

## EFFECT OF VARIOUS STABILIZERS ON TITRE OF LYOPHILIZED LIVE-ATTENUATED PESTE DES PETITS RUMINANTS (PPR) VACCINE

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### ABSTRACT

Lyophilization stabilizes the biological materials by using two overlapping drying procedure i.e. primary drying by sublimation of the ice crystal from frozen material and secondary drying or desorption by evaporation of the free water adsorbed into the dried product. Three different stabilizers i.e. lactalbumin hydrolysate-sucrose, Weybridge medium and lactalbumin hydrolysate-manitol were used to lyophilize the Peste des petits ruminants (PPR) vaccine. Titre of live-attenuated PPR cell culture experimental vaccine was studied after lyophilization which revealed that PPR vaccine lyophilized with Weybridge medium was more stable and maintained the virus titre longer than rest of stabilizers used in the study.

**Key words:** Peste des petits ruminants, vaccine, lyophilization.

### INTRODUCTION

Peste des petits ruminants (PPR) is a highly contagious viral disease of sheep and goats characterized by high morbidity and mortality (Khan *et al.*, 2007). It is caused by a virus, Peste des petits ruminants virus (PPRV), which is classified in the genus morbillivirus within the family paramyxoviridae (Gibbs *et al.*, 1979).

The use of rinderpest vaccine to protect small ruminants against PPR is contraindicated because it produces antibodies to rinderpest, which compromises serosurveillance for rinderpest, and thereby the global rinderpest eradication programme (Anonymous, 1999). The only way to control PPR disease is the use of homologous vaccine (OIE, 2004).

Different stabilizers i.e., lactalbumin hydrolysate-sucrose (LS), Weybridge medium (WBM), buffered gelatin-sorbitol (BUGS) and trehalose dihydrate (TD) are used to prepare the lyophilized vaccines. However, LS and TD are more stable than rest of the stabilizers to lyophilize PPR vaccine (Sarkar *et al.*, 2003). LS stabilizer can maintain the protective titre of vero cell adopted rinderpest vaccine upto 4 hours at ambient temperature if reconstituted with 0.85% sodium chloride and 1M magnesium sulphate (Mariner *et al.*, 1990).

One batch of PPR cell culture vaccine (live) was prepared for research purpose by using different stabilizers at Veterinary Research Institute, Lahore, Pakistan. This study was conducted to compare the efficacy of various stabilizers on the maintenance of titre of a conventionally lyophilized newly produced live-attenuated PPR cell culture vaccine.

### MATERIALS AND METHODS

#### Cell line and virus

African green monkey kidney (Vero) cells were maintained in Minimal Essential medium (MEM) supplemented with nystatin, penicillin and streptomycin sulphate and 10% foetal calf serum. PPR virus Nigeria 75/I (PPR 75-1 LK 6 Vero 75) obtained from Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD) France, was used as seed virus to prepare the live attenuated PPR cell culture vaccine at Veterinary Research Institute, Lahore, Pakistan.

#### Vaccine and stabilizers

A batch of PPR cell culture was experimentally prepared for research purpose. Three different stabilizers i.e., lactalbumin hydrolysate-sucrose (LS), Weybridge medium (WBM) and lactalbumin hydrolysate-manitol (LM) were used in the study. LS stabilizer consisted of 5% lactalbumin hydrolysate and 10% sucrose; the WBM consisted of 2.5% lactalbumin hydrolysate, 5% sucrose and 1% sodium glutamate, while LM consisted of 5% lactalbumin hydrolysate and 10% manitol. The vaccine vials containing different stabilizers were then lyophilized simultaneously under identical conditions to compare the titre loss during lyophilization. Vaccine vials were then sealed.

#### Estimation of virus content

For each stabilizer, three vaccine vials were taken, reconstituted separately in MEM and subjected to micro titration assay (OIE, 2004). Log<sub>10</sub> median tissue culture infective dose (log<sub>10</sub> TCID<sub>50</sub>) was calculated on day 15 by using spearman-Karber method (Anonymous, 1996). Virus titrations were carried at day 0 and then at 4, 8, 12 and 16 months.

## RESULTS AND DISCUSSION

There is no published data available with respect to use of different stabilizers in the preparation of PPR vaccine. Therefore, findings of this study have been compared with a similar vaccine against rinderpest (RP). Log<sub>10</sub> median tissue culture infective dose per lyophilized PPR vaccine vial stabilized with LS at day zero and month 4, 8, 12 and 16 were 5.3, 5.3, 5.2, 5.2 and 5.1, respectively. Corresponding values for WBM were 5.3, 5.3, 5.3, 5.3 and 5.3, respectively, while for LM at these values were 4.9, 4.9, 4.9, 4.7 and 4.5, respectively.

Among LM and LS stabilizers, the former stabilized the initial titre of the vaccine for longer time than latter (LM:<12 months, LS:<8 months). Nayak *et al.* (1995) studied the thermostability of vero cell adopted rinderpest vaccine and reported that LS and LM-stabilized tissue culture vaccines reached recommended titres in 68.51, 25.26 and 3.58 days and in 70.00, 13.83 and 2.20 days at 20, 37 and 45°C, respectively. These workers stated that in a statistical assessment of stability, LM-stabilized vaccine was superior to LS-stabilized vaccine.

The results of the present study are contrary to the findings of Sarkar *et al.* (2003). Their study revealed that vaccine stabilized with either LS or trehalose dihydrate was more stable than that stabilized with buffer gelatin-sorbitol and Weybridge medium at 4, 25 and 37°C. The difference may be due to storage temperature, as in the present study the vaccines were stored in the freezer (-20°C).

The present study revealed loss of virus titre in LS-stabilized vaccine from month 4 to 16 (5.3 log<sub>10</sub> TCID<sub>50</sub> to 5.1 log<sub>10</sub> TCID<sub>50</sub>). However, Mariner *et al.* (1990) reported that LS stabilizer can maintain the protective titre on vero cell adopted rinderpest vaccine. The variation could be due to different viruses.

The titre of PPR vaccine stabilized by Weybridge medium remained constant throughout the study i.e. 5.3 log<sub>10</sub> TCID<sub>50</sub>. This is in accordance to OIE (2004), which concludes that Weybridge medium is a stabilizer of choice for freeze-drying of PPR cell culture vaccine to save the vaccinal titre.

Keeping in view results of the present study it can be concluded that PPR vaccine lyophilized with Weybridge medium was more stable and maintained the virus titre longer than other two stabilizers used in the study.

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