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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-