Comparative Efficacy of Stabilizers on the Thermostability of Peste des Petits Ruminants Vaccine

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Abstract

Peste des petits ruminants (PPR) is an acute and highly contagious fatal disease of small ruminants mainly affecting sheep and goats, and camels. PPR is endemic in many developing and tropical countries including Pakistan, and vaccine is currently the only means of disease control. However, lack of established infrastructures and high temperature, maintaining cold chain for effective delivery of the vaccine is a challenge. All vaccines that are currently being applied for the control of PPR are thermo-labile and require cold chain for stability. In this study, we assessed the efficacy of different stabilizers on the thermostability of live attenuated vaccine of PPR virus (PPRV). Five formulations of four thermo-stabilizers including lactalbuminhydrolysate sucrose (LS), trehalose dihydrate (TD), weybridge medium (WBM) and buffered gelatin sorbitol (BUGS) were used to prepare vaccine using Nigeria 75/1 strain of PPRV virus. Results revealed that vaccine having LS proved maximum stability as it reduced minimal viral infectivity (0.42 log_{10}TCID_{50}) during lyophilization as well as three time higher shelf life (3.69 days) as compared to commercial vaccine (1.23 days) at 37°C. Moreover, it was also revealed that PPR vaccine lyophilized with LS, WBM and TD, was more stable than commercial vaccine having shelf life of 206-304 days at 4°C, 8-10 days at 25°C and 2.5-3.7 days at 37°C. Using regression analysis on the shelf life of vaccine preparations in different thermo stabilizers, we can demonstrate that BUGS provided minimal stability as well as maximum viral infectivity loss (1.49 log_{10}TCID_{50}) after lyophilization. Taken together, we provide foundation studies to improve the thermostability of PPRV to facilitate the immunization of susceptible animals in situations where meeting cold chain is not feasible. Efforts are required to genetically select strains that confer protection at higher temperature without compromised immunogenicity which is potentially feasible with the established reverse genetics.

Key Words:

Peste des petits ruminants; Attenuated vaccine; Thermo stabilizers; Thermostability

Introduction

Peste des petits ruminants (PPR) is caused by PPR virus (PPRV) which is a member of the genus morbillivirus in the family paramyoviridae [1]. The PPR is an acute, highly contagious and fatal disease of small ruminants and camels. The disease is characterized by pyrexia, stomatitis, pneumonia, oculo-nasal discharge and diarrhea. The transmission of PPR is mainly through aerosol route between animals living in close vicinity [2]. This disease is endemic in various regions of Asia and Africa [3-5]. In Pakistan, PPR was documented in 1991 but confirmation of PPR virus was done through PCR in 1994 [6]. Moreover, by using antigen and antibodies detection, PPR has been reported in wildlife breeding center of Faisalabad, Pakistan and Sindh Ibex in Sindh province [7]. It can be controlled by classical methods including, controlling the movement of small ruminants from affected areas, proper quarantine during import of animals, slaughtering of infected animals, cleaning of infected area with lipid solvent solutions. However, the most efficient way to control disease is by vaccination. Currently, live attenuated vaccines are being used to immune the susceptible and at risk small ruminant population. By their nature, these are heat labile and is primary cause of vaccine failure especially during transportation and storage in laboratories where there is no or poor infrastructure [8]. Moreover, due to current energy crisis in the country, maintain cold chain is a challenge even in moderately equipped labs. It is thus required to explore alternative means of virus stabilization. Several efforts have been made to stabilize the PPRV with variable outcomes and success. Mariner and colleagues have improved the thermostability of TCRV through freeze-drying technology [9]. Thermostability of PPR vaccines has also been tried using heavy water [10]. Two thermostable vaccines (PPR/Revati and PPR/Hansi) were claimed at the IVRI, India. Thermal degradation profile of these vaccines using stabilizer E (trehalose, calcium chloride and magnesium chloride) and LS appeared encouraging. Sarkar and his colleagues also compared the efficacy of different chemical stabilizers such as LS, WBM, BUGS and TD on the thermostability of a live attenuated PPR vaccine using PPRSungrdi/96 [11].

Beside these initial reports, there remains a need to improve thermostability of vaccines in local laboratory setting to meet the domestic and national demands. This study was designed to evaluate the effectiveness of thermostabilizer on the PPRV in an effort to improve thermostability at local laboratory conditions.

Materials and Methods

Nigeria 75/1 strain was used for preparation of PPR vaccine. The virus was propagated on Vero cell line. Vero cells were cultivated on Dulbeco’s Modified Eagle’s Medium (DMEM) along with penicillin and
streptomycin at the dose rate of 100 IU/ml and 100 µg/ml respectively. The medium was supplemented with 10% fetal bovine serum (FBS) and serum concentration was reduced to 2% after completion of monolayer. The monolayer was infected with viral virus at multiplicity of infection of 0.01 virus/cells. The flasks were incubated at 37°C in CO2 incubator and examined regularly till the development of cytopathic effects (CPE). On the development of 80-90% CPEs, the virus was harvested by a cycle of freeze and thawing to break the cells and stored at -80°C till further use. Five formulations of four different types of stabilizers such as lactalbuminhydrolysate sucrose (LS), weybridge medium (WBM), buffered gelatinsorbil (BUGS) and trehalose dihydrate (TD) were prepared to evaluate the thermostability of vaccine. These stabilizers were prepared according to standard protocols as described previously [11]. The composition of these thermostabilizer used is given in Table 1.

| Stabilizer | Composition                                                                 |
|------------|-----------------------------------------------------------------------------|
| LS         | 5% lacalbuminhydrolysate (LAH) and 10% sucrose in Hank’s balanced salt solution (HBSS) at pH 7.2. |
| WBM        | 5% sucrose, 1% sodium glutamate and 2.5% LAH in HBSS at pH 7.2.              |
| BUGS       | 3.5% D-sorbitol and 3.5% hydrolyzed gelatin in 0.1 M potassium phosphate buffer at pH 6.2. |
| TD         | 5% TD in distilled water                                                     |
|            | 10% TD in distilled water                                                   |

Table 1: Composition of different thermostabilizers.

TD was used in two concentrations. TD concentration mentioned onwards indicates the final concentration in vaccine. Lyophilization was carried out using Edwards Modulyo 4K freeze-drier. PPR virus and stabilizers were mixed in equal volumes and 300 µL of solution was dispensed in vaccinal vials and sealed by vented rubber stoppers. After keeping the vaccine vial at -80°C for overnight, the vaccine vials were lyophilized at a condenser temperature of -60°C at vacuum of 0.06 mbar. The efficiency of each thermostabilizer was checked by exposing vaccine vials at 4°C in refrigerator, 25°C and 37°C in incubator and 45°C in hot air oven. The vaccine samples were checked for infectivity at 4°C on days 3, 7, 10, 14, 21, 25, 36, and 45. From incubators titration of vaccine was done at 25°C on days 3, 6, 9, 12, 15, 18, and 20 while at 37°C vaccine efficiency was tested on days 1, 3, 5, and 7. Finally titration of thermostable vaccine was done at 45°C at 6 hour interval up to 24 hours. After exposing vaccine vials at different temperatures; reconstitution was done into 1 ml distilled water prior to performing titration on Vero cells. Two vaccinal vials against each stabilizer were titrated and their log10TCID50 was calculated following Reed and Muench method [12].

Results and Discussion

Vaccine was prepared by using five different thermostabilizers and lyophilized using conventional techniques to compare the virus titer before and after lyophilization. The loss of virus titer ranged from 0.37 to 1.49 log10TCID50 for tested stabilizers. Among five thermostabilizers, vaccine with 2.5%TD showed minimum virus titer loss (0.37 log10TCID50 per ml) while the virus titer loss against LS, WBM, 5%TD, and BUGS was recorded 0.42, 0.44, 0.59 and 1.49 log10TCID50 respectively. The results are summarized in Table 2. Three thermostabilizers (LS, TD, and WBM) significantly increased the shelf life as well as half-life of vaccine. However, WBM found to be superior as it increased three fold shelf life (304 days) and half-life (65 days) as compared to commercial vaccine (UVAS-PPR-VAC). LS, 2.5% TD and 5% TD increased vaccine shelf life up to 208, 206 and 191 days respectively at 4°C among the tested stabilizers (Table 2).

| Stabilizer | (log10TCID50) |
|------------|---------------|
|            | Before lyophilization | After lyophilization | Loss |
| LS         | 6.08          | 5.66              | 0.42 |
| 2.5% TD    | 6.08          | 5.71              | 0.37 |
| 5% TD      | 6.08          | 5.49              | 0.59 |
| WBM        | 6.08          | 5.64              | 0.44 |
| BUGS       | 6.08          | 4.59              | 1.49 |
| Commercial Vaccine | 6.08          | 5.66              | 0.42 |

Table 2: Effect of different chemical stabilizers on viral loss during lyophilisation.

Sarkar et al. [11] had reported almost the same findings showing TD and LS provided higher protection to the vaccine after lyophilization. However, the thermostabilizers BUGS and WBM provided insufficient protection during freeze drying [11] and this finding is contradict to present study. Trehalosedihydrate is a sugar (disaccharide) used for the dehydration of PPR vaccine to combat the environmental stress [13]. In present study, TD thermo stabilizer induced a loss ranged 0.37 to 0.59 log10TCID50 after lyophilization and these findings are similar to Sarkar et al. results (0.32-0.48 log10TCID50). Vaccine with TD stabilizer showed two fold higher shelf life (206 days) and half-life (42 days) than commercial vaccine shelf life (99 days) and half-life (20 days) (Table 2). WBM showed more titer loss (0.44) as compared to TD (0.37) but shelf life was found to be highest among tested stabilizers. Both shelf life and half-life of WBM was found higher than commercial vaccine at 4, 25, 37 and 45°C, these findings are in favour of Asim et al. [14]. While at these same temperatures BUGS showed the least shelf life but the half-life of BUGS stabilized vaccine found higher than that of commercial vaccine. It was due to the loss of infectivity titer of BUGS stabilized vaccine during lyophilization. Infectivity titers of vaccines having different thermostabilizers were analysed when exposed to 4°C, 25°C, 37°C and 45°C for varying time intervals and regression analysis was also applied. Results showed gradual decrease in infectivity titers at 37°C for all thermo stabilized vaccines (Figure 1), while, drastic loss in titer was recorded at 45°C (Figure 2). Moreover, graphical representation revealed inverse relationship between infectivity titer and temperature at 37°C as well as 45°C. Half-life (time required for loss of half the original titer, i.e. 0.30 log10TCID50 based on the degradation constant) was also calculated using regression equation (Table 2). Regression equation showed that temperature negatively affects the vaccine titer, by increase in temperature vaccine titer decreases. At refrigerator temperature (4°C), no significant loss in infectivity titer was recorded for all thermo stabilized vaccines as well as for commercial vaccine up to 45 days. Although the vaccines showed a decline trend in titers with respect to exposure period and this decline was found non-significant. Moreover, linear equation was developed to find infectivity titer of vaccines at specified day with fair amount of accuracy.

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Figure 1: Regression line expressing degradation of lyophilized PPR vaccine with different thermo stabilizers at 37°C. Regression analysis was carried out using MS Excel program.
Figure 2: Regression line expressing degradation of lyophilized PPR vaccine with different thermo stabilizers at 45°C. Regression analysis was carried out using MS Excel program.

Vaccines when exposed at 25°C for different days, maximum protection up to 20 days was recorded for LS and WBM thermo stabilizers and least protection up to 12 days in commercial vaccine. No infectivity titer was recorded later than 18 days for BUGS and TD (2.5% and 5%) thermo stabilizers. Similarly when vaccines were exposed at 37°C for 1, 3, 5, and 7 days. The titer consistently dropped up to 5 days for all thermo stabilizers but after 5 days no infectivity titer was found except for LS and WBM. Both of them sustained titer up to 7 days. Lowest protection was observed for commercial vaccine up to 3 days. Similar trend was observed when thermo stabilized vaccines were exposed to 45°C for 6, 12, 18 and 24 hours. This temperature is not uncommon in summer (Mid April to Mid-September) in several part of Pakistan. Irrespective of drastic decline in infectivity titer, commercial vaccine, BUGS and TD (2.5% and 5%) were able to retain titer up to 12 hours. However, minimum degradation was recorded for LS and WBM and titer was detectable up to 18 hours. These findings reported herein showed the improvement of thermal stability of lyophilized vaccine using the thermo stabilizers.
Overall results revealed that LS and WBM were found to be superior in providing protection as compared to other stabilizers. More or less similar finding were observed that LS and TD proved superior to BUGS and WBM (Table 3) [11].

| Temp (°C) | Stabilizera | Initial Titer (log_{10} TCID_{50}/ml) | Nb | Regression equation | Student’s t-test | Shelf Lifec (days or hours) | Half Lifed (days) |
|-----------|-------------|-------------------------------------|----|---------------------|-----------------|--------------------------|----------------|
| 4         | LS          | 5.66                                | 9  | y=5.6144-0.0116x    | P<0.05          | 208 days                 | 30 days         |
|           | BUGS        | 4.59                                | 9  | y=4.5086-0.0069x    | P<0.05          | 45 days                  | 43.47 days      |
|           | WBM         | 5.6                                  | 9  | y=5.6000-0.0046x    | P<0.05          | 304 days                 | 65.22 days      |
|           | TD 2.5%     | 5.71                                | 9  | y=5.6626-0.0071x    | P<0.05          | 206 days                 | 42.25 days      |
|           | TD 5%       | 5.48                                | 9  | y=5.4636-0.0066x    | P<0.05          | 191 days                 | 45.45 days      |
|           | Commercial  | 5.64                                | 9  | y=5.6736-0.0149x    | P<0.05          | 99 days                  | 20.13 days      |
| 25        | LS          | 5.66                                | 8  | y=5.7649-0.1471x    | P<0.05          | 10.63 days               | 2.0 days        |
|           | BUGS        | 4.59                                | 8  | y=4.8897-0.1692x    | P<0.01          | 4 days                   | 1.77 days       |
|           | WBM         | 5.6                                  | 8  | y=5.6876-0.1459x    | P<0.05          | 10.19 days               | 2.05 days       |
|           | TD 2.5%     | 5.71                                | 8  | y=5.9680-0.2118x    | P<0.05          | 8 days                   | 1.37 days       |
|           | TD 5%       | 5.48                                | 8  | y=5.7798-0.1990x    | P<0.05          | 5.94 days                | 1.129 days      |
|           | Commercial  | 5.64                                | 8  | y=8.8439-0.2656x    | P<0.01          | 2.42 days                | 1.129 days      |
| 37        | LS          | 5.66                                | 5  | y=5.7314-0.4142x    | P<0.05          | 3.69 days                | 17.28 h         |
|           | BUGS        | 4.59                                | 5  | y=5.3475-0.6204x    | NS              | 1.84 days                | 11.6 h          |
|           | WBM         | 5.6                                  | 5  | y=5.7858-0.4618x    | NS              | 3.43 days                | 15.6 h          |
|           | TD 2.5%     | 5.71                                | 5  | y=6.1485-0.7532x    | P<0.05          | 2.58 days                | 9.55 h          |
|           | TD 5%       | 5.48                                | 5  | y=6.1029-0.7484x    | NS              | 2.54 days                | 9.62 h          |
|           | Commercial  | 5.64                                | 5  | y=5.2326-0.8364x    | NS              | 1.23 days                | 8.60 h          |
| 45        | LS          | 5.66                                | 5  | y=6.392-0.215x      | P<0.05          | 10.19 h                  | 1.39 h          |
|           | BUGS        | 4.59                                | 5  | y=5.462-0.2295x     | P<0.05          | 5.49 h                   | 1.31 h          |
|           | WBM         | 5.6                                  | 5  | y=6.508-0.2078x     | NS              | 11.1 h                   | 1.44 h          |
|           | TD 2.5%     | 5.71                                | 5  | y=5.62-0.2608x      | P<0.05          | 5.44 h                   | 1.15 h          |
|           | TD 5%       | 5.48                                | 5  | y=5.862-0.258x      | P<0.05          | 6.44 h                   | 1.16 h          |
|           | Commercial  | 5.64                                | 5  | y=5.282-0.2463x     | P<0.05          | 4.39 h                   | 1.21 h          |

NS, not significant
*aStabilizers, LS, lactalbuminhydrolysate–sucrose; WBM, Weybridge media; BUGS, buffered gelatin sorbitol; TD, trehalosedihydrate.
*bSample Size
*cTime required to reach 4.2 log_{10} TCID_{50} in a 300 dose vaccine preparation, calculated from the regression equation.
*dTime required for the loss of the original titer of vaccine for about 0.30 log_{10} TCID_{50} based on the degradation constant.

Table 3: Effect of Temperature on the degradation values of PPR vaccine with different stabilizers.

BUGS was prepared following [9] and it failed to protect the viral viability during exposure at 25°C, 37°C and 45°C. The possible reason of this viral loss might be due to Lower pH of BUGS (pH 6.2) [11]. Trehalosedihydrate (TD) was used with two concentrations 2.5% TD and 5% TD which gave 0.37 and 0.59 titer loss respectively. Keeping in view the results it could be concluded that Trehalosedihydrate with 2.5% concentration performed well at 4°C and 25°C. However, shelf life at 37°C against 2.5% and 5% TD was recorded 2.58 and 2.54 days respectively. Interestingly, shelf life at 45°C against 5%TD was found higher (6.44 hr) as compared to 2.5%TD (5.44 hr). This showed that higher temperature efficacy of 5%TD thermostabilizer is higher as compared to 2.5%TD (Table 2). Results are in favour of researchers, who developed two thermostable vaccines in India and tested ther thermal degradation profile. The novel thermostable vaccines developed were also tested after reconstitution. The PPR/Revati and PPR/Jhansi vaccines showed shelf life 7.62 and 3.68 days at 37 and 40°C respectively, whereas the native Sungri/96 vaccine had a shelf life of 1.58 days at 37°C [15].
Conclusions

Current study concluded that TD and WBM produced satisfactory result to protect vaccine from harsh conditions. Moreover, use of thermostable vaccine is a better opportunity in developing countries having energy crisis to enhance the livability of vaccine during storage.

Conflict of Interest Statement

The authors declare no conflict of interest.

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