THE POTENTIAL USE OF GUINEA PIGS AND MICE AS AN ALTERNATIVE TO SHEEP AND GOATS FOR SAFETY TESTING OF PESTE DES PETITS RUMINANTS LIVE VACCINE

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ABSTRACT

Three sterile, potent and identified separate batches of the locally manufactured live pestes des petits ruminants virus (PPRV) vaccine were subjected to safety testing in rodents (Guinea pigs and mice) as well as in small ruminants (sheep and goats). For each vaccine batch, three susceptible animals of each of sheep and goats, including one pregnant animal per species were inoculated subcutaneously, each with 1ml of the vaccine containing 5 $\log_{10}$ $TCID_{50}$ of the reconstituted randomly selected, statistically representative samples per batch. Same number & status of animals were held as contact control, inoculated S/C, each with the same volume of normal physiological saline solution as a placebo. Corresponding tests in rodents were done using 10 young and 6 pregnant Guinea pigs as well as 10 unweaned and 6 pregnant mice for each of the three vaccine batches. Five young and 3 pregnant Guinea pigs received an intramuscular dose of 0.5 ml of 6 $\log_{10}$ $TCID_{50}$/ml per head/batch. The same dose was given intraperitoneally per head of the rest half number of animals. Ten unweaned and 6 pregnant mice received an intraperitoneal dose of 0.1 ml of 6 $\log_{10}$ $TCID_{50}$/ml per head per batch. A similar number of control rodents were given the same dosing volume of normal physiological saline solution per corresponding routes of inoculations, as a placebo.

All tested small ruminants as well as rodents remained absolutely healthy throughout a three weeks observation post inoculateions. Pregnant animals gave birth to normal healthy suckling offsprings. Non lactating rodents, sacrificed for post-mortem examinations, were absolutely negative to gross pathological findings.

Results obtained would be considered a convincing evidence encouraging the orientation to test the locally produced PPRV vaccine safety in rodents as an alternative to

sheep and goats. This alternation might save a lot of expenses, time and effort spent in performing one criterion of the quality control integration system.

INTRODUCTION

Peste des petits ruminants (PPR) is an acute contagious viral disease of small ruminants caused by a Morbillivirus in the family Paramyxoviridae **(Gibbs et al., 1979)**. PPRV virus is transmitted by aerosols between animals living in close contact (Lefevre and Diallo, 1990). Infected animals show clinical signs of fever, oculonasal discharges, stomatitis, diarrhoea and pneumonia **(Taylor et al., 1990)**. The disease occurs in most African countries south of the sahara and north of the equator **(OIE, 2004)**, and in nearly all Middle Eastern countries up to turkey **(Furley et al., 1987; Lefevre et al., 1991; Perl et al., 1994 and Taylor et al., 1990)**. PPR is also wide-spread in India and south-west Asia **(Shaila et al., 1989)**. The morbidity rate can be up to 100% and in severe outbreaks, with 100% mortality. In milder outbreaks, the mortality rate may not exceed 50% **(OIE, 2004)**.

The OIE International Committee endorsed the use of homologous live PPRV-vaccine (PPRV 75/1) **(Diallo et al., 1989)** in countries that have decided to follow the "OIE Pathway" for epidemiological surveillance for rinderpest in order to avoid confusion when serological surveys are performed **(OIE, 2000)**. Safety testing of this vaccine is done in rodents (Guinea pigs and mice) (OIE, 2004).

Nevertheless, the corresponding locally produced PPRV-vaccine is still tested for safety in small ruminants. Hence, the object of the study presented was aiming at performing an evaluative comparison of the safety test as carried out in both rodents and small ruminants for three separate batches of the PPRV-vaccine, locally produced for exportation purposes.

MATERIAL AND METHODS

Live PPRV-vaccine batches:

Three separate batches of this vaccine were manufactured as routinely produced. The substrate was vero cells **(Yasumura and Kawatika, 1963)** and the inoculum was the vero cell-attenuated PPRV, that was derived from a local isolate designated Egypt-87 **(House, 1987)**. Vaccine batches were stored lyophilized: into a (-20°C) cabinet. They were subjected to identity, sterility and potency testing through recommended evaluative parameters **(OIE, 1996; 2000; 2002 and 2004)**.

Safety testing:

In small ruminants:

Randomly selected, three susceptible, heads per each of the two species, sheep and goats including a pregnant animal per species for each of the three vaccine batches were inoculated subcutaneously each with 1ml containing 5 $\log_{10}$ $TCID_{50}$ of the reconstituted randomly selected, statistically representative samples for each vaccine batch. Three heads per species status were held as contact control inoculated S/C. each with a similar volume of normal physiological saline solution as a placebo.. All animals were ascertained seronegative to PPRV through proven freedom of their sera samples collected just prior to inoculations of PPRV-antibodies. Parameter used was the virus neutralization test (VNT) (**OIE, 1996, 2000 and 2004**). They were kept under keen daily clinical observation throughout a three weeks post inoculations, after which time all animals were bled and their serum samples were subjected to VNT (Descriptive details are found in table 2).

In rodents: safety test was performed according to (OIE, 2004: with modifications of the number of animals):

Guinea pigs:

Five young as well as 3 pregnant animals for each of the three vaccine batches were inoculated I/M, each with 0.5ml of 6 $\log_{10}$ $TCID_{50}$/ml of the reconstituted randomly selectively, representative sample for each vaccine batch. A similar number and status of animals were inoculated I/P with the same vaccine dose.

Mice:

Ten unweaned as well as 6 pregnant mice for each of the three vaccine batches were inoculated I/P, each with 0.1ml of 6 $\log_{10}$ $TCID_{50}$/ml of the reconstituted randomly selected, statistically representative samples for each vaccine batch.

Corresponding number and status of both guinea pigs and mice were held as control, inoculated, per corresponding routes, each with a similar volume of normal physiological saline solution as a placebo (descriptive details are found in table 2).

All test rodents were keenly observed for 3 weeks post inoculations: after which time: non lactating animals were sacrificed, subjected to post-mortem examination.

The methods followed for P.M. examinations were essentially those mentioned in (**Thomson's Special Veterinary Pathology, 1995**).

RESULTS

Sterility and potency of three PPRV-vaccine batches:

Table 1 shows an absolute negativity to microbiological contaminants as tested for the three vaccine batches. It shows, also a $TCID_{50}$ PPRV titres ranging between 6.0 and 6.3 log10 per ml of reconstituted vaccine for the 3 batches.

Safety of the three PPRV-vaccine batches:

In small ruminants:

It was found that all animals included in the test remained absolutely healthy through a 3 weeks observation period post inoculations. Pregnant animals gave birth to normal healthy suckling offsprings. Virus inoculated animals seroconverted. Control ones remained seronegative.

In rodents:

It was revealed that not a single sign of ill-health could be detected in any animal throughout an observation period of 3 weeks post inoculations. Pregnant animals gave birth to normal healthy suckling offsprings. Gross pathological lesions were completely absent in sacrificed non-lactating animals.

Results of safety testing of the three PPRV-vaccine batches are given in table 2.

DISCUSSION

With an expanding global population, the demand for foodstuffs in the future will become ever greater, resulting in increased pressures on the agriculture and livestock industries for higher levels of production. In the case of livestock, control of the major epizootic diseases will be a prime requirement if increased production is to come from making use of the potential for animal husbandry in the developing world. Veterinary vaccines are a major factor in programmes to bring the economically important diseases under control.

Vaccination is a major weapon in the control of many viral diseases of humans and their domestic and pet animals **(Brown, 1990)**. There is no doubt that vaccines have made an enormous impact on the health and consequently the productivity of the recipients **(Brown, 1997)**.

The locally produced live PPRV-vaccine, as derived from a local isolate, designated (Egypt-87),

was and still enjoying much interest, as millions of doses are being exported to countries of the Arabian gulf area **(VSVRI records, 1996-2005)**. At the time of its initial production, scientific quality control committee endorsed the utilization of susceptible small ruminants for safety testing of PPRV-vaccine batches. Since that time, several tens of such vaccine batches have successfully passed the quality control measures applied per batch **(Records of the CLEVB, 1996-2005)**.

In view of the fact that each vaccine batch is subjected to quality control criteria testing for identity, sterility, potency and the safety test is done in small ruminants; it was a good idea to think for application of the safety test in recommended rodents which are guinea-pigs and mice **(OIE, 2004)**. Cognition of the factual identification of the master as well as the working seed virus strain as a prerequisite for perfect vaccine manufacture coupled with the nature of soleness of source; encouraged the orientation to the trend of rodents as an alternation to small ruminants. Such an orientation is not extra-ordinary in its kind, since it is supported by international recommendations **(OIE, 1996, 2000 and 2004)**.

In the present study, susceptible sheep and goats exposed to a S/C PPRV-vaccine dose as massive as 100 times (5 $\log_{10}$ $TCID_{50}$) the field applied dose (3 $\log_{10}$ $TCID_{50}$) failed to display the least sign of ill-health, disease syndrome or side reactions. Moreover, contact control animals remained seronegative, denoting a status of non-virus shedding from inoculated animals which seroconverted. These results were found with the three vaccine batches that were manufactured and tested at separate occasions. Such a reproducibility was found previously with several tens of batches of this vaccine **(CLEVB, 1996-2005)**.

On applying the safety of the PPRV-vaccine batches in rodents, pregnant animals were deliberately included in the test for the three batches, and the total number of both Guinea pigs and mice was multiplied for more convenience in interpretating the obtained results. It was of interest to find out that all pregnant rodents gave birth to normal suckling offsprings. Moreover, not a single sign of ill health, side reactions or disease syndrome could be detected in inoculated rodents, even though receiving doses as drastic as 5 $\log_{10}$ $TCID_{50}$ of the reconstituted PPRV-vaccine. These results as reproduced with three vaccine batches manufactured at different occasions would encourage the reliance on rodents for safety testing of this vaccine. It is worthy to mention that the standard operating procedures described in FAO-Animal Production and Health paper, 118 (1994), gave a detailed description of the methods for safety testing of rinderpest vaccine in rodents (guinea-pigs and mice). These methods are exactly the same, to the most fine details, as those produced in Manual of Diagnostic Tests and Vaccines for Terrestrial Animal, 5th edition, 2004, for safety testing of PPRV-vaccine in the same species of rodents, which

were also followed through carrying out the present work. It is well recognized that both rinderpest and PPR viruses are morbilliviruses sharing a strong antigenic relationship **(Gibbs et al., 1979)**.

Provost et al. (1987) demonstrated the procedures for safety testing of contagious bovine pleuropneumonia vaccine in guinea pigs and mice. These procedures are very approximating those mentioned above for both rinderpest and PPR vaccines.

It is worth mentioning that the safety of the locally produced PPRV-vaccine as performed in small ruminants, is amply documented **(Khodeir and Mouaz, 1998; Mouaz et al., 1998; Abeer, 1997; Afaf, 1998; Hanan, 1998; Hanan, 2000; Nahed et al., 2000; Samia et al., 2000; Nahed et al., 2004 and Laila et al., 2005)**.

The results obtained through the present work would be considered as a convincing evidence on the reliability of rodents as an alternative to small ruminants for PPRV-vaccine safety testing.

Table 1. Sterility and potency testing results of three PPRV-vaccine batches (live)

PPRV-vaccine batches	* Microbiological sterility testing	** Potency (log_{10})
	Absolute negativity to:	
1	bacteria,	6.3
2	fungi and	6.0
3	mycoplasma	6.2

*: as carried out according to standard operating procedures (FAO, 1994).

**: designated as geometric mean $TCID_{50}$ virus titre per ml of reconstituted randomly selected, statistically representative samples per vaccine batch (FAO, 1994).

Table 2. Collective results of safety testing of three batches of PPRV-vaccine (live) in small ruminants as well as in rodents

PPRV-vaccine (live)	Safety testing per batch in:			
	6, small ruminants		32, rodents	
	3, Sheep	3. Goats	16, guinea pigs	16, mice
	including one pregnant animal/species		10. young 6. pregnant	10. unweaned 6. pregnant
	5 $\log_{10}$ $TCID_{50}$ S/C dose/head. 3. heads/species status. contact control		5 (5 $\log_{10}$ $TCID_{50}$, I/M. dose/head. per 5, young and 3, pregnant. Same dose I/P per head/rest half. Similar No./status. control	5 $\log_{10}$ $TCID_{50}$ I/P dose/head. Similar No./status. control
Three separate batches	1. All animals remained absolutely healthy throughout an observation period of 3 weeks post inoculations. 2. Pregnant animals gave birth to normal healthy suckling offsprings. 3. Inoculated animals seroconverted. 4. Control ones remained seronegative		1. Not a single sign of ill-health could be detected in any animal throughout an observation period of 3 weeks post inoculations. 2. Pregnant animals gave birth to normal healthy suckling offsprings. 3. Post-mortem examinations revealed absolute negativity to gross pathology.	

I/M: intramuscular S/C: subcutaneous I/P: intraperitoneal

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الملخص العربى

إمكانية إستخدام خنازير غينيا والفئران كبديل للأغنام والماعز لاختبار سلامة اللقاح الحى لطاعون المجترات الصغيرة الحى

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تم فى هذا البحث إجراء إختبار السلامة (Safety) لعدد ثلاثة دفعات (sterile identified potent) منفصلة من اللقاح الحى لمرض طاعون المجترات الصغيرة - المنتج محلياً - وذلك فى المجترات الصغيرة (أغنام وماعز) وكذلك فى القوارض (خنازير غينيا وفئران) وقد خصص لكل دفعة لقاح عدد (٣) ثلاثة رؤوس من كل من الأغنام والماعز (susceptible) مشتملة على أنثى حامل بكل منهما - حقنت كل حيوان تحت الجلد بجرعة مقدارها (6 $\log_{10}$ $TCID_{50}$/ml) فى ١ ملليليتر من معلق اللقاح الممثل إحصائياً بعينة عشوائية لدفعة اللقاح المختبر - وكذلك خصص لكل دفعة لقاح عدد (١٠) عشرة حيواناتْ يافعة من خنازير غينيا وعدد (٦) ستة من الإناث الحوامل - حقن نصف عدد كل منهما فى العضل بجرعة مقدارها ٥ر٠ ملليليتر من (6 $\log_{10}$ $TCID_{50}$/ml) لكل حيوان وحقن نصف العدد الآخر فى البريتون بذات الجرعة لكل رأس - وقد خصص لكل دفعة لقاح أيضاً عدد (١٠) عشرة فئران رضيعة وعدد (٦) ستة من الإناث الحوامل - حقنت كل فأر منها فى البريتون بجرعة مقدارها ١ر٠ ملليليتر من (6 $\log_{10}$ $TCID_{50}$/ml) من ذات معلق فيروس اللقاح الممثل للدفعة المختبرة، وقد احتفظ بعدد مماثل من جميع هذه الحيوانات كماً ونوعاً - كضوابط حقنت بمحلول الملح الفسيولوجى المعقم بذات الحجم وطريق الحقن النظيرين لما تم تنفيذه بمعلق اللقاح - وقد أبقيت الحيوانات قيد التجارب تحت الملاحظة الإكلينيكية لثلاثة أسابيع متصلة عقب الحقن وقد خلصت النتائج إلى أنه بكل من الحيوانات المحقونة باللقاح وضوابطهما :

أولاً : في المجترات الصغيرة :

١- بقيت جميعها بحالة صحية جيدة ولم يستدل على حدوث أية أعراض مرضية أو آثار جانبية أو ظواهر غير طبيعية.

٢- أنتجت حواملها مواليد طبيعية وبحالة صحية جيدة وتناولت غذائها من أثداء الأمهات بصورة طبيعية.

٣- بقيت الضرابط Seronegative بينما حدث تحول فى الحيوانات المحقونة بفيروس اللقاح (PPRV neutralizing antibody seroconversion).

ثانياً : فى القوارض :

١- بقيت جميعها بحالة صحية جيدة ولم يستدل على حدوث أية أعراض مرضية أو آثار جانبية أو ظواهر غير طبيعية.

٢- أنتجت حواملها مواليد طبيعية وبحالة صحية جيدة وتناولت غذائها من أثناء الأمهات بصورة طبيعية.

٣- بإجراء الصفة التشريحية للحيوانات من غير الأمهات ومواليدها - لم يستدل على وجود أية ظواهر باثولوجية.

وقد خلصت هذه الدراسة إلى أنه يمكن الاعتماد على إستخدام القوارض (خنازير غينيا وفئران) بدلاً من المجترات الصغيرة (أغنام وماعز) لتنفيذ إختبار السلامة (safety) كأحد أركان إختبارات السيطرة النوعية للقاح طاعون المجترات الصغيرة - الحى والمنتج محلياً - إستهدافاً لتقليص التكلفة الاقتصادية وضغط الانفاق وتحجيم الجهد وتوفير الوقت - واستشرافاً لمسايرة الاتجاهات العلمية الحديثة.