PRODUCTION AND EVALUATION OF CELL CULTURE ATTENUATED GOAT POX VACCINE

By

Michael, A. *; Shalaby, M. A. **; Olfat E. Nakhla* and Daoud, A.M. *

** Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.*

*** Virology Dept., Fac. Vet. Med., Cairo Univ.*

ABSTRACT

In this study, production and evaluation of a cell culture attenuated goat pox vaccine was conducted. The obtained results indicated that the "Held strain" of goat pox virus was adapted on three types of cell cultures, lamb testicle, Vero and BHK-21. Clear cytopathic effect (CPE) appeared nearly on the 5th day post inoculation for these types of cells. The tissue culture adapted goat pox virus on Vero cells was selected for vaccine production due to its advantages and it was lyophilized after addition of equal volume of lactalbumin sucrose stabilizer. The lyophilized vaccine had a final titre $10^{6.1}$ $TCID_{50}$/ml in cell culture and $10^{5.0}$ GID_{50}/ml in susceptible goats. Keeping quality and field dose was determined and the humoral antibody level in the serum of vaccinated and control kids were estimated by virus neutralization test (VNT) and enzyme linked immunosorbent assay (ELISA). The antibodies against goat pox could be detected from the 1st week up to the 4th week post vaccination and remained 3 weeks post challenge with the virulent goat pox virus (VGPV). The produced vaccine gave a solid protection against infection up to 9 months after vaccination (Period of study). Attenuated goat pox vaccine was proved to be sterile, safe and potent in protection of kids against pox infection.

INTRODUCTION

Goat pox is a highly contagious disease of goats. It is endemic in most of Africa, the Middle East and Asia. It was first described by **Hansen** in Norway in **(1879)**. High mortality is generally associated with pneumonia (**House, 1992**). The morbidity rate was higher in the adults than the kids and the affected kids had lower body weight gains (**Nagpal *et al.*, 1990**). Goat pox may be mechanically transmitted by insects and control without annual vaccination is extremely difficult in endemic areas (**Kitching *et al.*, 1986 and Carn, 1993**).

Goat pox virus can be propagated on different cell cultures as chorioallantoic membrane of chicken embryo (**Joshi *et al.*, 1996**), kidney and testicle cells of sheep and goats (**Joshi *et al.*, 1994**), sheep thyroid (**Anandan *et***

***al.*, 1976**); embryonic goat lung (**Dubey and Sawhney, 1975**) and on cell lines as Vero cell culture (**Maity *et al.*, 1997**).

The present investigation is the first trial in Egypt to produce a specific goat pox vaccine needed to provide good and virtually long protection against capripox infection.

MATERIAL AND METHODS

Material:

1. Kids:

Thirty-nine susceptible kids, 3-4 months of age, apparently healthy were previously screened for goat pox antibodies by virus neutralization test. They were divided as follows:

- Two kids for titration of the prepared goat pox vaccine.
- Twelve kids for detection of the suitable field dose.
- Eight kids for testing the safety of goat pox vaccine.
- Fifteen kids for estimation of the potency and duration of immunity of the prepared vaccine.
- Two kids for preparation of hyperimmune serum.

2.Virus strains:

a. Reference goat pox virus (Held strain):

It was kindly supplied from Foreign Animal Disease Diagnostic Laboratory (FADDL), Plum Island, USA through Dr. J. House. Its titre was $10^{2.8}$ $TCID_{50}$/ml.

b. Egyptian virulent goat pox virus:

It was obtained from the Pox Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo. It was used for challenge test. Its titre was 10^4 GID_{50}/ml.

3. Cell cultures:

a. Primary cell culture:

Lamb testicles were aseptically obtained from freshly slaughtered young lambs. These cells were used in the adaptation and propagation of goat pox virus.

b. Cell lines:

African Green Monkey Kidney cells (Vero):

Vero cell line was obtained from FADDL, Plum Island, USA. These cells were used in the adaptation and evaluation of goat pox virus.

Baby Hamster Kidney cells (BHK-21):

These cells were used for goat pox virus adaptation and evaluation. It was obtained from VSVRI, Cairo, Egypt.

4. Cell culture media:

The medium used for cell cultures was minimum essential medium (MEM). It was supplied by Sigma Chemical Company, USA. Preparation of growth and maintenance media was done according to **United State**

Environmental Protection Agency (USEPA, Manual of Methods for Virology, 1984). Growth medium was supplemented with 10% newborn calf serum and maintenance medium was provided with 2%.

5. Biological reagents:

a. Bovine serum (Newborn calf serum):

Virus and mycoplasma screened newborn calf serum produced by PAA Laboratories GmbH, Austria. It was used for media supplementation.

b. Hyperimmune serum against goat pox virus:

It was prepared according to **Kalpana *et al.*, (1995)**.

c. Goat pox antigen:

It was prepared according to **El-Bana, (1978)**.

d. Anti-goat IgG peroxidase conjugate:

It was supplied by Sigma, USA and used in solid phase ELISA.

e. Serum samples:

Blood samples were collected at weekly intervals from vaccinated and control kids before and after vaccination and challenge.

6. Stabilizer:

Lactalbumin sucrose stabilizer was prepared according to **OIE Manual Volume 1 (1989)** as 5% lactalbumin hydrolysate and 2.5% sucrose.

7. Antibiotic stock solution:

It was prepared according to USEPA, Manual of Methods for Virology (1984).

Methods:

1. Adaptation of goat pox virus on different cell cultures:

a. On lamb testicle cells:

Goat pox virus was adapted on lamb testicle cells using the technique of **El-Zein *et al.*, (1983)**. Twenty passages were done and titrated on lamb testicle cells till reaching a fixed titre for several successive passages.

b. On Vero cells:

According to **Prakash *et al.*, (1994)**, the adapted goat pox virus on lamb testicle cells which recorded the highest titre was inoculated into confluent sheet of Vero cells.

c. On BHK-21 cells:

The adapted goat pox virus on lamb testicle cells was inoculated onto confluent sheet of BHK-21 cells according to **Kirubaharan *et al.*, (1993 a)**.

2. Growth kinetics of goat pox virus on Vero cell line:

This experiment was done according to **Onar (1973)** and conducted to determine the cell free and cell associated virus titres in Vero cell cultures infected with goat pox virus.

3. Preparation and lyophilization of tissue culture goat pox vaccine:

According to **OIE Manual Volume 1 (1989)**, the virus fluid was mixed with equal volume of the stabilizer and after addition of antibiotics, this mixture

was distributed as 2ml in each 10ml sterile neutral glass vials, then submitted to freeze drying process.

4. Titration of tissue culture goat pox virus vaccine:

a. In Vero cell culture:

The produced lyophilized vaccine was titrated according to **Tiwari and Negi, (1995)**.

b. In susceptible kids:

It was applied according to **Wang and Jiang, (1988 a)**.

5. Selection of the field dose:

It was applied according to **Wang and Jiang, (1988 b)**, in which, twelve kids were used. They were divided into 4 equal groups. The used dilutions of the lyophilized vaccine were (10^{-1}, 10^{-2} and 10^{-3}). Each dilution was inoculated as a field dose in one group (1, 2 and 3) respectively (0.5ml by I/D injection in the ventral aspect of the tail) and the 4th group was left as non-vaccinated control. All groups were challenged with the virulent goat pox virus one month post vaccination (0.5ml in the inner side of the thigh containing 100 virus particles) and were kept under observation for 3 weeks.

6. Evaluation of the produced tissue culture goat pox vaccine:

a. Sterility test:

This test was carried out according to **OIE manual Volume 1 (1992)**.

b. Safety test:

It was applied according to **OIE Manual Volume 1 (1992)**.

c. Keeping quality test:

Vials from the lyophilized goat pox vaccine were preserved at different temperatures (37, +4, -4 and -20°C) and retitrated at different intervals (1, 3, 5, 7 and 9 months) in Vero cell cultures.

d. Potency test and duration of immunity:

According to **Wang and Jiang, (1988b)**, fifteen susceptible kids were divided into 2 groups, the first group of 10 kids were vaccinated with the selected field dose I/D in the ventral aspect of the tail and the second group of 5 kids left as non-vaccinated controls till challenge time. The duration of immunity was estimated for 9 months (period of study). Challenge test was firstly carried out one month post vaccination on 2 vaccinated and one control kids, then repeated every two months with another group of kids till the end of this study.

8. Serological assays:

a. Virus neutralization test (VNT):

It was applied according to the method described by **Martin *et al.*, (1975)**.

b. Solid phase enzyme linked immunosorbent assay (ELISA):

It was done according to the method described by **House *et al.*, (1990)**.

RESULTS

1. Adaptation of goat pox virus on different cell cultures:

a. On lamb testicle cells:

The cytopathic effect was not clear for two blind passages and began to appear by the 8th day post inoculation and this incubation period decreased gradually by serial passages till reaching 5 days after 13 serial passages where the titre was increased and reached $10^{6.2}$ $TCID_{50}$/ml.

b. On Vero cell line:

The cytopathic changes began to appear by the 7th day post inoculation, but after 9 serial passages, it appeared after 4-5 days and reached a titre of $10^{6.5}$ $TCID_{50}$/ml.

c. On BHK-21 cells:

The cytopathic effect began to appear on the 6th day post inoculation, but after 8 serial passages, it appeared 5 days post inoculation and the titre was $10^{5.5}$ $TCID_{50}$/ml.

2. Growth kinetics of tissue culture adapted goat pox virus on Vero cell line:

The titres of both cell free and cell associated virus increased gradually from 60 to 96 hours post inoculation and reached their maximum titres at 120 hours ($10^{6.4}$ $TCID_{50}$/ml) for cell free virus and ($10^{6.0}$ $TCID_{50}$/ml) for cell associated virus as recorded in Table (1).

3. Titration of tissue culture goat pox vaccine:

a. Titration of the vaccine in Vero cell culture:

The titre of the lyophilized goat pox vaccine in tissue culture was $10^{6.1}$ $TCID_{50}$/ml.

b. Titration of the vaccine in susceptible kids:

The titre of the goat pox vaccine in susceptible kids was $10^{5.0}$ GID_{50}/ml.

4. Selection of the field dose:

The results indicated that the dilution 10^{-2} (containing 1000 virus particles) of goat pox vaccine considered the field dose. It was the lowest vaccine dilution which gave 100% protection without unfavourable post vaccinal reaction.

5. Evaluation of the produced tissue culture goat pox vaccine:

a. Sterility, safety and keeping quality results:

The prepared vaccine was proved to be sterile, safe and can be preserved at -4°C or -20°C for 9 months.

b. Results of potency tests:

1. Clinical effect of vaccination:

The post vaccinal reactions appear as slight increase in body temperature for 2 days, and reddish nodular swellings at the site of inoculation which started to diminish 7 days after vaccination and disappeared within 15 days.

2. Clinical effect of challenge:

It was found that the vaccinated kids gave no local or systematic reactions (resisted the challenge), while the control non-vaccinated kids showed the typical symptoms of pox infection. These symptoms were associated with rise in the body temperature which reached 41.2°C and finally, pustules were formed.

6. Duration of immunity:

Results of the challenge were obtained in Table (2), it showed that kids vaccinated with goat pox vaccine were 100% protected against the challenge with VGPV for 9 months.

DISCUSSION

In goat, pox is one of the most serious contagious diseases causing high mortality (80% in adults and reach 100% in kids) and economic losses among susceptible animals as referred by **Sabban, (1960); Saha *et al.*, (1985) and Joshi *et al.*, (1999)**.

Firstly, the "Held strain" of goat pox virus was adapted on lamb testicle cell cultures and gave the same observations of **El-Zein *et al.*, (1983) and Talhouk and El-Zein, (1986)**.

Titration of the adapted goat pox virus in lamb testicle cells revealed that the highest titre of the virus reached ($10^{6.2}$ $TCID_{50}$/ml) and these findings were correspondent to that of **Katiyar and Soman, (1987)**.

Although lamb testicle cells seemed sensitive but it is difficult to maintain and may transmit any other caprine organisms and were not economic for vaccine production. Therefore, the use of cell lines became necessary for vaccine production.

The adapted goat pox virus on lamb testicle cultures was transferred to Vero and BHK-21 cell lines and serial passages were undergone. The results showed that the highest virus titre was $10^{6.5}$ $TCID_{50}$/ml in Vero cells and $10^{5.5}$ $TCID_{50}$/ml in BHK-21 which agreed with the results obtained by **Kirubaharan *et al.*, (1993 b), Prakash *et al.*, (1994) and Maity *et al.*, (1997)**.

The titration of cell free (CF) and cell associated (CA) goat pox virus (Table 1) revealed that the maximum infectivity titres reported 120 hours post inoculation and was $10^{6.4}$ $TCID_{50}$/ml for CF and 10^{6} $TCID_{50}$/ml for CA which agreed with the results of **Rao and Malik, (1982) and Singh and Rai, (1991)**.

The titration of the lyophilized vaccine in Vero cells and in susceptible kids showed that the highest titre of goat pox vaccine was $10^{6.1}$ $TCID_{50}$/ml in Vero cells and in susceptible kids was $10^{5.0}$ GID_{50}/ml which conclude that the titration results in susceptible kids were lower than that obtained in tissue culture which was referred to the self reaction of the living animal and the response of its immune system, but this decrease did not affect the potency of the prepared vaccine.

The prepared vaccine when inoculated on specific media proved negative to any contaminants. The animals inoculated with 100 folds of the field dose showed no unfavourable reaction and withstood the challenge with the virulent virus while the contact control kids and the isolated control animals showed clinical and typical pox infection. These results meaning that the prepared goat pox vaccine was safe and did not spread the virus to in-contact kids. It is in agreement with **Guo *et al.*, (1986); Wang and Jiang, (1988 a) and Agrawal *et al.*, (1995)**.

Challenge test considered as the direct method used for measuring the immunity induced by vaccination (**Fassi-Feri *et al.*, 1984 and Mahmood *et al.*, 1993**).

Moreover, Table (2) revealed that the kids vaccinated with the produced goat pox vaccine were completely protected against the challenge with the virulent virus for 9 months, while the control ones showed severe reactions which was in agreement with **Patnaik, (1986); Rajendran and Kalaimathi, (1992); Guler *et al.*, (1994) and Joshi *et al.*, (1999)**.

From Table (3), it was observed that the neutralizing antibodies of vaccinated kids were detected from the 14th day and reaching its highest level on the 21th day and persisted up to the 28th day post vaccination. The previous results agreed with **Deshmukh and Gujar, (1992) and Rizkallah, (1994)** and disagreed with **Sharma *et al.*, (1987)** who mentioned that neutralization indices after 21 days indicates no rise in antibody concentrations.

As shown in Table (4), ELISA test proved that there was a significant increase in the antibody titres of the vaccinated kids from the first week post vaccination, which gradually increased and at the 3rd week, the antibody titres reached its maximum level that agreed with **Carn *et al.*, (1994); Tiwari *et al.*, (1996) and Tiwari and Negi, (1996)**.

It was revealed that the ELISA was sensitive and detected antibodies from the 1st week post vaccination which agreed with **Williams, (1987); Carn *et al.*, (1994); Tiwari and Negi, (1996 b) and Tiwari *et al.*, (1996)**.

The obtained results concluded that the cell culture attenuated goat pox vaccine proved to be sterile, safe and potent. We recommended the production of this vaccine for mass vaccination of kids and for studying its cross-immunity with other members of capri-pox.

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Table (1): Titration of cell free and cell associated virus of goat pox in Vero cell line.

Time post inoculation in hours	Virus titre expressed in log_{10} $TCID_{50}$/ml	
	Cell Free Virus	Cell Associated Virus
2	3.8	2.9
6	2.5	1.7
12	2.0	1.7
24	2.0	2.8
36	2.4	3.6
48	2.7	3.8
60	3.0	4.0
72	3.5	4.6
84	4.7	4.8
96	5.3	5.3
108	6.4	5.9
120	6.4	6.0
132	6.0	5.5
144	5.9	5.6

N.B. The titre of the original virus (inoculum) was $10^{6.5}$ $TCID_{50}$/ml.

Table (2): Duration of immunity detected by vaccination and challenge.

No. of vaccinated kids	Control kids	Challenge		
		Months post vaccination	No. of vaccinated reacted kids	No. of control reacted kids
2	1	1	None	1
2	1	3	None	1
2	1	5	None	1
2	1	7	None	1
2	1	9	None	1

N.B. Tissue culture goat pox vaccine protected kids from infection for 9 months post vaccination (period of study).

Table (3): Neutralization indices of vaccinated and challenged kids.

Tested kids	Animal Number	Before vaccination	Days Post vaccination				Days post challenge		
			7	14	21	28*	7	14	21
Vaccinated	1	0.1	0.8	1.4	2.2	2.0	1.9	2.0	2.0
	2	0.3	1.0	1.6	2.2	2.2	2.2	2.2	2.0
Safety	1	0.1	1.0	1.7	2.2	2.0	2.0	2.1	2.0
	2	0.0	0.9	1.5	2.0	1.9	1.9	2.1	2.0
Contact control	1	0.2	0.4	0.6	0.5	0.5	1.2	1.8	2.0
	2	0.3	0.3	0.3	0.4	0.4	1.4	2.0	2.1
Isolated control	1	0.1	0.3	0.5	0.5	0.5	1.1	2.0	2.2
	2	0.0	0.1	0.3	0.3	0.5	1.2	1.8	2.2

N.B. Neutralizing index (NI) ≥ 1.5 considered as the lowest protective mean against goat pox virus (**Cottral, 1978**).
* Challenge time.

Table (4): Optical densities of vaccinated and challenged kids as measured by ELISA.

Tested kids	Animal Number	Before vaccination	Days Post vaccination				Days post challenge		
			7	14	21	28*	7	14	21
Vaccinated	1	0.07	0.74	1.80	1.90	1.34	1.67	1.80	1.84
	2	0.14	0.91	1.66	1.97	1.69	2.13	2.45	1.89
Safety	1	0.14	0.80	1.46	2.05	1.77	2.25	2.25	1.84
	2	0.11	0.90	1.70	2.00	1.87	2.11	2.30	2.00
Contact control	1	0.06	0.07	0.10	0.09	0.09	1.25	1.57	2.18
	2	0.09	0.13	0.11	0.17	0.10	1.23	2.00	2.25
Isolated control	1	0.02	0.04	0.90	0.12	0.08	2.08	2.18	2.16
	2	0.09	0.15	0.15	0.16	0.16	1.26	1.63	1.90

N.B. Sample positive ≥ one consider protective (**Williams, 1987**).

الملخص العربي

إنتاج وتقييم لقاح جدري الماعز النسيجي المستضعف

عزيز ميخائيل أسحق* محمد عبد الحميد شلبى**

ألفت إسطفانوس نخلة* أحمد محمود داود*

* معهد بحوث الأمصال واللقاحات البيطرية-بالعباسية-القاهرة.

** قسم الفيرولوجى – كلية الطب البيطري – جامعة القاهرة.

اهتمت هذه الدراسة بإنتاج وتقييم لقاح جدري الماعز النسيجي المستضعف. وقد أفادت النتائج المتحصل عليها أنه تم أقلمة "عترة هيلد" لفيروس جدري الماعز على ثلاث أنواع من الخلايا النسيجية وهى خصية الحملان، كلية القرد الأفريقي الأخضر (فيرو)، كلية العرسة السورية. وظهر التأثير المرضى بوضوح عند اليوم الخامس بعد الحقن في جميع أنواع الخلايا تقريبا. تم اختيار الفيروس المؤقلم على خلايا الفيرو لإنتاج اللقاح بسبب مميزات هذه الخلايا وقد تم التجفيد بعد إضافة مثبت لاكت البيومين سكروز بكمية مساوية للسائل الفيروسي. وكانت عيارية اللقاح المجفد $10^{6,1}$ / ملى على خلايا الزرع النسيجي، 10^{5}/ملى في الجديان القابلة للعدوى. وتم تقدير مواصفات حفظ اللقاح والجرعة الحقلية كما تم قياس مستوى المناعة المصلية المكتسبة في سيرم الجديان المحصنة وذلك باختبارات التعادل المصلى والاليزا وأمكن قياس الأجسام المناعية لجدري الماعز ابتداء من الأسبوع الأول وحتى الأسبوع الرابع بعد التحصين وأستمر وجودها حتى الأسبوع الثالث بعد التحدي بفيروس جدري الماعز الضاري. اللقاح المنتج أعطى حماية تامة ضد العدوى حتى تسعة أشهر بعد التحصين (فترة الدراسة). وثبت أن لقاح جدري الماعز المستضعف المنتج نقى، آمن، وينتج عنه حماية الماعز ضد العدوى بالجدري.