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PREPARATION OF UNIVALENT AND BIVALENT CAPRIPOX VACCINES

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SUMMARY

In the present study four vero cell based live attenuated sheep pox (Kenyan and Romanian strains), goat pox (Held strain) and bivalent (Romanian and Held strains) vaccines were prepared, lyophilized and laboratory evaluated.The results concluded that the three capripox viruses were successfully propagated on the African green monkey kidney (vero) cells with different titres in which the Kenyan strain reached to $10^{5.8}$ $TCID_{50}$/ml; Romanian strain reached $10^{5.1}$ $TCID_{50}$/ml and Held strain $10^{5.0}$ $TCID_{50}$/ml on vero cells. The bivalent SP and GP fluid (RSP & HGP) was prepared by mixing viruses fluids in equal volumes of the same virus titre and its titre before lyophilization was $10^{5.4}$ $TCID_{50}$/ml. The 10% trehalose stabilizer was added in ratio 1:1 (v:v) to the four prepared virus fluids for preparation of the vaccines; then they were lyophilized by freez-drying and retitrated on vero cells after lyophilization, in which a mild losses in their titre, differ between the different vaccines ($10^{5.4}$ $TCID_{50}$/ml for KSP vaccine; $10^{4.8}$ $TCID_{50}$/ml for RSP and HGP and10^{5} $TCID_{50}$/ml for the bivalent SP and GP), that equivalent or overcome the acquired field dose of log $10^{2.5}$ $TCID_{50}$/ml.The prepared attenuated vaccines were tested for sterility to detect any possible contaminant and the results proved that the four prepared vaccines were negative to any contaminating agents as bacteria, moulds and fungi when inoculated on specific media.

INTRODACTION

Sheep pox and goat pox result from infection by sheep pox virus (SPV)or goat pox virus (GPV), closely related members of the Capripox genus of family pox viridae. Most isolates are host specific, with SPV mainly causing disease in sheep and GPV predominantly affecting goats **(Babiuk et al., 2009a).** They are phylogenetically distinct and can be differentiated by molecular tools **(Kargar Moakhart et al., 2007).** Sheep pox and goat pox diseases are endemic in Egypt and both of them are Office International des Epizooties (OIE)

notifiabile diseases, owing to its severity and economic loss in enzootic countries, they are include in list A., **(OIE, 2010)**.

The use of a single sheep pox vaccine is practiced in Egypt for many years but goat pox vaccine, are not commercially available, shepherds occasionally rely either on heterologous sheep pox vaccine, or ovination, which is colligated with rapid spread of virulent virus (**Bhanuprakash et al., 2006**). Goat pox vaccine also are not available in any Arabian or African countries in spite of the fact that goats is highly existing in Egypt and in these countries. Attempts to protect either goats with sheep pox virus vaccines or sheep with goat pox virus vaccines are largely unsuccessful **(Prasad and Datt, 1973, Agrawal and Soman, 1997)**

The combination of sheep pox and goat pox as a combined vaccine has not been tried. There is the potential to use a bivalent vaccine to protect sheep and goats.Preservation of vaccines, viruses and other biological is one of the enormous tasks in maintaining the quality of the products from manufacture until they reach the end users. They usually require cold-chain maintenance during storage and distribution. A number of stabilizers are currently used to help retain the quality of live attenuated viral vaccines, during transit **(Sen et al., 2009)**.

Trehalose has previously been reported to have a protective effect on biomolecules (protein and nucleic acids), cells and simple multicellular organisms against thermal shock **(Carninci et al., 1998; Kaushik and Bhat, 2003 and Litamoi et al., 2005)**. Trehalose was selected to use in preparation of sheep pox, goat pox and bivalent caprine vaccines in this study.Accordingly the aim of the present study is the preparation of bivalent attenuated sheep pox and goat pox vaccine for small ruminants.

MATERIALS AND METHODS

1.Viruses

1. 1. Kenyan sheep pox virus

The reference O180 Kenyan sheep pox (KSP) virus strain had been supplied from foreign animal disease diagnostic laboratory (FADDL), Plum Island, USA. As freeze dried preparation, originally isolated from an outbreak of sheep pox in Kenya by Dr. J. Davies **(Davies, 1976).**

1.2. Romanian sheep pox virus

Romanian sheep pox virus strain was supplied from pox Department, Veterinary Serum and Vaccine Research Institute, Egypt.

1.3. Held goat pox virus

Reference goat pox virus "Held strain" (HGP), originated in Turkey, had been supplied from FADDL, plum Island –N.Y.–USA.

2. Cell culture: African green monkey Kidney cell line (Vero)

It was kindly supplied by Dr. J. House, FADDL, Plum Island, USA.

3. Media

3.1. Minimum Essential Medium (MEM)

The media used for cell cultures was Minimum Essential Medium (MEM) with Earle's salts and L-glutamine without Sodium bicarbonate. It was supplied by Sigma Chemical Company U.S.A. It was prepared according to the manufacturer's instructions. Preparation of growth and maintenance media was done according to United State Environmental Protection Agency **(USEPA, Manual of Methods for Virology, 1984).** Growth media was supplemented with 10% newborn calf serum and maintenance media was provided with 2%. The final pH was adjusted to approximately 7.2 - 7.4 using 7.5% Sodium bicarbonate solution. It was sterilized by filtration.

3.2. Nutrient agar:

It was obtained from Oxoid Ltd., England. It was used for the cultivation of aerobic organism to detect the sterility of propagated virus. It was prepared by suspending 28 gm/liter of DDW and boiling it to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes and PH adjusted to 7.4.

3.3. Thioglycolate medium:

It was obtained from Oxoid Ltd., England. It was used for the cultivation of both aerobic and anaerobic organisms. Put 29.5 gm/liter DDW, boiled to dissolve completely and sterilized by autoclaving at 121°C for 15 minutes. Mix well and cool to approximately 25°C.

3.4. Sabouraud-Dextrose agar

It was obtained from El- Nasr pharmaceutical chemical Company, Egypt. It was used for the cultivation of fungi. Suspend 65 gm/liter of DDW and bring to boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes.

4. Biological reagents

4.1. Bovine Serum:

Virus and mycoplasma screened newborn calf serum (of less than 14 days old) produced by Gibco Laboratories, New Zealand lot 6834217D sterile A. It was used for media supplementation as 10% for growth media and 2% for maintenance media.

5. Buffers and solutions:-

5.1. Bicarbonate solution:

For adjusting the PH of media, 7.5% sodium bicarbonate solution was prepared in double distilled water. It was sterilized by filtration and Kept at $4^{o}C$.

5.2. Antibiotic stock solution:

It was prepared according to **USEPA, Manual of Methods for Virology (1984).** It was added to cell culture media, 1 ml of stock solution was added to each 100 ml of tissue culture media to yield a final concentration of 100 I.U. penicillin, 100µg streptomycin, 2.5 mg neomycin and 50 I.U. nystatin /ml of the medium.

5.3. Trypsin solution:

It was purchased from Difco Lab. Michigan, USA and prepared according to **Lennette (1964).**

5.4. Physiological buffer saline (0.85% - PH 7.2):

It was prepared by dissolving 8.5gm of sodium chloride in 1000ml distilled water.

6. Chemical reagents-Stains

6.1. Methyl violet stain

It was used for staining of tissue culture cells grown in microtiter plates (either normal or after virus inoculation or titration), it was prepared according to **Clayden (1971).**

6.2. Harris haematoxylin and eosin stain:-

According to **(Harris, 1898)** it was used for staining cells infected with sheep pox or goat pox virus, to reveal the cytopathogenic changes and appearance of the intracytoplasmic eosinophilic inclusion bodies.

7. Microplates

7.1. Tissue culture plates:-

Sterile microplates (Nunc) Inter Med, 96 well, were used in titration of sheep pox or goat pox virus and vaccines.

8. Stabilizer:-Trehalose

Trehalose (α-D- glucopyranoside dehydrate) was obtained from SERVA Electrophoresis Company. It was used as 10% concentration in preparation of stabilizer **(Christine et al. 2010);** it was sterilized by autoclaving according to **Kitching et. al. (1986b).**

Experiments and Results

I.1. Propagation of Caprine pox viruses in African green monkey kidney cell (VERO cells)

Sheep pox viruses "Kenyan and Romanian strains" and goat pox virus Held strain propagated in Vero cells according to **Singh and Rai (1991) and Prakash et al. (1994).** The virus was inoculated on confluent sheet Vero cells, incubated for one hour at 37°C to allow the virus to be adsorbed on the cells with occasional tilting, covered with maintenance medium, then incubated at 37°C with daily observation for appearance of CPE. The inoculated cultures were exposed to three cycles of freezing and thawing and centrifuged at 3000 rpm for 10 minutes. The supernatant fluid was titrated on Vero cell culture. According to **Vegad and Sharma (1973) and Rizkallah (1994),** a change in Vero cells infected with every strain of sheep pox or goat viruses were studied.

The cytopathic changes after inoculation with Kenyan SP virus began to appear 24 hours post inoculation and practically the cells become increasingly rounded and granular until 80-90% on the 4th day. There was a restricted slow detachment of the degenerated cells, but the majority appeared to be still present. It was remarkable that the detachment of the degenerated cells from the sheet was poor. Many of the cells show finally granular intracytoplasmic acidophilic matrices with basophilic inner bodies, often in the shape of broken arc or continuous hoop towards the periphery of the cell.The cells contained one to several intracytoplasmic inclusions of varying size and the affected cells become rounded and shrunken.

The cytopathic changes from Romanian SPV began to appear 3rd day post inoculation and reach the maximum (80-90%) on the 5th day. Affected cells were granular, shrunken and rounded in appearance,

characterized with an increase in the size of individual foci and create irregular holes. In general no great difference in cytopathic changes between Kenyan and Romanian sheep pox viruses in Vero cells. The previously adapted goat pox virus "Held strain" was propagated on Vero cell culture according to **Prakash et al. (1994) and Olfat (2000).** The cytopathic changes began to appear on the 4th day post inoculation, as a foci of rounded and refractile cells, then forming of syncytia accompanied with intracytoplasmic inclusion bodies.

I.2.Detection of the harvestation time of the different sheep pox and goat pox viruses adapted on Vero cells

According to **Tozzini et al. (1987),** prescription bottles containing confluent sheet of Vero cell cultures were inoculated with Kenyan, Romanian SPV and Held GPV, then kept for 7 days under examination, daily harvestation and titration on Vero cell cultures.

Results

Inoculation of different sheep pox viruses (seed virus) "K. and R. strains" and Held goat pox virus "H. strain" on Vero cell line was characterized by appearance of CPE from the 1st day post inoculation of K strain; on the 3rd day for R strain and on the 4th day for H strain by changing in the normal cell figure and this CPE increased day by day and became complete at the 4th, 5th and 6th days respectively. It was detected that the CPE occurred while the cells were poorly detached from the wall of the bottles.By titration of the daily harvested virus, it was found that the average titre were $10^{5.8}$; $10^{5.1}$ and $10^{5.0}$ $TCID_{50}$/ml respectively at the 6th day post inoculation where nearly 90% CPE was present and then began to decrease.

I.3. Titration of both Kenyan and Romanian sheep pox viruses and a Held goat pox on Vero cell culture

The harvested pox viruses were titrated in Vero cell culture according to **House (1989) and Tiwari and Negi (1995)** as follows:Serial ten fold dilutions of the adapted pox viruses were prepared from 10^{-1} to 10^{-7}.100µl of each dilution was inoculated into each of five wells of microtitre plates having monolayer Vero cells. Five control non infected microtitre plate wells were involved in the experiment and the plates were incubated at 37°C in Co_2 incubator for one hour, then 100µl of maintenance medium was added to each well and reincubated at

37°C and examined daily to detect CPE specific for each virus. The virus infectivity titre was calculated according to the method described by **Reed and Muench (1938).**

Results

The average titre of the viruses fluids of K, R and H strains (table 1) reached to $10^{5.8}$ $TCID_{50}$/ml; $10^{5.1}$ and $10^{5.0}$ respectively without great change between the titre of three passages.

Table (1): The average titre of Kenyan and Romanian sheep pox viruses and Held goat pox virus on Vero cell cultures before lyophilization:

Batch number	Titre in log 10 (TCID50/ml)		
	Kenyan	Romanian	Held
1	5.6	5.0	4.8
2	5.7	5.2	5.0
3	6.0	5.2	5.2

TCID50 = Tissue culture infective dose fifty.

2. Preparation and titration of different pox vaccines formulas

2.1. Preparation of virus fluid

Five milliliters of the adapted sheep pox (KSPV and RSPV) and goat pox (HGPV) seed viruses each was inoculated in Roux bottles containing confluent growth of Vero cell cultures. The inoculated bottles were incubated for one hour at 37°C to allow virus adsorption, and then 75 ml of maintenance media were added. All bottles were incubated at 37°C and examined daily for 4-6 days for the development of CPE. The inoculated bottles were subjected to 3 cycles of freezing and thawing, harvested aseptically, and centrifuged at 3000 r.p.m. for 10 minutes. The supernatant fluid constitutes the virus fluid.

Results

The viruses fluids were collected separately and kept in deep freezer (-20°C) until they prove sterile, and then titrated before mixing with the different stabilizers.

2.2. Preparation of different pox vaccines:-

The two different sheep pox (KSPV and RSPV) and a goat pox (HGPV) vaccines were prepared by addition of stabilizer solution to the virus fluid of any attenuated live pox virus at the ratio 1:1 (v:v). The bivalent pox vaccine (RSPV and HGPV) was prepared by mixing

viruses fluids in equal volumes of the same virus titre, then 10% trehalose stabilizer was added in ratio 1:1 to mixed viruses fluid. The prepared vaccines were distributed in sterile neutral vials as 2ml of each vaccine / 10 ml glass vial, then the vials contents were lyophilized stoppered, capsulated and stored at -20°C.

3. Titration of different caprine pox vaccines before and after lyophilization:

Titration was applied directly before and after lyophilization according **to Rao and Malik (1982) and Tiwari and Negi (1995).** The titre of caprine pox virus vaccines was expressed by TCID50 and calculated according to the method of **Reed and Muench (1938),** for selection of the higher prophylactically effective titres.

Result

The caprine pox viruses vaccines titers before and after lyophilization were illustrated in table (2).

Table (2): Effect of lyophilization on different caprine pox vaccines:

Vaccine formula	Titration before lyophilization log10 (TCID50)*	Titration after lyophilization log10 (TCID50)*	Losses in titres log10 (TCID50)
KSPV	$10^{5.8}$	$10^{5.4}$	0.40
RSPV	$10^{5.1}$	$10^{4.8}$	0.30
HGPV	$10^{5.0}$	$10^{4.8}$	0.20
Bivalent (RSPV and HGPV)	$10^{5.4}$	$10^{5.0}$	0.40

* The average of the titre of 3 experimentally lots prepared.

$TCID_{50}$ = Tissue culture infective dose fifty.

4. Sterility test

This test was carried out according to **OIE Manual volume I (2004).**

Results:

Results of the four tested vaccines samples were negative to any contaminating agents, and thus it were passed to be used for other experiments.

DISCUSSION

Across the developing world, viral pathogens such as Capripox viruses (CAPV) place a huge disease burden on agriculture, in particular affecting small ruminant production and in turn increasing poverty in some of the poorest parts of the world.Vaccination has been considered

to be the cheapest and sustainable means of Capripox diseases control in enzootic situation **(Kallesh et al., 2009).** The use of single vaccine for each of sheep pox and goat pox was practiced in different countries but their combination as a bivalent vaccine has not yet been tried. Here we tried to control these diseases (SP and GP) through production of an efficient bivalent vaccine that could when used optimally, will reduce the need for expensive pharmaceutical intervention, and improve the health and welfare of animals, through antithetic the endemic SP and GP diseases, and this may promote a wider use of vaccination for their control in Egypt.

In this study, according to **Tozzini et al. (1987); Singh and Rai (1991) and Prakash et al. (1994)**, sheep pox viruses (Kenyan and Romanian) and Goat pox virus (Held strain) were inoculated on confluent Vero cell cultures. Cytopathic changes (CPE) began to appear early at 24 hours post inoculation, because they were previously activated by propagation on the Vero cells for different passages after activation through the primary cell cultures. CPE increased daily till reaching its maximum at 4-6 days. It was found that the highest titre was present in the bottles harvested on the 4th day post inoculation in case of Kenyan strain; on 5th day for Romanian strain and on the 6th day for held strain where the CPE was nearly 90%, these results supporting and agreed with the results obtained by **Singh and Rai (1991); Rizkallah (1994); Olfat (2000) and Christine (2008).**

Vero cell was selected for propagation of these viruses, it is the most commonly used cell system for Capripox viruses. Use of these cells is preferred mainly because of their ability to support the replication of a variety of Capripox isolates (**Singh and Rai, 1991; Prakash et al., 1994; Rizkalla, 1994; Maity et al., 1997; Olfat, 2000 and Hosmani et al., 2004a)** Vero cell line minimize animal use (lambs or kids), has necessitated investigating the suitability of continuous cell line. The primary and secondary cultures still have many of the detrimental, inherent characteristics, such as cell heterogeneity and the presence of potential contaminating elements such as endogenous viruses. The presence of a suitable continuous cell line susceptible to propagation of

capripox viruses, has invigorate the development, validation and wide spread use of virus isolation, replication, titration, serological detection, laboratory diagnosis and consequently vaccine production.

For preparation of viruses fluid the inoculated bottles that incubated at 37°C and examined daily for the appearance of CPE, and when the CPE reached about 90%, they were subjected to 3 cycles of freezing and thawing, harvested aseptically and centrifuged at 3000 r.p.m. for 10 minutes and the supernatant fluid constitutes the sterile virus fluid and it was titrated on Vero cells Table (1), in which the titre average reached to $10^{5.8}$ $TCID_{50}$ for KSP and $10^{5.1}$ $TCID_{50}$ for RSP and $10^{5.0}$ $TCID_{50}$ for HGP. The freezing, thawing and centrifugation is applied for preparation of pox virus fluid because the highest percentage of progeny virus persist inside the infected cells as intracellular virus, and this was in conform with **Reed and Munch (1938)** for assessment the number of infective viral particles in the vaccine, TCID50 assay in cell culture are commonly used **Srivastava and Singh (1980).**

It was in correspondence to **Kitching et al. (1987),** who reported that when 90% of the cells were showing cytopathic effect, and five days after infection with sheep pox virus, the culture was frozen at ---20°C and thawed. The suspension was centrifuged at 1000g for 10 minutes and the supernatant mixed with an equal volume of stabilizer. It was in agreement with **Talhouk and El-Zein (1986)**, who display that for the goat pox virus intracellular virus was much higher than extracellular virus The bivalent sheep pox and goat pox vaccine was prepared according to **OIE Manual volume 1 (2004),** by mixing of the attenuated Romanian SP and Held GP viruses fluids at the ratio 1:1 (v:v) of the equal titres (TCID50 10^5/ml) that containing the recommended doses of both viruses, and they mixed with the selected stabilizer solution (trehalose 10%) in an equal volume.

According to **Gary (2004),** combined vaccine consist of two or more separate immunogens combined in a single product and administered through the same syringe, for example of some of vaccines licensed in the united states were the influenza vaccine (in 1945) is a combination of three different strains of influenza virus. In the present study, live virus content has been evaluated by TCID50, for quality control of live

Capripox vaccines alone or in component of the bivalent vaccine.One of the object of the present study is to provide an improved stabilizer to have a prolonged store stability with diminished reduction in titre, for this reason we use trehalose 10% according to **Christine et al. (2010)** for preparation of four pox vaccines.

The mean loss of sheep pox virus titre following freeze-drying are given in table (2), Fig. (1). The results proved a significant difference in the losses of the different tested vaccines ranged from log10 (0.2 TCID50 in case of Held strain, 0.3 for Romanian strain and 0.4 for Kenyan and bivalent vaccines). According to **OIE (2004),** the four prepared vaccines, have a minimum titre log 4.5 TCID50/ ml after freeze drying that equivalent to a field dose of log10 $^{2.5}$ $TCID_{50}$/ ml, Table (2).Selection of trehalose 10% as a stabilizer was in agreement with **Christine (2008),** who founded that after preparation of experimental eight sheep pox vaccine formulas they were lyophilized in the same tray and under the same conditions and were titrated directly before and after lyophilization in order to induce the protective effect of stabilizers, the results proved a significant difference in the losses of the different tested vaccines following freeze-drying ranged from log10 0.3 TCID50 in case of 10% Trehalose stabilizer to $log10^{1.25}$ $TCID_{50}$ of 5%LA: 5%Sucrose (control vaccine), which was corresponded to **Sampedro et al. (1998)**, who reported that during freeze-drying and rehydration, the protective efficiency of carbohydrates was as follows: Trehalose > maltose > sucrose. **Joi and Rojiv (2003),** stated that Trehalose found to be very effective in the stabilization of labile proteins during lyophilization and exposure to high temperature in solution.

The prepared attenuated vaccines were tested for sterility to detect any possible contaminant and the results proved that the four prepared vaccines were negative to any contaminating agents as bacteria, moulds and fungi when inoculated on specific media.The results affirmed the preparation of a highly efficient bivalent vaccine against both of sheep pox and goat pox infection. Comparative immunological studies on the efficacy of the four prepared vaccines in susceptible small ruminant would be applied.

REFERENCES

Agrawal S.K. and Soman J.P.1997. Assessment of immune efficacy of attenuated live goat pox vaccine against sheep pox and contagious ecthyma. ***Ind. Vet. J., 74(12): 1016-1018.***

Babiuk S.; Bowden T.R.; Parkyn G.; Dalman B.; Hoa D.M.; Long N.T.; Vu, P.P.; Bieu D.X., Copps J., Boyle D.B. 2009a. Yemen and Vietnam capripoxviruses demonstrate a distinct host preference for goats compared with sheep.; ***J. Gen. Virol.; 90: 105-114.***

Bhanuprakash V.; Indrani B.K.; Hosamani M., Singh R.K.2006. The current status of sheep pox disease. ***Comp. Immuno. Microbio. and Infect. Dis.; 29(1): 27-60.***

Carninci P.; Nishiyama Y.; Westover A.; Itoh M.; Nagaoka S.; Sasaki N.; Okazaki Y.; Muramatsu M., Hayashizaki Y.1998. Thermostabilization and thermoactivation of thermolabile enzymes by trehalose and its application for the synthesis of full lenghth cDNA. ***Biochemistry.; 95(2): 520-524.***

Christine A. Mikhael .2008. Comparative studies on some stabilizers used in production of sheep pox vaccine. ***M.V.Sc. thesis (virology), Faculty of vet. Med., Cairo University, Egypt.***

Christine A. Mikhael; El-Sanousi A.A.; Reda I. M., Rizkhallah S.S.2010. Role of stabilizers in preparation of a potent sheep pox vaccine. ***Vet. Med. J., Giza.; 58(1):1-13.***

Clayden E.C.1971. Practical section culting and staining. ***5th ed. Edinburgh and London.*** **7:244-246.**

Davies F.G.1976. Characteristics of a virus causing a pox disease in sheep and goats in Kenya, with observations on the epidemiology and control. ***J. of Hyg. (Lond.); Cambridge, 76 (2): 163-171.***

Gary S. Marshall 2004. The vaccine handbook, a practical guide for clinicians. ***Lippincott Williams and Wilkins, A Walters Kluwer Company.***

Harris H.G.1898. Staining the cells by Haematoxyline and Eosin. ***J. Appl. Microb.*** **3:777.**

Hosamani M.; Nandi S.; Mondal B., Singh R.K.; Rasool T.J., Bandyopadhyay S.K. .2004*a.* A Vero cell-attenuated Goatpox virus provides protection against virulent virus challenge. ***Acta virol., 48(1):15-21.***

House J.A.1989. Unpublished information, USDA, APHIS, FADDL, Green Port, NY 11944 (Personal communication).

Joi K., Rojiv B.2003. Why is trehalose an exceptional protein stabilizer? ***J. Biol chem.;. 278(29): 26458 – 26465.***

Kallesh D.J.; Hosamani M.; Balamurugan V.; Bhanuprakash V.; Yadav V., Singh R.K.2009. Quantitative PCR : A quality control assay for estimation of

viable virus content in live attenuated goat pox vaccine. *Ind. J. Exper. Biol.; 47: 911-915.*

Kargar Moakhar R.; Madani R.; Golchinfar F., Shabnam T.M.2007. Purification of specific Capri pox virus protein (P32) for utilizing in ELISA Kit. *Animal and fisheries sci., 20(3)76: 104-108.*

Kaushik J.K., Bhat R.2003. Why is Trehalose an exceptional protein stabilizers? *J. Biol. chens., 278(29): 26458- 26465*.

Kitching R.P.; Mc Grane J.J., Taylor W.P.1986b. Capripox in the Yemen Arab Republic and the Sultanate of Oman. Trop. *Anim. Health. and Prod., 18(2): 115-122.*

Kitching R.P.; Hammond J.M., Taylor W.P.1987. A single vaccine for the control of capripox infection in sheep and goats. *Res. Vet. Sci., 12:53-60.*

Lennette E.H.1964. Diagnostic procedures for viral and rickettsial diseases. *An. Public Health Ass. Inc.3rd Broad way.*

Litamoi J.K.; Ayelet G. and Rweyemamu M.M.2005. Evaluation of the Xerovac process for the preparation of heat tolerant contagious bovine pleuropneumonia (CBPP) vaccine. *Vaccine., 23(20):2573-2579.*

Maity S.; Chattererjee A.; Mitra K.; Chakraborty J.; Nag N.C., Ganguly J.L. 1997. Studies on adaptation of goat pox virus (Uttar Kashi strain) in Vero cell. *Ind. Vet. J., 74(8): 700-702.*

OIE (Office International des Epizooties/World Organization for Animal Health)2004. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 5th ed.

OIE (Office International des Epizooties/World Organization for Animal Health) 2008. Manual of diagnostic tests and vaccines for terrestrial animals.

OIE Manual Volume I .004. Volume I Part I Section I.1. Sheep pox and goat pox. *Chapter 2.1.10. P: 211 – 220.*

OIE Terrestrial Manual 2010. Chapter 2.7.14 Sheep pox and goat pox in manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 1-12.

Olfat E. Nackhla 2000. Study on goat pox virus vaccine, Ph.D. Thesis, (Virology), Faculty of Vet. Med., Cairo University , Egypt.

Prakash V.; Chandra R.; Ras V.D.P.; Garg S.K.; Ved. Prakash, Rajesh Chandra 1994. Cultivation of goat pox virus in an established cell line. *Ind. J. Virol., 10(1): 60-63.*

Prasad I.J., Datt N.S.1973. Observation on the use of live and inactivated vaccines against goat pox. *Indian Vet. J., 50:1-10.*

Rao M.V.S., Malik B.S.1982. Behaviour of sheep pox, goat pox and contagious pustular dermatitis viruses in cell culture. *Ind. J. Compara. Microbiol. Immunal. and Infe. Dis.* 3(1): 26-33.

Reddy G.; Antony P., Srinivasan V.1997. Serological response to combined vaccine of cattle against foot and mouth disease, haemorhagic septicaemia and black quarter. *Indian. J. Anim. Sci.,* 67:585-586.

Reed L.I., Muench H.A.1938. A simple method for estimating fifty percent end point. *Amer. J. Hyge.; 27: 493-497.*

Rizkallah S.S. 1994. Further studies on sheep pox disease in Egypt. *Ph.D. Thesis, Vet. Med. College, Cairo University, Egypt.*

Sampedro J.G.; Guerra G.; Pardo J.P., Uribe S.1998. Trehalose- mediated protection of the plasma membrane H+ - ATPase from Kluyveromyces lactis during freeze-drying and rehydration. *Cryobiology.; 37(2): 131- 138.*

Sen A.; Balamurugan V.; kishor K.R.; Chakravarti S.; Bhanuprakash V., Singh R.K. 2009. Role of heavy water in biological sciences with an emphasis on thermostabilization of vaccines. *Expert review of vaccines vol. 8(11): 1587-1602.*

Singh M.P; Rai, A. 1991. Adaptation and growth of sheep pox virus in Vero cell culture. *Indian Veterinary Medical Journal. 15(4): 245-250.*

Srivastava R.N., Singh I.P.1980. A study on the role of cellular and humoral factors in immunity to sheep pox. *Ind. J. Anim. Sci., 50(10): 861-866.*

Talhouk R., El-Zein A.1986. Characterization of cell culture adapted goat pox virus, *J. Vet. Medi., B-Infectious D., 33: (7): 543-551.*

Tiwari A.K., Negi B.S. 1995. Neutralization ability precipitinogens of goat. *Ind. J. Compar. Microbiol. Immunal. and Infe. Dis, 16(1-2): 64-65.*

Tozzini F.; Salim H.A.; Abdijabar H.D., bdulcadir A.F.1987. Growth characteristics of vaccine strains of rinderpest virus and ovine pox-virus on subcultures of bovine fetal lung fibroblasts. *Archivio Veterinario Italiano, 38(1): 9-12.*

USEPA Manual of Methods for Virology 1984. United State Environmental Protection Agency Manual of Methods for Virology. *Cincinnati, Ohio 45368.*

Vegad J.L. and Sharma G.L. 1973. Cutaneous and pulmonary lesions of sheep pox. *Indian J. Anim. Sci., 43(12): 1061-1067.*

تحضير لقاحات أحاديه و ثنائيه للكابري الجدري

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في هذه الدراسه تم تحضير أربعه لقاحات مستضعفه علي خلايا ڤيرو وهي لقاحات جدرى الاغنام (العتره الكينيه، العتره الرومانيه) و جدرى الماعز (عتره هيلد) و اللقاح الثنائى (من العتره الرومانيه و عتره الهيلد)- و تم تجفيدها و تقيمها معمليا". أثبتت النتائج نجاح أقلمه الثلاث عترات الكابري جدريه على خلايا كليه القرد الاخضر الافريقى (ڤيرو) بقوي عياريه مختلفه حيث و صلت الى $^{٥.٨}$١٠/مللى للعتره الكينيه، $^{٥.١}$١٠/مللى للعتره الرومانيه ، ^{٥}١٠/مللى للعتره الهىلد ،اللقاح الثنائى لجدرى الأغنام و جدرى الماعز (من العتره الرومانيه و عتره الهيلد) تم تجفيدها بخلط مقادير متساويه من سوائل كلا" من الڤيروسين الذين لهما نفس العياريه و كانت عياريتها قبل التجفيد ^{٤} $^{٥.}$١٠/مللى لتحضير اللقاحات أضيف مثبت التريهالوز ١٠٪ بنسبه ١:١ حجما" الى سوائل الڤيروسات الأربعه، ثم تم تجفيدها و اعاده معايرتها بعد التجفيد و ذلك على خلايا ڤيرو، حيث وجد نقص طفيف في عياريتها و تختلف درجته فيما بين اللقاحات المختلفه (^{٤} $^{٥.}$١٠ للقاح جدري الأغنام العتره الكينيه، $^{٤.٨}$١٠ لكلا من للقاح جدري الأغنام العتره الرومانيه ، للقاح جدري الماعز العتره الهىلد، ^{٥}١٠/مللى للقاح جدرى الأغنام و جدرى الماعز الثنائى) و التي تساوي أو تفوق الجرعه الحقليه المطلوبه ($^{٢.٥}$١٠/مللى).تم اختبار النقاوه للقاحات المستضعفه المحضره وكانت خاليه من أي عوامل للتلوث عندما تم حقنها في ميديات متخصصه.