COMPARATIVE EVALUATION OF THE POTENCY OF THE ATTENUATED STRAIN OF PESTE DES PETITS RUMINANTS VIRUS (EGYPT-87) IN VERO CELL CULTURES AND IN SMALL RUMINANTS

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ABSTRACT

The object of the present study was to find out a correlation between the potency of the locally produced PPRV vaccine, as estimated in Vero cells and in susceptible sheep. In this respect, studying five batches of the vaccine produced as 1ml lyophilized product/vial/100 doses revealed that:

1. *Infectivity titres per 1ml/vial/100 sheep doses were found to be not less than 6 log10 $TCID_{50}$ on Vero cells, as estimated for the 5 batches of the PPRV vaccine.*
2. *Five serial ten fold dilutions (10^{-3}-10^{-7}) of batch 1 were used to inoculate (S/C), 5 susceptible sheep groups of 5 heads. Three weeks post inoculation, serum samples were VNT-positive as of 100%, 100%, 60%, 20% and 0% for the 5 respective groups. Corresponding geometric mean neutralizing antibody titres were 80.6, 80.6, 50.8, 8 and 0.*
3. *Five susceptible sheep groups of 3 heads were used to test the efficacy of inoculating a dose of 2 log_{10} $TCID_{50}$ per head, corresponding to the 5 batches / 5 groups. Twenty one days post inoculation, serum samples were VNT-positive for each individual animal. Geometric mean antibody titres were 32, 80.6, 80.6, 80.6 and 50.8 for the respective 5 groups.*

It was concluded that: 1 Vero $TCID_{50}$ is equal to 1 sheep ID_{50}. Potency testing of the PPRV vaccine in Vero cells is quite satisfactory and dependable to qualify the efficacy of the product. Such a procedure would be considered as a contribution in saving time, effort and money, through carrying out qualification of the PPRV vaccine.

INTRODUCTION

Peste des petits ruminants (PPR) is a viral disease of sheep and goat that included in the list A of the International Zoosanitary Code (**Anon, 1996**). In many cases, it is an acute disease that is characterized by an erosive stomatitis, a catarrhal inflammation of the ocular and nasal mucous

membranes, and profuse diarrhoea (**Lefevre and Diallo, 1990**). The mortality may reach up 60-70% (**OIE, 1996**). In the subacute form that is reported to be more frequent in sheep than in goats, the clinical symptoms are far less severe and in that case, usually the affected animal always recovers (**OIE, 2000**). Described for the first time in Cote d'Ivoire in 1942 (**Gargadennec and Lalanne, 1942**), PPR has been associated for a long time with West African countries (**Taylor, 1979**). However, with the help of new and specific diagnostic tests, the understanding of the geographical distribution of this disease has grown very quickly at the end of 1980's (**Lefevre et al., 1991**). To its historical endemic West African zone, are added all countries of Central Africa, North East Africa, The Middle East and South Asia (**OIE, 2002**). In all these areas, PPR seems to be the major constraint in sheep and goat production (**OIE, 2002**). The causal agent is a member of the Morbillivirus genus as the rinderpest virus (**OIE, 1990**). The closely relationship of these two viruses was demonstrated very early by cross-serological reactions and cross-protections against challenge (**OIE, 1992**). In the absence of a homologous vaccine, this property was exploited to control PPR with the rinderpest tissue culture vaccine over than 25 years period (**FAO, 1996**). However, with the success of the Global Rinderpest Eradication Programme (GREP) (**OIE, 1998**), the use of the rinderpest vaccine in all animals is discontinued (**FAO, 1999**). An effective PPR homologous attenuated vaccine was successfully developed late in the 1980s (**OIE, 1998**). It is now widely used for the control of PPR in domestic small ruminants (**FAO, 1999**). In Egypt, a homologous specific cell culture PPRV-vaccine was successfully produced and proved to be potent and safe (**Khodeir and Mouaz, 1998; Afaf, 1998; Mouaz et al., 1998; Abeer, 1997; Hanan, 1998; Hanan, 2000; Nahed et al., 2000; Ayad Samia et al., 2000 and Nahed et al., 2004**). Several batches of this vaccine have been issued for exportation and used to control PPR in some of the Arabian Gulf and African countries.

Over the 25 years period through which the cell culture rinderpest vaccine was used to control PPR, the immunizing dose for cattle was equally used for sheep and goats (**Scott et al., 1986**). A dose of 3 $\log_{10}$ $TCID_{50}$ was consistently recommended for both cattle and small ruminants through the vast majority of the published work in this respect (**Plowright, 1984**).

Based on comparative evaluation between $TCID_{50}$ titres in cell cultures and immunizing dose in cattle, the potency criterion of rinderpest vaccine was proven and approved to be estimated depending only on the result of infectivity titrations in cell cultures (**James and Rossiter, 1989; OIE, 1996; OIE, 1999**).

This study was aiming for correlating potency of the locally produced homologous PPRV vaccine in cell cultures and in sheep. The results of the study would be contributing in saving money, effort and time spent in carrying out potency tests of the PPRV vaccine in small ruminants.

MATERIAL AND METHODS

2.1. PPRV vaccine manufacture (Live):

Five batches of PPRV vaccine were produced utilizing the Vero cell adapted attenuated strain of PPRV **(Khodeir and Mouaz, 1998)** which was derived from a characterized PPRV strain (Egypt-87) **(House, 1987)**.

Actively growing Vero cells was infected at a multiplicity of infection (MOI) of 0.01 $TCID_{50}$/cell **(Yasumura and Kawatika, 1963)**. The method of virus production, stabilization, freeze drying and storage of the vaccine batches were essentially those recommended by **OIE (1996)**.

2.2. Virus titrations:

This was carried out according to methods described by **Rweyemamu et al. (1991)**.

2.3. Animals:

Forty three healthy 1 year old local breed sheep were used in the study, 25 for vaccine batch 1, 15 for potency of other 5 batches and 3 as control (Tables 1, 2). The animals required for the work were proven seronegative through screening their serum samples to both rinderpest and PPR viruses, just prior to experimentation, performing a virus neutralization test **(Rossiter and Jassette, 1982)**. Three weeks post inoculation, serum samples of test sheep were examined as for qualitative and quantitative estimation of neutralizing response using the same virus neutralization test.

2.4. Virus neutralizations:

The test was done as described by **Rossiter and Jassette (1982)**.

2.5. Comparative estimation of the value of PPRV vaccine batch 1 in Vero cell cultures and in susceptible sheep:

The virus content per vial of the lyophilized PPRV vaccine batch 1 was estimated according to recommended standard operating procedures **(Rweyemamu et al., 1991)**.

Twenty five susceptible sheep divided into groups (each group contain 5 sheep), each sheep was inoculated subcutaneously with 1ml of the respective virus dilutions of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. This procedure was done upon obtaining results of virus titrations. Twenty one

days post vaccination, neutralizing antibody response was estimated through a virus neutralization test (**Rossiter and Jessette, 1982**). Sheep immunizing dose 50 and Vero $TCID_{50}$ were mathematically compared.

2.6. Correlation study of the potency of 5 batches of PPRV vaccine in Vero cells and in susceptible sheep:

Potency estimation on Vero cells of 5 batches of PPRV vaccine including batch 1 was carried out as mentioned above.

Potency estimation of these batches was thereafter performed in susceptible sheep through S/C inoculation of a dose of 2 log_{10} $TCID_{50}$ per head of a 3 sheep groupings corresponding to the respective 5 batches of the vaccine (Table 2).

Twenty one days post vaccination, PPRV neutralization titres were estimated according to the method of **Rossiter and Jessette (1982)**.

RESULTS

3.1. Comparative estimation of the value of PPRV vaccine batch 1 in Vero cells and in susceptible sheep:

Estimation of virus content per vial of batch 1 of PPRV vaccine revealed a potency of 6.3 log_{10} $TCID_{50}$ per ml of undiluted virus suspension on Vero cells. Comparative estimation of the same batch in susceptible sheep showed an ID_{50} of 6.3 log10 for the same volume of virus suspension. Geometric mean neutralizing antibody titres, 21 days post vaccination were 80.6, 80.6, 50.8, 8 and 0, for respective dilutions of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} inoculated to corresponding groups of sheep. These results are given in (table 1).

3.2. Correlative results of studying potency of 5 batches of PPRV vaccine in Vero cells and in susceptible sheep:

Infectivity titres of 5 batches of the PPRV vaccine were respectively 6.3, 6.2, 6.3, 6.4 and 6.3 log_{10} $TCID_{50}$ per 1 ml of undiluted virus content per vial. Respective groups of sheep vaccinated with 2 log_{10} $TCID_{50}$ per head for corresponding batches were found to be 100% virus neutralization test-positive. Geometric mean neutralizing antibody titres, 21 days post vaccination was found to be ranging between 32 and 80.6. Obtained results are depicted in (table 2).

DISCUSSION

In view of consideration given to economy of vaccine manufacture and its quality control, the work presented was aiming to finding out a correlation between Vero cell $TCID_{50}$ and sheep ID_{50} for the locally produced live PPRV vaccine. Five batches of this vaccine were produced using Vero cells as the substrate and the attenuated strain of PPRV as the inoculum, which was derived from the local isolate: Egypt-87. It was found that PPRV content per 1 ml per vial (per batch), was not less than 6.0 $\log_{10}$ $TCID_{50}$ as estimated by Spearman-Karber formula **(OIE, 2000)** (table 2). Such a content per vial was supposed to form upon reconstitution, 100 doses/100 heads of sheep; meaning that 4.0 $\log_{10}$ $TCID_{50}$, at least could be received per head. However, it was found that 5 susceptible sheep being vaccinated with 1ml of the 10-4 dilution representing batch 1, elicited a 100% positivity to a VNT as well as a satisfactory geometric mean neutralizing antibody titre, 3 weeks later (Table 1). Such a result initiated the experimentation of a dose of 2 $\log_{10}$ $TCID_{50}$ in groupings of susceptible sheep, corresponding to the 5 vaccine batches.

Results were quite satisfactory in that such a small dose was sufficient to stimulate the acquisition of neutralizing antibody titres ranging between 32 and 80.6. Moreover, results obtained in this respect in a frequency of 5 times represents the reproducibility of the issue; a criterion known to be essential in such a study. Thus, it would be concluded that, as far as Vero cell infectivity titre which is representing potency in cells of a given PPRV vaccine batch is beyond 2 $\log_{10}$ $TCID_{50}$ per sheep dose, it could be considered as potency positive. Such a procedure would have a positive influence on the economic impact of vaccine quality control work.

It is noteworthy to mention that a considerable number of experiments have been carried out to find the relationship between the titre of the attenuated cell culture rinderpest virus, a quite very closely related virus, as determined by cytopathic effects on the one hand, and by immunizing potency for cattle on the other. The results have shown that there is a very close agreement between titres recorded in the two systems **(Plowright, 1968; Plowright, 1972; Plowright, 1984)**.

Recommendation put by **OIE (1990)** were those of 3 $\log_{10}$ $TCID_{50}$ per dose per head of the cell culture attenuated rinderpest vaccine, either for cattle or small ruminants **(OIE, 1990)**.

In addition, **OIE (2004)** called for a 3 $\log_{10}$ $TCID_{50}$ per dose per head of small ruminants of the PPRV vaccines. **OIE (2004)** norms require a neutralizing antibody titre of not less than 10 for PPRV vaccines.

It is evident that data obtained in the present work satisfy such requirements.

Table 1. Comparative estimation of the value of PPRV vaccine batch 1 in vero cell cultures and in susceptible sheep

Virus dilutions of PPRV vaccine batch No. 1 (log_{10})	Inoculum volume/vero cell culture tube or / head of sheep	No of vero cell culture tubes CPE positive/inoculated	% positive	No. of sheep VNT positive / inoculated	% positive	Geometric mean neutralizing antibody titres 21 days post vaccination of sheep **
10^{-1}	0.1 ml / tube or 1 ml / head	5/5 *	100	ND	-	-
10^{-2}		5/5	100	ND	-	-
10^{-3}		5/5	100	5/5	100	80.6
10^{-4}		5/5	100	5/5	100	80.6
10^{-5}		3/5	60	3/5	60	50.8
10^{-6}		1/5	20	1/5	20	8
10^{-7}		0/5	0	0/5	0	0
10^{-8}		0/5	0	ND	-	-

* $TCID_{50}$ titre on vero cells = 6.3 log_{10}; ID_{50} in sheep = 6.3 log_{10} i.e 1 vero $TCID_{50}$ = 1 sheep immunizing dose 50 calculated by **Spearman-Karber formula (1991).**

** Represented as the reciprocal of the last serum dilution inhibiting the appearance of CPE produced by 100 $TCID_{50}$ per 0.1 ml of PPRV on vero cells.

ND: Not Done.

Table 2. Correlative results of studying potency of 5 batches of PPRV vaccine in vero cells and in susceptible sheep

PPRV vaccine batch No.	Vaccine volume/vial	Supposed doses / vial	Potency in vero cells (virus content / vial)	Potency in sheep (2 log10 TCID50 per head of 3 sheep) seropositive / vaccinated	% positive	Geometric mean PPRV neutralizing antibody titres 21 days post vaccination of 3 susceptible sheep	Expected dose/head on field application (approximate) at first
1	1 ml	100	6.3 *	3/3	100	32 **	4 *
2			6.2	3/3	100	80.6	4
3			6.3	3/3	100	80.6	4
4			6.4	3/3	100	80.6	4
5			6.3	3/3	100	50.8	4
Unvaccinated control group of 3 susceptible sheep						0	

N.B. Least dose content per vial for 100 heads, issued from a manufacturing plant, as recommended by international organization is 5 log_{10} $TCID_{50}$ of PPRV, i.e. 3 log_{10} $TCID_{50}$ / dose / head (**OIE, 2000**).

* Log_{10} $TCID_{50}$ vero cell infectivity titres.

** Estimated as the reciprocal of the last serum dilution that inhibited the appearance of the cytopathic effect produced by 100 $TCID_{50}$ of PPRV on vero cells.

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الملخص العربى

مقارنة فاعلية العترة المستضعفة لفيروس لقاح طاعون المجترات الصغيرة فى خلايا فيرو وفى المجترات الصغيرة

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أجريت هذه الدراسة على عدد خمس دفعات من لقاح طاعون المجترات الصغيرة المستضعف والمنتج محلياً باستخدام عترة فيروس اللقاح المطوعة من العترة المحلية Egypt 87- والهدف من هذا البحث هو استنبط علاقة إرتباطية لما يسمى ب : Potency فى خلايا فيروس وفى الأغنام، وقد تبين من استقراء النتائج أن : (١) معيار فيروس لقاح طاعون المجترات الصغيرة فى الزجاجة المحتوية على ١ مل من المنتج المجفد هو مالا يقل عن 6 $\log_{10}$ $TCID_{50}$ (١٠٠ جرعة / ١٠٠ رأس من الأغنام). (٢) بتجريب حقن عدد خمسة تخفيفات متتالية (١٠-٣، ١٠-٤، ١٠-٥، ١٠-٦، ١٠-٧) للدفعة رقم "١" فى مجموعات خماسية من الأغنام القابلة للعدوى، تبين إيجابية نتائج إختبارات التعادل التى أجريت لأمصال هذه الحيوانات بعد مضى ٢١ يوماً على حقنها بنسب مئوية ١٠٠، ١٠٠، ٦٠، ٢٠، صفر - على الترتيب وبمتوسط معيار مناعى ٦ر٨٠، ٦ر٨٠، ٨ر٥٠، ٨، صفر - على التوالى، وقد تم عمل تجريب آخر بحقن جرعة مقدارها 2 $\log_{10}$ $TCID_{50}$/head والتى تمثل جرعة فى الأغنام، وذلك من كل دفعة من الدفعات الخمس فى خمس مجموعات ثلاثية من الأغنام القابلة للعدوى على الترتيب، وكانت نتائجها إيجابية إختبار التعادل الذى أجرى لكل حيوان منها على حدة بعد مضى ٢١ يوماً على الحقن وبمتوسط معيار مناعى ٣٢، ٦ر٨٠، ٦ر٨٠، ٦ر٨٠، ٨ر٥٠ للمجموعات الخمس على الترتيب .

وقد خلصت نتائج هذا البحث إلى أنه : (١) 1 vero $TCID_{50}$ = 1 sheep ID_{50} (٢) يمكن الاعتماد كليتا على نتائج إختبارات Potency فى خلايا فيرو للحكم على فاعلية اللقاح، وانتفاء حتمية اللجوء إلى إجراء إختبار Potency فى الأغنام، لإيجابية عوائد ذلك من حيث توفير الوقت والجهد والمال اللازم لعمل إختبار Potency فى الأغنام.