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Thermostability of rinderpest vaccine virus after adaptation to Vero cells*

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ABSTRACT

Stability of Vero-cell-adapted rinderpest vaccine virus with 2 different stabilizers, viz. 5% lactalbumin hydrolysate (LAH) plus 10% sucrose (LS stabilizer) and 5% LAH plus 10% lactose (LL stabilizer), was tested at various temperatures. At 4° C, no loss in infective titres was found after 3 months of storage. At 25° C on storage for 29 days, loss of infectivity was nonsignificant. A significant ($P < 0.05$) linear decline in the titres was observed when stored at 37° C for 21 days as well as at 45° C for 8 days. Based on declining degradation curves observed at different temperatures, the vaccine is expected to reach the minimum W.H.O. requirement of 2.5 $\log_{10}$ $TCID_{50}$ after storage for approximately 305 days (LS) and 123 days (LL) at 25°C; 28 days (LS) and 48 days (LL) at 37° C; and 3 days (LS) and 7 days (LL) at 45° C. Stabilizer LL was found to be marginally superior to LS at 37° and 45° C.

Key words : Thermostability, Rinderpest vaccine virus, Vero cells

The Office International des Epizooties (OIE) has recommended (OIE 1989) limited trial of vaccine derived from Vero-cell-adapted rinderpest virus. Mariner *et al.* (1990) have already reported better thermostability of Vero-cell-adapted rinderpest vaccine by using improved lyophilization cycle. The present investigation was undertaken to look into the stability aspects of the conventionally lyophilized Vero-cell-adapted rinderpest virus at various temperatures as may be encountered under field conditions.

MATERIALS AND METHODS

Vaccine

The attenuated RBOK vaccine master seed (Plowright and Ferris 1962) at 101st passage level in bovine kidney cells was passaged 4 times on Vero cells. The fifth passage was carried out on Vero cells grown in Roux-flasks and harvested for vaccine production at 90 % CPE on day 7. Glasgow-modified Eagle's minimum essential medium (GMEM) supplemented with 10 % bovine serum was used as the growth medium.

Infected cultures in Roux-flasks were pooled together after a cycle of freezing and thawing, and centrifuged at 2 000 run for 15 min to remove the cell debris. The supernatant containing virus was used for vaccine preparation.

To produce 2 different vaccines containing different stabilizer combinations, viral fluid was made into 2 parts. Aliquots of the virus were combined with equal volume of each of the stabilizers LS and LL. Stabilizer LS consisted of 5% LAH and 10% sucrose; and stabilizer LL consisted of 5% LAH and 10% lactose. At the time of lyophilization at least two 1 ml samples were taken for virus titration. Lyophilization was carried out in glass ampoules with 0.5 ml of vaccine. Primary drying was carried out for 20 hr duration. Condenser temperature was maintained at an average of -44.5° C. Initial vacuum level in the machine was 0.09 Torr; subsequently 0.08 Torr of vacuum was maintained till the primary drying was completed. Secondary drying under vacuum was carried out for 5 hr. Ampoules were flame sealed and were kept at -20° C .till further use.

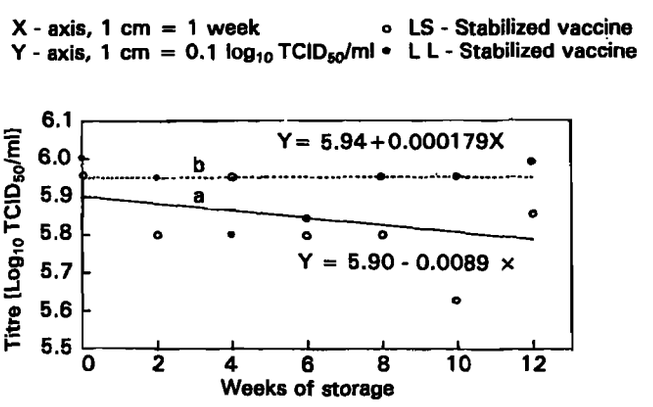

Fig. 1. Degradation curves for freeze-dried Vero-cell-adapted rinderpest vaccine exposed at 4° C (a) LS-stabilized vaccine, (b) LL-stabilized vaccine.

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Stability tests of the reconstituted vaccine

Vaccine ampoules in sufficient numbers were placed in incubators/drying oven, maintained at different temperatures. In this study, temperatures of 4°, 25°, 37° and 45° C were opted. Two ampoules were taken at each time point and stored at -20° C until titration.

Samples were taken from the refrigerator at fortnightly intervals, from the 25° C incubator on days 2, 4, 8, 13, 17, 25 and 29; from 37° C incubator on days 2, 4, 8, 11, 13, 17 and 21; and from 45° C drying oven on days 1, 2, 3, 4, 6, 8, 11, 18 and 22.

Virus titrations

Virus titrations were carried out in test-tube cultures of Vero cells. The content of a vaccine ampoule containing 0.5 ml of virus suspension was reconstituted in GMEM and then 10-fold dilutions were made in GMEM. Four tubes were infected with 1 ml of each dilution and a set of 4 tubes were kept as control in which 1 ml of GMEM was dispensed. Incubation was carried out at 37° C. The observations were recorded on day 7 and 10 post-infection. The presence of CPE was taken as the end point.

Calculation of virus titre and regression

Titre of the virus was calculated as per the method of Reed and Muench (1938).

For the calculation of regression and assessing the significance of regression coefficient, standard statistical techniques were applied (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

The infective titres obtained during differnt storage periods after exposing the vaccines to various temperatures were analysed and the results are summarized in Table 1. Degradation values at 4°, 25°, 37° and 45° C are graphically represented in Figs 1, 2, 3 and 4 respectively. Based on Student's 't' test, the decline in the infective titres was found to be nonsignificant at 4° and 25° C and significant ($P<0.05$) at 37° C for both the vaccines. However, at 45° C the decline was found to be less significant ($P<0.05$) for LS-stabilized vaccine compared to the vaccine containing LL stabilizer ($P<0.01$).

Based on declining degradation curves observed at different temperatures (Figs 2, 3, 4) under the described experimental conditions, the titre of the vaccines used in the present work will reach the minimum WHO requirement (2.5 $\log_{10}$ $TCID_{50}$) after a storage period of approximately 305 (LS) and 123 (LL) days at 25° C, 28 (LS) and 48 days (LL) at 37° C, and 3 (LS) and 7 (LL) days at 45° C. At 4° C, there was no real change in the infectivity of the vaccines over the period of observations as can be inferred from Fig. 1 and hence the vaccines were not considered to calculate shelf-life with respect to WHO requirement.

Using a freeze-dried TCRP vaccine produced in calf kidney cultures, Languet *et al.* (1985) conducted stability testing at 5°, 20°, 37° and 45° C. These workers reported that their vaccine

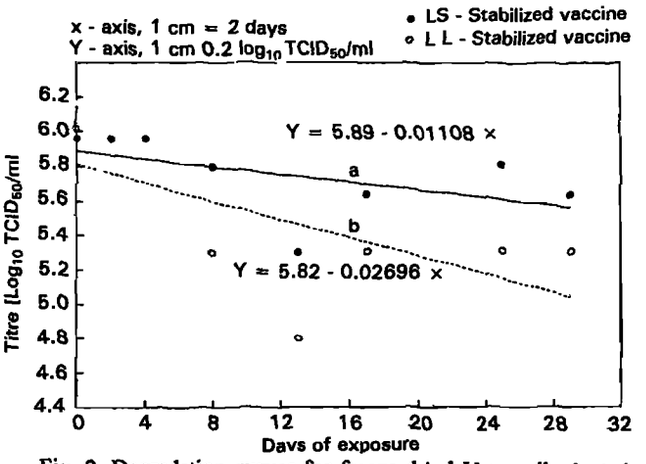

Fig. 2. Degradation curves for freeze-dried Vero-cell-adapted Rinderpest vaccine exposed at 25° C (a) LS-stabilized vaccine; (b)

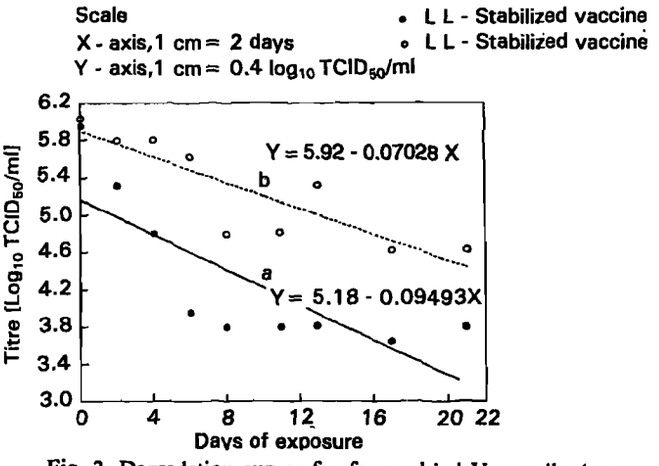

Fig. 3. Degradation curves for freeze-dried Vero-cell-adapted Rinderpest vaccine exposed at 37° C (a) LS-stabilized vaccine; (b) LL-stabilized vaccine;

stabilized with LS would reach the titre of 2.5 $\log_{10}$ $TCID_{50}$ after storgage of about 56 days at 20° C, 7 days at 37° C and 3.5 days at 45° C. On comparison of the results obtained in present study with that of above the mentioned authors as well as others (Plowright *et al.* 1970, Provost and Borredon 1972), it was evident that LS-stabilized Vero cell-adapted vaccine has a superiority over TCRP vaccine in terms of thermostability at higher temperatures. This superiority may be due to high initial titre. However, there is an indication that it was not merely the initial difference in the vaccine titre that contributed to the marked superior keeping quality of the vaccine at 25° and 37° C. Residual moisture (RM) of the LS-stabilized vaccine was estimated to be 6.15%. In spite of that, it had higher keeping quality than that of LS-stabilized TCRP vaccine, which opens up the possibility that adaptation of TCRP virus to Vero cells could have resulted in evolution of the virus with more thermostable property at 25° and 37° C. This means that the vaccine produced in Vero cells, even if it is not maintained under ideal refrigeration conditions, could

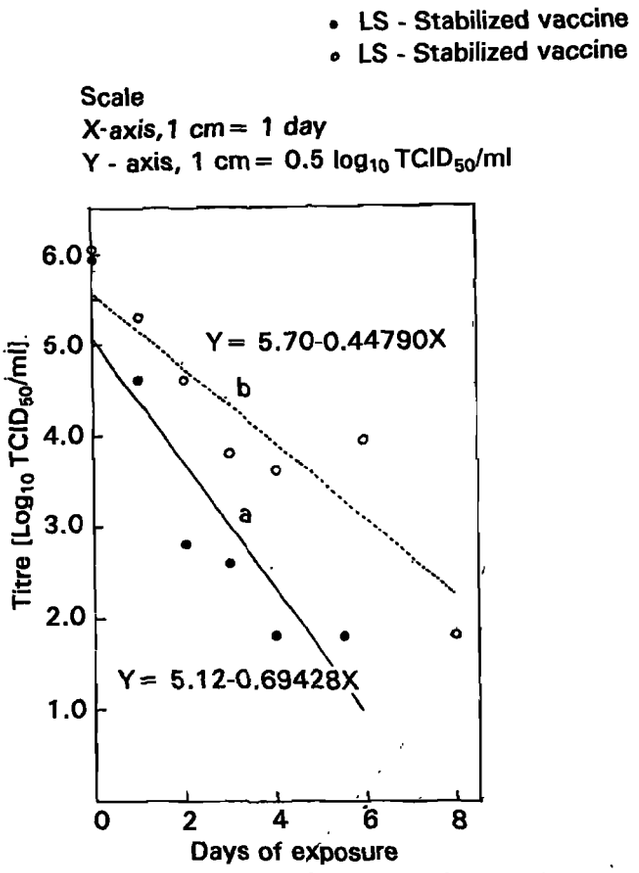

Fig. 4. Degradation curves for freeze-dried Vero-cell-adapted Rinderpest vaccine exposed at 45° C (a) LS-stabilized vaccine; (b) LL-stabilized vaccine.

have an appreciably good keeping quality, provided it is not exposed to a temperature beyond 37° C.

At 45° C, it was found that Vero-cell-adapted vaccine had inferior keeping quality as reported by Languet *et al.* (1985).

Comparative efficacy of 2 different stabilizers, LS and LL, at various temperatures was tested. At 37° and 45° C, significant degradation in titre was observed with both the stabilizers. However, LL stabilizer was found to be better than LS with respect to the time required to reach 2.5 $\log_{10}$ $TCID_{50}$. It is therefore possible to deduce that LL stabilizer can be a good substitute for LS stabilizer at higher temperatures. This finding is in contrast to that of other workers (Plowright *et al.* 1970, Mariner *et al.* 1990) who reported LS to be an ideal stabilizer. Although RM content of the vaccines could be an important factor to have a bearing on the keeping quality of the vaccine, it is difficult to account for the marginal superior stability of the vaccine with LL stabilizer in the absence of the data regarding RM of the LL stabilized vaccine. However, at 25° C LS stabilizer provided more stability than LL stabilizer. This tempts us to speculate that the chemical structure of lactose may have some role to play in increasing the keeping quality at higher temperature.

Influence of RM on the viability of freeze-dried vaccines has been reported by Precausta *et al.* (1980). Studies by Mariner *et al.* (1990) showed that this is equally applicable for the stability of freeze-dried Vero-adapted RP vaccine. The reported RM for different batches of vaccines was between 1.1 and 3.5%. These workers reported a half-life of 11.5 days at 45°C for LS-stabilized vaccine having RM of 1.1%. In the present study the conventional freeze-drying technique was employed. When RM content of LS-stabilized Vero-adapted vaccine was determined, it was found to contain 6.15% of RM. Based on regression analysis, this vaccine had a half- life of only 10 hr at 45° C. This appreciable difference in the keeping quality from that reported by Mariner *et al.* (1990) clearly confirmed that the RM content forms one of the importnat factors that contributes to the keeping quality. Conventional systems of freeze drying do not always have the option of attaining low level of RM. This will require a reliable state of the art freeze drier with vacuum level control and good temperature regulation to maintain the viability of the virus in the vaccine.

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