COMPARATIVE STUDY ON THE USE OF DIFFERENT SOLVENTS AND THEIR EFFECT ON THE STABILITY OF PPR ATTENUATED VACCINE

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ABSTRACT

Peste des petits ruminants (PPR) virus as one of Morbillivirus (Paramyxoviridae) is a heat labile virus. In Egypt, as a hot-country-in general we are urgently in need to find out a suitable solvent that may be act as heat tolerant. In this work, we tried to select the best solvent which provid a longer stabillity of reconstituted PPR vaccine six new solvents were studied in a comparative with the traditional control solvent (physiological saline solution). These solvent solutions included physiological saline solution, 1 M magnesium sulphate solution ($MgSO_4$), 2.5% lactalbumin hydrolysate solution, a mixture of lactalbumin hydrolysate 2.5% and sucrose 5% solution, 5% sucrose solution, a mixture of 1 M magnesium sulphate and saline solution and dimethyl sulfoxide 2.5% solution. By using these solvents solutions, the reconstituting PPR vaccine was kept on ice and at 37°C, titration of the virus infectivity on Vero cells was carried out at 0, 1 and 2 hours post reconstitution. It was revealed that the best solvent solution was the mixture of 2.5% lactalbumin hydrolysate and 5% sucrose that could keep the loss in the original virus litre at the most minimum.

INTRODUCTION

Peste des petits ruminants (PPR) virus is a highly contagious Morbillivirus infecting sheep, goats and other small ruminants (**OIE, 1998**).

PPR is a weak virus and could be affected by surrounding temperature. **Appel et al. (1981)** noted that inactivation of PPRV at various temperatures varies greatly depending on the substrate, viral concentration and the virus had half lives of 2-3.4 minutes at 56°C, 10 minutes at 45°C, 1-3 hours at 37°C and 9-11 days at 4°C. They added that the addition of sodium or magnesium sulphate delayed heat inactivation of the virus.

It is well known that vaccination is the best means for animal protection against infectious diseases.

Live attenuated virus vaccines were developed to protect sheep and goats against PPR disease **(Couacy et al., 1995; Khodeir and Mouaz, 1998 and afaf, 1998)**.

The main goal of the present work was targeted to the potentiation of validity-span of time for the reconstituted live PPR vaccine at ambient temperatures, through determination of a most suitable protective solvent to be used for reconstitution of the vaccine.

MATERIAL AND METHODS

1. Vaccine solvent solutions:

Six different solvent solutions were experimented to reconstitute the PPRV vaccine. They were: (1) Physiological saline solution, (2) 1 M magnesium sulphate solution, (3) 2.5% lactalbumin hydrolysate solution, (4) a mixture of 2.5% lactalbumin hydrolysate and 5% sucrose solution, (5) 5% sucrose solution, (6) a mixture of 1 M magnesium sulphate and physiological saline solution (7) 2.5% dimethyl sulfoxide solution (DMSO).

These solutions were prepared and sterilized by filtration and were kept cold at 4°C till time of use.

2. PPRV vaccine:

The locally produced PPRV live vaccine was used to test its stability after being reconstituted in seven different solvents. This vaccine was derived from a local PPRV isolate designated as Egypt-87 **(House, 1987)**. Required number of vaccine vials were randomly collected from a routinely produced batch.

3. PPRV titration:

Infectivity titrations were performed on vero cell **(Yasumura and Kowatika, 1963)** for the PPRV vaccine batch as well as for each of the seven reconstituted vaccine samples dissolved in seven different solvent solutions.

The geometric mean infectivity titre was expressed in log10 $TCID_{50}$ per ml as calculated by

Reed and Muench Formula

4. stability testing of the live PPRV dissolved in seven different solvents as kept on ice:

This experiment was carried out through reconstituting the PPR vaccine vial content per each of the seven aforementioned diluents kept on ice for the subsequent 2 hours. Representative samples per each of them were titrated for PPRV infectivity on vero cells at 0, 1 and 2 hours post

reconstitution. The geometric mean virus titre of three samples per solvent was taken and each virus titration result was calculated by **Reed and Muench (1938).**

5. **Stability testing of the live PPR vaccine dissolved in seven different solvents as kept at 37°C:**

This test was done as mentioned above except that the reconstituted vials in different diluents were held at 37°C for 2 hours.

RESULTS

1. **Stability testing of the live PPR vaccine reconstituted in seven different solvents kept on ice:**

Results of such testing are given in table (1) and fig. (1).

2. **Stability testing of the live PPR vaccine reconstituted in seven different solvents held at 37°C:**

Results of these experiments are shown in table (2) and fig. (2).

DISCUSSION

The main inconvenience of most of the Morbillivirus live vaccines, is their heat instability after being reconstituted PPRV live vaccine, being manufactured in Egypt for exportation purposes, is not an exception. Hence, it was of interest to find out a suitable solvent solution that might be used as a heat tolerant reconstituent for this vaccine. In this aspect, seven different solutions were tried being experimented first through keeping the dissolved vaccine on ice (Table 1), thus, simulating the field practical recommended conditions and secondly through holding it at 37°C (Table 2) which might be the case of unavailability of ice in some field occasions. Among these solvents the mixture of 2.5% lactalbumin/5% sucrose was found to be an efficacious solvent solution, in that only 0.5 $\log_{10}$ $TCID_{50}$ was lost after a period of 2 hours on ice (Table 1).

Moreover, only one log loss was found after a period of 2 hours at 37°C. Thus, such a reconstituting solution proved to be an adequate diluent that could support a reasonable PPRV titre over a period of 2 hours exposure either on ice or at 37°C (Tables 1, 2).

On reviewing the correlated literature, it was found that the object of prolongation of the validity span of a reconstituted Morbillivirus vaccine was targeted by several authors **(Languat et al., 1985; Plowright, 1972; Mouaz et al., 1998 and Abeer, 1993).** It is noteworthy to mention that the live PPRV vaccine vial manufactured to contain 5 $\log_{10}$ $TCID_{50}$ per ml of the lyophilized

product is sufficient to vaccinate 100 heads of sheep or goats. However, it is delivered arbitrarily for only 50 heads. So, each animal will receive (2×10^3) $TCID_{50}$ although only $10^{2.5}$ $TCID_{50}$ is required for each animal (OIE, 2004).

Thus, in view of such a requirement, a PPR virus titre of 4.5 $\log_{10}$ $TCID_{50}$ post exposure for 2 hours on ice is quite satisfactory to vaccinate 50 animals.

On the other hand, even though a PPR virus titre of 4 $\log_{10}$ $TCID_{50}$ will satisfy the need to vaccinate 50 animals which is the case with holding the dissolved vaccine at 37°C. The inherent reason which lies behind the efficacy of the lactalbumin sucrose mixture might be attributable to the biochemical nature of both sugars and proteins. Thus, working in a combination to protect virus particles against the influence of temperature. It could be concluded that results obtained in the present study would be a good contribution to the available knowledge in this concern.

Table (1): Results of stability testing of the live PPR vaccine reconstituted in seven different solvents kept on ice.

No	Solvent	Hours post reconstitution		
		0	1	2
1	Physiological saline solution	5.0 *	4.5	4.0
2	1 M mag. sulf. solution	5.0	4.0	4.0
3	2.5% lactalbumin hydrolysate solution	5.0	4.0	3.5
4	A mixture of 2.5% lact. alb., 5% sucrose solution	5.0	4.5	4.5
5	5% sucrose solution	5.0	3.5	2.5
6	A mixture of 1 M mag. sulf. solu. And saline solution	5.0	3.5	3.5
7	2.5% dimethyl sulfoxid solution (DMSO)	5.0	4.0	3.0

* PPRV titres expressed as $\log_{10}$ $TCID_{50}$ per ml.

Table (2): Results of stability testing of the live PPR vaccine reconstituted in seven different solvents held at 37°C.

No.	Solvent	Hours post reconstitution		
		0	1	2
1	Physiological saline solution	5.0 *	4.0	3.5
2	1 M mag. sulf. solution	5.0	3.0	3.0
3	2.5% lactalbumin hydrolysate solution	5.0	4.0	3.0
4	A mixture of 2.5% lact. alb., 5% sucrose solution	5.0	4.0	4.0
5	5% sucrose solution	5.0	3.0	2.0
6	A mixture of 1 M mag. sulf. solu. And saline solution	5.0	3.0	3.0
7	2.5% dimethyl sulfoxid solution (DMSO)	5.0	3.0	2.5

*PPRV titres expressed as $\log_{10}$ $TCID_{50}$ per ml.

Fig. (1): Results of stability testing of the live PPR vaccine reconstituted in seven different solvents kept on ice

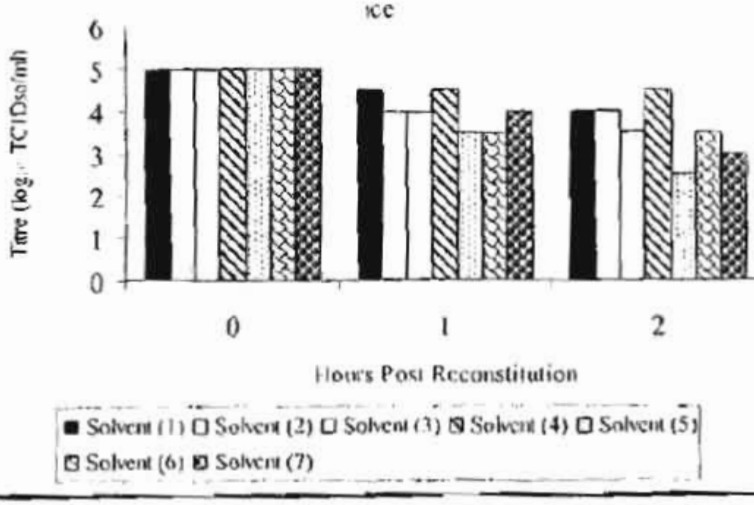
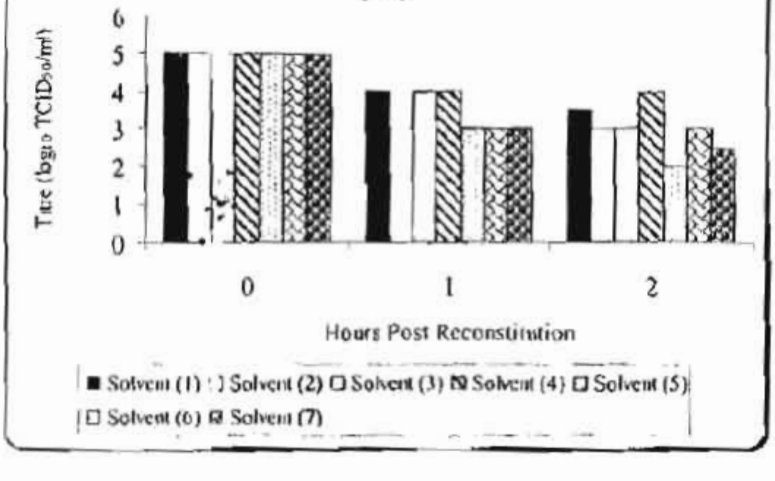


Fig. (2) Results of stability testing of the live PPR vaccine reconstituted in seven different solvents held at 37°C



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الملخص العربى

طاعون المجترات الصغيرة كأحد أفراد مجموعة الموربلى عائلة البارامیکسوفیریدی یعتبر فیروس سهل التأثر بالحرارة، ولما كانت مصر من البلاد ذات الطبيعة الحارة فكان من الضرورى إيجاد مذيب للقاح طاعون المجترات الصغيرة يمكنه من تحمل درجات الحرارة السائدة، وفى هذا العمل أجريت محاولة لإيجاد أفضل المذيبات التى توفر أقصى حماية للقاح بعد الإذابة وهذه المذيبات تتكون من ٦ أنواع بالإضافة للمذيب التقليدى للقاح وهى : محلول ملح فسيولوچى، محلول ملح كبريتات الماغنسيوم الجزئى، خليط من حجمين متساويين من المستحضرين السابقين، محلول سكروز ٥٪؛ محلول لاكت البيومين هيدروليزات ٥ر٢٪؛ خليط من حجمين متساويين من المستحضرين السابقين؛ ومستحضر دايميثيل سلفوكسيد ٥ر٢٪.

باستخدام هذه المذيبات واستبقاء اللقاح الذاب عند درجة حرارة ٣٧٪ درجة مئوية وكذلك على الثلج ويعمل معايرة لكل منهم وعلى فترات مختلفة (بعد الإذابة مباشرة وبعد ساعة ثم بعد ساعتين).

وجد أن أفضل مذيب للقاح يحافظ على قوته العيارية لأطول فترة ممكنة هو خليط من محلول لاكت البيومين ٥ر٢٪ ومحلول سكروز ٥٪.