Evaluation of stability of reconstituted live attenuated Peste des Petits Ruminants (PPR), Sheep Pox and Goat Pox vaccines

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ABSTRACT

In the present study, the thermostability of live attenuated Peste des Petits ruminants (PPR), sheep pox and goat pox vaccines were assessed for their stability at 5±3°C, 25±1°C and 36±1°C in reconstituted form. All the vaccine batches maintained the minimum infectious titre in their reconstituted form (2.5 $\log_{10}$ $CCID_{50}$/dose for PPR and 3 $\log_{10}$ $CCID_{50}$/dose for sheep/goat pox) when stored at 36±1°C for at least 20 hrs. These live attenuated vaccines can be used within 8 hrs of reconstitution for immunization of animals in tropical field situations or during cold chain failures by delivering the required quantity of vaccine dose as they are found to be stable in their reconstituted form at ambient as well as at higher temperatures.

Key words: Peste des petits ruminants virus, Sheep pox virus, Goat pox virus, Vaccines, Stabilizers, Reconstitution, Thermostability

The diseases caused by Peste des Petits Ruminants virus (PPRV), Sheep pox virus (SPV) and Goat pox virus (GPV) cause substantial loss to farming community throught the world. Very effective vaccines are available against PPR and Capripox to provide strong and long lasting immunity. It is necessary that each animal vaccinated against PPR should receive a minimum recommended dose of $10^{2.5}$ cell culture infective dose 50 ($CCID_{50}$) whereas a minimum infectious dose of 10^3 $CCID_{50}$ is recommended for Sheep pox virus (SPV) and Goat pox virus (GPV) (Indian Pharmacopoeia 2018). However, one of the key issues in effective implementation of the existing live PPR vaccine is limited thermotolerance that requires the maintainance of a continuous cold chain (Baron *et al*. 2017). In contrast, Capripox viruses are generally considered to be thermoresistant and have differing sensitivity to heat between isolates (Rao and Bandyopadhyay 2000).

In India, with respect to PPR and Capripox viral diseases, the control strategy is mostly aimed at vaccination using the conventional live attenuated vaccines. Most of these live attenuated vaccines lose their potency if not stored under a controlled cold chain which remains a major hurdle

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in mass immunization programs (Kumru *et al.* 2014). Under such situations, studies on thermal stability of PPR and Capripox virus vaccines is important when these vaccines are to be used in tropical climatic conditions and in areas lacking cold chain infrastructure. Therefore, the present study focuses to evaluate the thermostability of PPR, sheep pox and goat pox vaccine viruses in reconstituted form at various temperatures which are usually encountered during storage and transport under field settings.

The vaccine batches of PPRV, SPV and GPV were prepared by propagating the viruses on Vero cell line. The virus was harvested at appropriate time and mixed with the stabilizers. The composition of PPR and SPV vaccine formulation consisted of 5.1 % lactalbumin hydrolysate (LAH, BD), 5.05 % sucrose (Himedia) and 0.63% gelatin hydrolysate (Himedia), whereas, the formulation for GPV vaccine consisted of 5% LAH,10% Sucrose, 1% sodium glutamate and 0.63% gelatin hydrolysate. One millilitre of virus-stabilizer mixture was dispensed in sterile 2 ml capacity glass vials and lyophilized using automated bench top freeze-dryer (Labocon, LFD-BT-102). The vaccine vials were tested for vacuum by spark test and moisture by Karl Fischer volumetric titration. All the vaccine vials used in the study showed to contain the vacuum as tested by vacuum spark test. The residual moisture (RM) levels observed in the vaccine vials ranged from 1.8-2.2%, which was within the acceptable limit of 3%.

For stability studies of reconstituted PPRV, SPV and GPV vaccine, the freeze dried vaccine vials was reconstituted in 100 ml of diluent representing 100 doses of vaccine stored at respective stability temperatures of 5 ± 3°C, 25±1°C and 36±1°C. The sample from each vial was titrated on 0, 4, 8, 12, 16, 20 and 24 hours interval and infectivity titres were calculated using Spearman-Karber method.

The reconstituted PPR vaccine when stored at 5±3°C retained titre of 2.75 $\log_{10}$ $CCID_{50}$/dose at the end of the study period of 24 hrs, whereas, reconstituted SPV and GPV vaccines retained an infectivity titre of 3.5 $\log_{10}$ $CCID_{50}$/dose at the end of 24 hours of storage at 5 ± 3°C (Table). The PPR vaccine lost 0.75 $\log_{10}$ $CCID_{50}$ virus titre at the end of 24 hrs of exposure at 25±1°C, whereas, reconstituted SPV and GPV vaccine maintained infectivity titre of 3.25 $\log_{10}$ $CCID_{50}$ /dose till 24 hrs of incubation at 25°C. Similar results were observed with reconstituted thermo-adapted PPR vaccine virus (Ta PPRV Jhansi/2003) where it maintained the required titre for 48 hrs at 25±1°C (Riyesh *et al*. 2011).

Reconstituted PPR vaccine virus titres rapidly dropped during storage at 36 ±1°C from an initial titre of 3.5 $\log_{10}$ $CCID_{50}$/dose to 2 $\log_{10}$ $CCID_{50}$/dose within the span of 24 hours. However, the SPV and GPV vaccine viruses could able to retain an infectivity titre of 3 $\log_{10}$ $CCID_{50}$/dose at 36±1°C till 24 hrs of observation period (Table). Both SPV and GPV vaccine viruses were found to be marginally superior to PPRV in terms of their thermostable nature.

Storage of reconstituted live attenuated vaccines beyond 6 hours at ambient temperature or higher is usually

not recommended since reconstitution affects both the safety and effectiveness of vaccines (WHO 2000). In the present study, all the reconstituted vaccine batches showed stability beyond 16 hrs at 36 ±1°C. However, we would recommend to store the reconstituted live attenuated PPR, SPV and GPV vaccines upto the point of use at 5±3°C and to vaccinate animals within 8 hours of reconstitution and the left over vaccine should be discarded at the end of each immunization session. Even if the reconstituted vaccines are exposed to higher temperatures for a brief period, the vaccines tend to be effective.

REFERENCES

Baron M. D, Diop B, Njeumi F, Willett B J and Bailey D (2017). Future research to underpin successful peste des petits ruminants virus (PPRV) eradication. Journal of General Virology. **98** (11) : 2635-44.

Indian Pharmacopoeia. (2018). 8th Edition. Indian Pharmacopoeia Commission (IPC). Ministry of Health and Family Welfare. Government of India.

Kumru O S, Joshi S B, Smith D E, Middaugh C R, Prusik T and Volkin D B (2014) Vaccine instability in the cold chain: mechanisms, analysis and formulation strategies. Biologicals. **42**(5):237-59.

Rao T V S and Bandyopadhyay S K (2000) A comprehensive review of goat pox and sheep pox and their diagnosis. Animal Health Research Reviews. **1:** 127-136.

Riyesh T, Balamurugan V, Sen A, Bhanuprakash V, Venkatesan G, Yadav V and Singh R K. (2011) Evaluation of efficacy of stabilizers on the thermostability of live attenuated thermo-adapted Peste des petits ruminants vaccines. Virologica Sinica. **26(5):**324.

World Health Organization (WHO) (2000) Vaccines and Biologicals Update. Vol. 34. https://www.who.int/immunization/documents/updat34e.pdf

Table 1 Stability of reconstituted PPR, sheep pox and goat pox vaccines at various temperatures

Vaccine	Temp (°C)	Virus titre (log_{10} $CCID_{50}$/dose)						
		Time (hrs) 0	4	8	12	16	20	24
PPRV	5 ± 3	3.5	3.25	3.5	3.25	3.5	3.5	2.75
	25 ±1	3.5	3.5	3.5	3.5	3.5	3.25	2.75
	36 ±1	3.5	3.5	3.25	2.75	2.75	3	2
SPV	5 ± 3	3.75	3.75	3.75	3.75	3.75	3.5	3.5
	25 ±1	3.75	3.75	3.5	3.5	3.5	3.25	3.25
	36 ±1	3.75	3.5	3.5	3.25	3.25	3.25	3
GPV	5 ± 3	4	3.75	3.75	3.75	3.75	3.5	3.5
	25 ±1	4	3.5	3.5	3.5	3.5	3.5	3.25
	36 ±1	4	3.5	3.5	3.5	3.5	3.25	3