initial titer of the vaccine on day 0 [15].

The half-life was calculated as the time taken to lose 0.3 $\log_{10}$ $TCID_{50}$ based on the degradation constant k:

$$(0.3\log_{10}\mathrm{TCID}_{50})/k$$

The *corrected shelf life* was calculated as the time taken for the titer to fall from the value of the intercept of the linear component to the minimum titer per vial. Assuming a 25-dose pack size and a minimum required titer of 2.5 $\log_{10}$ $TCID_{50}$ per dose, the required titer per vial was 3.9 $\log_{10}$ $TCID_{50}$. Thus, the formula for the *corrected shelf life* was:

$$\text{Shelf-life} = (\text{Intercept} - 3.9\log_{10}\mathrm{TCID}_{50})/k$$

The relationship between the three degradation constants estimated for Batch 5 at 37, 45 and 56 °C was analyzed using the Arrhenius equation:

$$\log_{10}(k) = -(\Delta\mathrm{Ha}/2.303\mathrm{R})(1/T)$$

where ΔHa is the heat of activation, R is the gas constant and T is the temperature at which the degradation test were conducted expressed in degrees Kelvin.

Essentially, the temperature data are transformed to an inverse Kelvin scale and the degradation constants to the log scale to give a linear relationship, the Arrhenius plot. Regression analysis is completed and the slope (K) and intercept allow estimates of corrected k for the temperatures at which the experiments were conducted, interpolation of the degradation constants at intermediate temperatures not tested and extrapolation to lower temperatures where the same underlying degradative processes can reasonably be assumed [8,1].

3. Results

The accelerated stability test results at 37 °C are summarized in Table 1 and the degradation curve for each individual batch is illustrated in Figs. 1–5. The degradation curves for the lyophilized vaccines exhibited a biphasic degradation consisting of a period of rapid initial loss followed by a period of more gradual linear decline for all lyophilized vaccines. The Xerovac degradation exhibited a straight linear decline.

The LS stabilized vaccine in batches 1, 4 and 5 had shelf lives at 37 °C of 177.6, 105.0 and 148.9 days, respectively. The averages of the shelf-lives of these three batches at 37 °C as 25, 50 or 100 dose presentations were 143.8, 98.5 and 53.8 days, respectively.

In the comparisons of LAH-sucrose and trehalose without LAH stabilized vaccines lyophilized in the same run, the degradation constants 'k' were found to be equal (0.0196) but the initial losses in the first phase were larger for the trehalose preparations. In the case of the LAH-sucrose and LAH-trehalose comparison, the 'k' constant was larger for the LAH-trehalose (0.0108 vs. 0.0140, respectively) and the initial loss in the first component of the degradation was also larger. The estimated shelf lives at 37 °C of the two trehalose preparations with and without LAH was 0 days as 25 dose presentations.

The degradation constant 'k' of the Xerovac lot was 0.0810 and the shelf life at 37 °C of a 25-dose presentation was 22 days. The final titer of the product at day 21 at 37 °C was 4.1 $\log_{10}$$TCID_{50}$.

The results of the testing of Batch 5 at 45 and 56 °C and the Arrhenius analysis are presented in Table 2 and Fig. 6. At 56 °C, the degradation constant was −0.1177, the shelf life for a 25-dose vial was 13.7 days and the half-life was 2.4 days. The projected half-life at 25 °C was 1.3 years.

4. Discussion

This goal of this work was to compare different approaches to thermostabilizing PPR vaccine and document PPR vaccines that were suitable for commercialization and use without a cold chain. Thermostability is a relative term referring the rate of degradation as a function of temperature. Virtually all substances degrade and degradation or change is accelerated by increased temperature. For the purposes of this discussion we define thermostability as the ability of the product to retain the required minimum dose at ambient temperatures for a period of time that facilitates practical fieldwork without a cold chain. In the case of rinderpest, 30 days was found to be sufficient for teams to work on the field without a cold chain. Average ambient temperatures rarely exceed 25 °C in any inhabited regions on earth. Daily temperatures in some locations can reach 45 to 50 °C. The product must be able to resist average ambient temperature and temperature fluctuations for a defined period with a wide margin of safety.

The World Health Organization defines the half-life of a vaccine as the time taken to lose half of its original potency [7]. This definition assumes simple linearity. Given the evidence of biphasic degradation processes for formulations of measles, RP and now PPR, more explicit measures are appropriate. In this paper, half-life has been defined as the time taken for loss of one-half of the vaccine's potency (0.3 log10 TCID50) based on the degradation constant and independent of original potency. This quantity is more reproducible and allows comparison of the relative stability of different formulations that is independent of the initial batch conditions and takes into account the shape of the degradation curve.

The method used in the production of thermostable rinderpest vaccine resulted in a vaccine with a shelf life of up to 150 days at 37 °C when the full protocol was implemented. The vaccine also resisted temperatures of up to 56 °C for 13.7 days. This level of thermostability is on the same order of magnitude as that obtained for rinderpest (up to 240 days) and is sufficient for use of the vaccine without a cold chain for up to 30 days.

The thermostable vaccine resulted from producing vaccine with a good initial titer and drying it to low residual moisture in a manner that fully preserves the stable frozen structure of the vaccine. The principal technical requirements are: 1) a strong cell and virus production system that results in harvest titers greater than 6 $\log_{10}$ $TCID_{50}$/ml, 2) a harvest procedure that minimizes loss of titer, and 3) freezing the product to below its eutectic point and conducting the freeze-drying process in a slow, gentle manner that maintains the product temperature below its eutectic point during the differ-

Table 1
Summary of accelerated stability test results at 37 °C for all vaccine batches and formulations.[a]

Batch	Stabilizer[b]	Harvest titer[c]	Product titer[d]	n[e]	k[f] $\log_{10}$TCID$_{50}$/day	Intercept[g]	Loss on lyophilization[h]	Initial Loss[i]	37 °C shelf-life in days[j]
1	LS	6.60	6.60	18	−0.0051	4.80	0.00	1.80	177.6
2	LS	5.95	5.60	8	−0.0196	4.61	0.35	0.99	36.4
	TD	5.95	5.72	8	−0.0196	3.82	0.23	1.90	0
3	LS	6.60	5.98	14	−0.0108	4.39	0.62	1.59	45.8
	LT	6.60	5.93	14	−0.0140	3.69	0.67	2.24	0
4	LS	6.66	6.35	13	−0.0118	5.14	0.31	1.21	105.0
5	LS	6.72	6.30	16	−0.0060	4.79	0.42	1.51	148.9
Xero	TD	6.10	5.76	6	−0.0810	5.70	0.40	NA	22.2

[a] All values are $\log_{10}$ TCID$_{50}$ unless otherwise indicated.
[b] Stabilizer preparation: LS = 2.5% lactalbumin hydrolysate and 5% sucrose, TD = 5% trehalose dehydrate, LT = 2.5% lactalbumin hydrolysate and 5% trehalose dehydrate.
[c] Titer of pooled virus after one freeze-thaw cycle.
[d] Titer per vial after lyophilization.
[e] Number of time point include in the regression analysis.
[f] Slope of the regression line or degradation constant.
[g] Y-intercept of the degradation line.
[h] Difference between the harvest and product titer.
[i] Difference between the product titer and the intercept of the regression line.
[j] Time interval that the vaccine maintains the minimum required dose for 25-dose vial (3.9 $\log_{10}$ TCID$_{50}$).

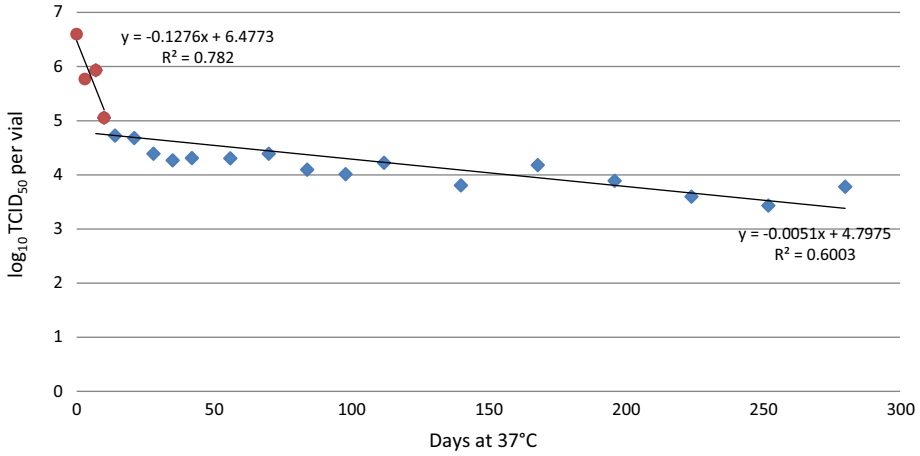

Fig. 1. Degradation curve for PPR vaccine batch 1 (LS Stabilizer) at 37 °C: A biphasic degradation curve is evident with an initial period of rapid loss lasting approximately 7 days followed by a more gradual linear decay.

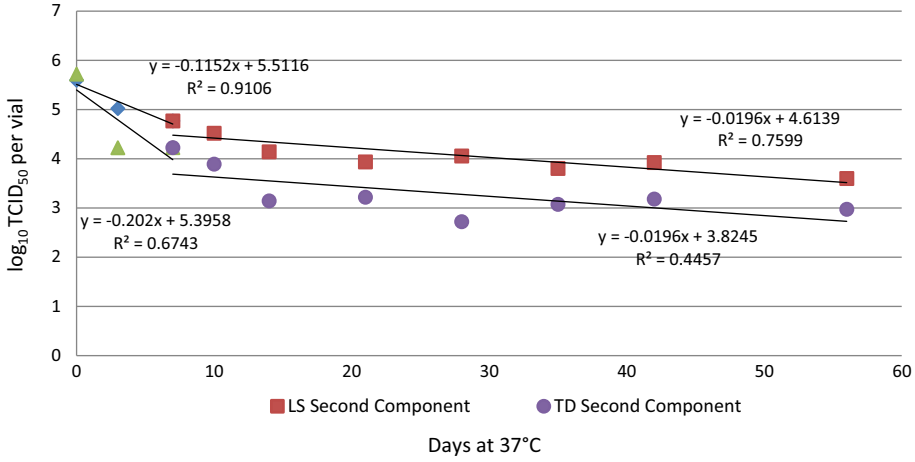

Fig. 2. Degradation curve for PPR vaccine batch 2 comparing preparations stabilized with LS and TD preparations at 37 °C: Note that the LS vaccine retained more virus during the initial period of rapid loss leading to a higher titered vaccine throughout the second component of the degradation. The degradation constants for the two preparations were essentially equal during the second component.

ent stages of the drying process. The final residual moisture of the product should be in the range of 1–1.5%. In this work, stabilizers were prepared in concentrate and added to virus in a 1:4 ratio in batches 4 and 5 to reduce the loss of titer due to dilution. For all harvests, cells were mechanically detached, stabilizer added, and then the cell-virus-stabilizer suspension was directly frozen at

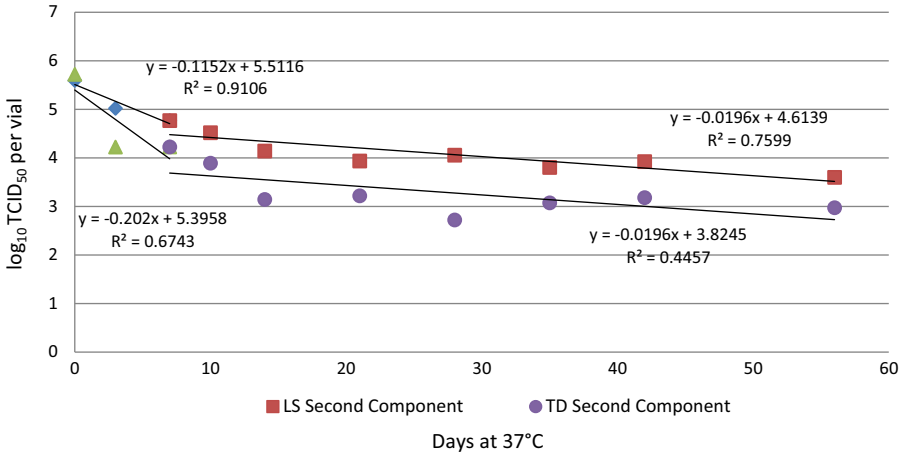

Fig. 3. Degradation curve for PPR vaccine comparing preparations stabilized with LS and LT at 37 °C: The LS vaccine retained more virus during the initial period of rapid loss, yet the degradation constants for the two preparations were essentially equal during the second component. The LS preparation maintained a higher level of potency throughout the period of analysis.

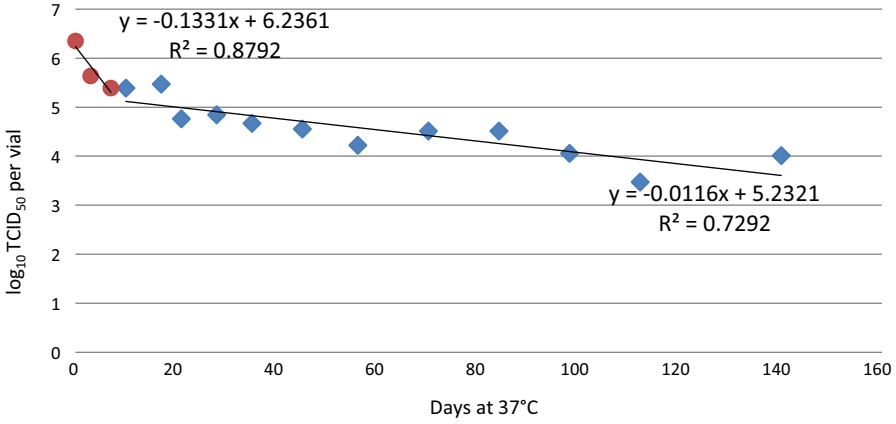

Fig. 4. Degradation curve for PPR vaccine batch 4 (LS stabilizer) at 37 °C: This curves presents the least stable of three batches produced using the rinderpest method and the LS stabilizer. The degradation constant was 0.0116 leading to a shelf-life estimate of 105 days at 37 °C.

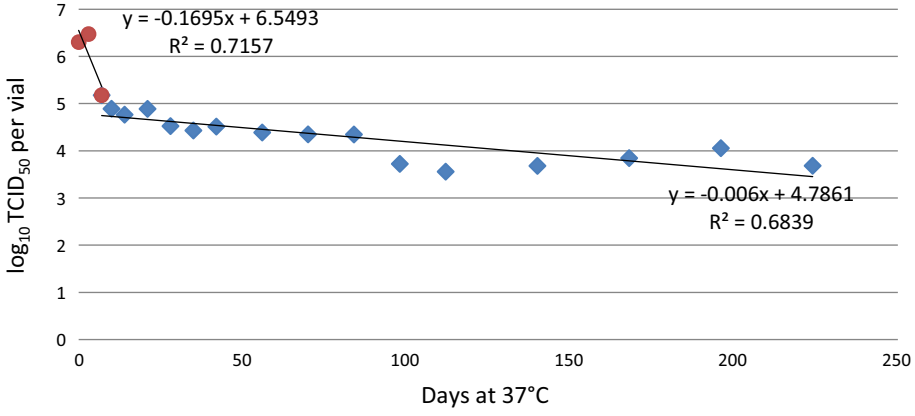

Fig. 5. Degradation curve for PPR vaccine batch 5 (LS stabilizer) at 37 °C: The degradation parameters (intercept and slope) of the second component of the degradation curve of batch 5 resulted in a vaccine with a shelf-life of 148 days at 37 °C.

−70 °C in a single step to minimize losses due to repeated freezing and thawing. The key to implementing thermostable vaccine production is to fully integrate thermostability and the factors that contribute to thermostablity into the quality assurance process [12,10].

Sakar reported much lower levels of stability for LAH-sucrose stabilized PPR vaccines in the lyophilized state [24] in a set of comparisons similar to those done by Mariner and co-workers for rinderpest [15]. The residual moistures of the products in their comparisons were as high as 5%. A vaccine cake with this level of

Table 2
Arrhenius analysis of thermostable PPR vaccine.

Temperature[a] (°C)	n[b]	Experimental k[c] ($log_{10}TCID_{50}$/day)	Shelf-life[d] (Days)	Corrected k[e] ($log_{10}TCID_{50}$/day)	Half-life[f]
4	–	–	–	0.000009014	91.2 years
10	–	–	–	0.000032435	25.3 years
25	–	–	–	0.000635661	1.3 years
37	16	−0.0060	148.9	0.005584193	53.7 days
45	18	−0.0200	26.7	0.021704389	13.8 days
56	12	−0.1177	13.7	0.126012139	2.4 days

[a] Degradation temperature: Experiments were conducted at 37, 45 and 56 °C. Stability values at 4, 10 and 25 °C are projected from the regression analysis.
[b] Number of time points included in the regression estimates of the experimentally determined k.
[c] Degradation constants (k) measured at the respective temperatures.
[d] Time taken for the vaccine titer to fall to the minimum required dose for a 25-dose vial from the y-intercept of the regression line.
[e] Degradation constant estimated from the slope of the Arrhenius line.
[f] Vaccine half-life calculated using the corrected k.

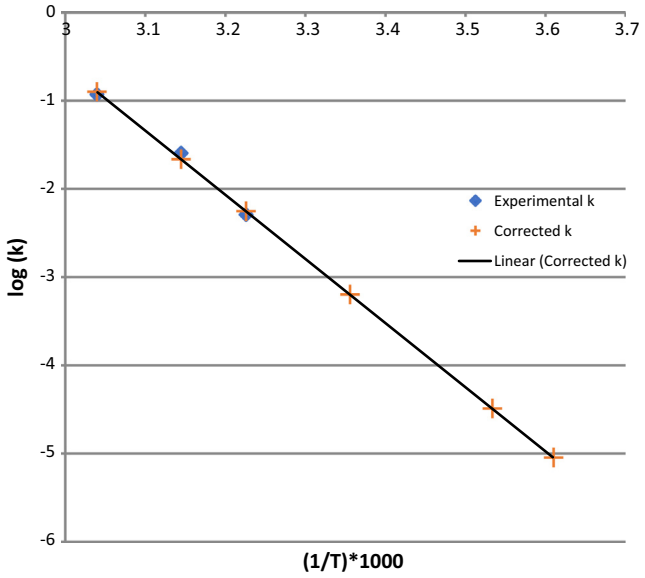

Fig. 6. Arrhenius plot of the degradation constants for batch 5 between 4 and 56 °C: The plot illustrates the three values measured experimentally as blue diamonds and the points predicted from the regression analysis as red plus signs. The degradation constants were predicted from the line at 25, 10 and 4 °C.

residual moisture is in a deformable plastic state where the constituents are free to flow and water is readily available. These are conditions that elevate degradative reaction rates. Residual moistures on the order of 4–5% are inappropriate, even for non-thermostable vaccines.

In the comparisons of disaccharide components of the stabilizer when using the rinderpest protocol, sucrose was superior to trehalose. The results indicate there is no advantage to the use of trehalose in the manufacture of thermostable PPR vaccines by lyophilization. The data therefore point to the suitability of using cheap and easily acquired LAH-sucrose as a stabilizer to offer thermal protection of PPR vaccines.

The only published or public information on the thermostability of PPR vaccine produced using Xerovac technology is the paper by Worrall and co-workers [28]. The paper presents the results of three batches, of which only the final one described is indicated by the authors to be thermostable (Batch 3). This batch was tested at 45 °C and the titer per vial was reported to be 4.15 and 3.1 log_{10}-$TCID_{50}$ per vial after 8 and 14 days storage at 45 °C, respectively. The thermostable batch was not repeated. For the sake of comparison to the vaccine produced using the rinderpest method described in the present paper, this would suggest a shelf life of about 7 days at 45 °C as 25 dose presentation.

The batch of Xerovac produced as part of the present research was evaluated at 37 °C and the two different temperatures of analysis complicate comparison. On the other hand, a shelf-life of 22 days at 37 °C compared to a shelf-life of about 7 days at 45 °C, suggest that the batch of Xerovac produced as part of this research had similar levels of thermostability to the best batch reported by Worrall and co-workers.

Batch 5 of the vaccine produced using the rinderpest method had a shelf life of 26.7 days at 45 °C or about 4 times of the life that can be estimated from the data provided by Worrall et al. [28] on their best batch. Further, when comparing shelf lives of vaccine produced by the rinderpest method with the one batch of Xerovac produced in the present research, the rinderpest method resulted in a vaccine with about 5–7 times the stability of Xerovac (100–150 days vs. 22 days).

The main advantage of rinderpest method over Xerovac is in terms of the suitability of the method for scale-out to commercial-scale production. Although it was indicated that Xerovac was 'shorter, cheaper and easier', this has proved not to be the case [28]. Attempts to commercialize Xerovac production have found high batch failure rates and considerable intra-batch variability. The result is considerable loss of reagents, time and opportunity costs for production plant infrastructure. On the other hand, the rinderpest method was established in three commercial laboratories in the 1990s and 10s of millions of doses of cost effective vaccine were produced to complete rinderpest eradication. Production was discontinued as part of the process of cessation of vaccination and sequestration of rinderpest virus upon completion of the global eradication [22].

Work on enhancement of PPR production in Ethiopia reported on the thermostability of the final product [25]. They tested formulations at 37 and 45 °C for up to 4 days and found that a trehalose-based vaccine maintained the minimum titer required for one dose of vaccine over the 4 days at 45 °C. Final titers were not reported. These results are comparable to those found for trehalose-stabilized vaccine in the work presented here, but do not constitute sufficient evidence that the process can result in a practical, multi-dose presentation of commercial vaccine with sufficient stability for use with a reduced cold chain in the field.

The rinderpest method of thermostabilization stabilized the PPR vaccine virus to practical levels equivalent to those obtained with rinderpest. This was to be expected given that thermostabilization in physico-chemical process and that all the morbillivirus are essentially identical at the structural level. The vaccine has suitable stability for use without a cold chain for up to 30 days. As with rinderpest, this is sufficient stability for vaccination teams to travel in the field without refrigeration or ice.

The Global Rinderpest Program used a few practical guidelines regarding thermostable rinderpest vaccine and no vaccine failures were noted. First, the Pan African Veterinary Vaccine Centre

PANVAC) independently tested all lots of thermostable vaccine for thermostability and potency by conducting the potency test on vials of vaccine after 2 weeks storage at 45 °C. All national RP eradication programs were required to purchase PANVAC certified vaccine. Secondly, once vaccine was removed from the cold chain for field use, it should be used or destroyed. No vaccine should be returned to the depot to avoid confusion. Thirdly, as with any medicine, the vaccine should be protected from direct sunlight and extremes of heat. A practical training message for vaccination personnel is to treat the thermostable vaccine the same way you would a bottle of oxytetracycline. Once reconstituted, the vaccine should be used within two hours. The reconstituted vaccine will retain the required dose when room temperature normal saline is used as the diluent [24].

In addition to reducing the need for cold chain infrastructure, this level of stability frees vaccination programs from the requirement for vehicles, which together with *per diem* is the principal cost of vaccine delivery and vaccination as a whole [9]. It also facilitates the integration of community-based vaccination programs that are critical to obtaining good herd immunity levels [11,4] and extending the reach of livestock health and disease eradication programs to remote and politically unstable areas [13].

One of the advantages to thermostabilization of existing strains vaccines through improved manufacturing systems is that the underlying immunogenic agent has not been altered. All of the changes described above fall within the OIE norms for existing PPR vaccines. As with rinderpest, the next step for PPR should be to adapt production to commercial scale lots and pilot the use of the vaccine in practical field programs. As a matter of due diligence, every step of the scaling out process should be monitored including vaccine potency and sero-conversion in the field.

5. Conclusion

The method used in the lyophilization of thermostable RP vaccine and a lactalbumin and sucrose stabilizer when applied to PPR vaccine resulted in a vaccine with levels of thermostability that were comparable to those obtained with RP and superior to the other options tested with PPR vaccine virus. This level of thermostability is sufficient for use in the field without a cold chain for up to one month, as was done in the global eradication of RP. The rinderpest method of thermostabilization has been successfully commercialized in the past indicating that the commercialization of thermostable PPR vaccine using the rinderpest method is feasible and can greatly facilitate the eradication of PPR.

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