Preparation of trivalent vaccine against lumpy skin disease using different capripox viral strain

***Kafafy, M. H., ** El Soally, S.A., *Aboul-Soud, E. A., ***Zaghloul, M.A. and ***Christine A. Mikhael**

***Veterinary serum and Vaccine Research Institute, Pox department, Abbasia, Cairo**

****Military Veterinary Hospital. Cairo**

*****Central Laboratory for Evaluation of Veterinary Biologics**

Abstract

Vaccination is considered the most effective measure to control lumpy skin disease, Various types of Capripoxvirus vaccine strains are used in vaccination programs. In Egypt, since the appearance of LSD in 1988, various live attenuated sheep pox vaccines were used. The aim of this study was preparation and evaluation of a new live attenuated tissue culture trivalent vaccine from two sheep pox viruses (Romanian, Kenyan sheep pox) and one goat pox viruses (Held goat pox virus) as a trial to improve vaccination against LSD. Capripox viruses were titrated in Vero cell, and were mixed with each other in equal volumes (1:1:1) with equal titers ($4.5\log_{10}TCID_{50}$/ml). The experiment was performed using 24 calves of 6-12 months, They were divided into three groups; the first group of 12 animals were vaccinated with the new trivalent vaccine, second group of 12 animals were vaccinated with monovalent Romanian sheep pox vaccine and the third group of animals were left as non-inoculated contact controls. The cellular immunity of all animals were estimated using lymphocyte blastogenesis measured by XTT assay and the humoral immunity were evaluated by serum neutralization test and ELISA. The results revealed that the trivalent vaccine showed higher level of cellular immunity for longer perioed as compared to the monovalent Romanian sheep pox vaccine. The level of antibodies measured by SNT and ELISA was protective in animals vaccinated with both vaccines. Challenge test was applied using 0.5 ml of virulent LSDV ($5\log_{10}TCID_{50}$/ml) every 3 months till the end of experiment (12 months post inoculation). The trivalent vaccine was protective in 100%, 100% , 66% of vaccinated animals at, 6, 9,12 months post vaccination respectively, while the potency of Romanian Sheep Pox Vaccine

was 100%, 100%, 33% at the same periods No post vaccinal reaction appeared on all vaccinated animals .

In conclusion the current study proved that a trivalent vaccine (Romanian, Kenyan sheep pox and goat pox viruses) was safe, potent, high immunogenic and provide long duration of immunity.

1. Introduction

Sheep pox virus (SPV), goat pox virus (GPV) and Lumpy skin disease virus (LSDV) comprise the Capripoxvirus genus within family *Poxviridae* **(Buller et al., 2005).** Sheep pox (SPV) and goat pox (GPV) are endemic in northern and central Africa and in large parts of Asia. Lumpy skin disease (LSD) occurs across Africa and has recently been aggressively spreading in the Middle East, despite excessive vaccination campaigns carried out in the region. Because SPV and GPV do not occur in southern Africa, only attenuated LSDV vaccines are used against LSDV. Whereas, in central and northern Africa and in the Middle East, where the distribution of SPV, GPV and LSD overlap, attenuated SPV vaccines, such as KSGP O-240, Yugoslavian RM65 and Romanian SPV strains, have been used for the control of LSDV **(Brenner et al., 2009; Davies, 1991; Somasundaram, 2011).** The three virues share common major antigens, responsible for the induction of neutralizing antibodies **(Kitching, 1986).**

The efficacy of LSDV vaccine was superior when compared with the RM65 SPPV (10X) vaccine **(Gera et al., 2015)**.

The Ethiopian Neethling and KSGP O-180 (Kenya sheep and goat pox vaccine) vaccines failed to provide protection in cattle against LSDV, whereas the Gorgan GPV vaccine protected all the vaccinated calves from development of clinical signs of LSD **(Gari et al., 2015)**.

The capripox viruses strain were antigenically identical. Sequence analysis indicated that field skin LSDV isolate is more related to tissue culture adapted LSDV/Ismailyia88 strain than to vaccinal SPV/ Kenyan strain and the skin

isolate of SPV is more closely related to field skin isolate of LSDV than to SPV/Kenyan vaccinal strain **(El-Kenawy, and El-Tholoth 2010),**

GPV and LSDV are more closely related to each other than to SPV **(Le Goff C et al., 2009)**

The Kedong strain has been used as a vaccine for cattle against LSDV in Kenya **(Coakley and Capstick, 1961).**

CaPV infecting all three species: sheep, goats and cattle. The major difference between the African and the Middle Eastern and Indian SPV and GPV strains seems to be the wider host range of the African isolates **(Davies, 1976).** The Kenyan sheep-1 (KS-1) strain is derived from the attenuated KSGP O-240 vaccine strain **(Chand et al., 1994).**

Recent molecular studies have reported a close relationship between the KS-1 and LSDV, suggesting that KS-1 is actually LSDV **(Tulman et al., 2002).**

Sheep pox virus vaccines have been widely used for cattle against lumpy skin disease virus Due to cross-protection within the Capripoxvirus genus **(Eeva, 2014).**

Combined immunization including two types of vaccines may be more effective than either immunogen alone **(Hu SL et a1., 1991).**

Aim of this study is to improve the control strategies against LSD in Egypt. So this paper describes the evaluation of prepared trivalent vaccine used to control lumpy skin diseases in Egypt.

2. Material and Methods

2.1. Animals:

24 cross breed apparently healthy susceptible calves of about 6-12 months old were previously screened for free of specific antibodies against LSD virus. They were used for vaccination with different prepared vaccines and challenge test.

2.2-Viruses and vaccines:

2.2.1. Romanian sheep pox virus and vaccine (RSPV):

The virus was cultivated and propagated on African green monkey kidney cells (Vero cells) and the vaccine is produced according to **(Rizkallah ,1994).** And had a titer of $log10^{5.5}$/ml $TCID_{50}$.

2.2.2 Kenyan sheep pox virus (KSPV):

The virus had been supplied from Foreign Animal Disease Diagnostic laboratory (FADDL), plum Island, USA. It was passaged on fetal bovine lung for successive passages then adapted on Vero cell (African green monkey kidney cells) In Egypt (**Rizkallah, 1994).** And had a titer of $log10^{5}$/ml $TCID_{50}$.

2.2.3. Held goat pox virus (HGPV):-

Reference goat pox virus "Held strain" (HGP), originated in Turkey, had been supplied from Foreign Animal Disease Diagnostic laboratory (FADDL), plum Island –USA. It was passaged two times on lamb testicle cells and for one passage on sheep choroid plexus cells. In Egypt, the virus adaptation was completed by **Olfat (2000)**, for another sixteen passages on lamb testicle cells and for fourteen passages in Vero cells, And had a titer of $log10^{4.5}$/ml$TCID_{50}$.

2.2.4. Lumpy skin disease virus (LSDV): (A virulent challenge virus)

Virulent LSD local isolate Ismailia strain, had supplied from Foreign Animal Disease Diagnostic laboratory (FADDL), plum Island–USA, it is titer was $log10^{5}$/ml $TCID_{50}$ (**Aboul Soud 1995).**

2.2.5-Preparation of trivallent Romanian and Kenyan SP and Held GP experimental vaccine:-

The attenuated RSP, KSP and HGP viruses strains fluids were mixed with each other's at the ratio of 1:1:1 (v:v) of equal titers ($10^{4.5}$ $TCID_{50}$/ml for each one) one vial 1ml for 50 animal(50dose).

2.3.Cells culture:

2.3.1. African Green Monkey Kidney cell line (VERO) cell: were used for RSP, KSP & HGP viruses propagation, titration and serological tests.

2.3.2.Madin-Darby bovine kidney cell line (MDBK) cell: were used for LSD virus propagation, titration and serological tests.

2.4. Stabilizer:

The stabilizer used was the Lactalbumin-Sucrose, in which the lactalbumin hydrolysate (5%) was added to sucrose (2.5%) in double distilled water **(OIE 2010)**, the mixture was sterilized by filtration.All the experimental vaccine batches were prepared by mixing stabilizer solution (lactalbumin sucrose) with the virus fluids. To each 100ml vaccine 100IU/ml penicillin and 100µg/ml streptomycain sulfate; were then submitted to lyophilization and stored at -20° C.

2.5. Chemical and biological reagents:

Heparin, Foetal calf serum, Trypan blue stain used in the lymphocyte blastogenesis assay.

2.6. Kits

2.6. 1. ELISA KIT:ID Vet Capripox ELISA Kit, France.

2.6. 2.XTT Cell Viability Assay Kit: The kit was supplied by (AppliChem) and used in the lymphocyte blastogenesis assay.

2.7- Titration of Capripox vaccines before and after lyophilization:

It was applied according to **Tiwari and Negi (1995)**. The titre of any of these vaccines was expressed by $TCID_{50}$ and calculated according to the method of **Reed and Muench (1938)**.

2.8. Sterility test:

It was carried out according to **OIE (2010).**

2.9. Animal Vaccination and experimental challenge:

As described by **Sabban (1960))** 24 apparently healthy susceptible calves of about 6-12 months old were divided into 2 groups, each of nine calves in group 1 were vaccinated intradermally in the tail fold with 1ml of the field dose of the attenuated RSPVV (available vaccine), nine calves in group 2 were vaccinated intradermally in the tail fold with 1ml with trivalent (RSP, KSP& HGP); Beside another 6 calves as a control (non vaccinated).

2.10, Sample Collection:

Serum samples: were collected from calves just before and weekly after vaccination interval, each group were divided into subgroups; for 6 month in subgroup (1), 9 month in subgroup (2) and 12 month in subgroup (3) and from the control. Samples were stored at -20°C until examined by serological test.

Whole blood: samples were collected on heparin (heparin sodium) containing syringe then directly tested for estimation of the cellular immunity on day 0, 1, 3, 7, 10, 15, 20, 30 and 40 post vaccinations.

2.11. Evaluation of the cell mediated immune response:

Assay of lymphocyte blastogenesis (XTT):

It was applied according to the method adopted and modified by **El Watany et al. (1999**).

2.12. Evaluation of humoral immune response:

2.12.1. Serum Neutralization Test (SNT):

This was applied according to the method described by **House et al. (1990)** the neutralizing index (NI) was calculated according to **Reed and Muench (1938)**.

2.12.2. Indirect ELISA:

It was applied according to **Babiuk et al. (2009)** by using ID Vet Capripox ELISA Kit **.**

2.13. Challenge test

Both the vaccinated and unvaccinated groups were housed together prior to challenge using virulent LSD strains. Viral challenge was performed in both vaccinated and non vaccinated animals using a 0.5 ml intradermal injection of the (10^5 $TCID_{50}$/ml) **(Bowden et al., 2008).**

The animals were grouped in GP1:8 calves (3 vaccinated with RSP vaccine, 3 vaccinated with trivalent vaccine & 2 control) challenged after 6 months, GP2:8 calves (3 vaccinated with RSP vaccine, 3 vaccinated with trivalent vaccine & 2 control) challenged after 9 months and GP3:8 calves (3 vaccinated with RSP vaccine, 3 vaccinated with trivalent vaccine &2 control) challenged after 12 months. All animals were observed daily, with clinical signs recorded

throughout the study. Rectal temperatures were measured daily for up to 21 days post challenge (DPC).

3. Results

1-Evaluation of the cell mediated immune response of calves:

Assay of lymphocyte blastogenesis (XTT):

The results were illustrated in figure (1) disclosed that all vaccinated calves with different capripox vaccines had variable cellular immune responses depending on the vaccine used and they reached to the maximum nearly on the 10th day post vaccination in RSPV then decreased at 21 DPV ; while in trivalent vaccine the peak was at 7th , 10th and 15th DPV, then decreased at 40 DPV.

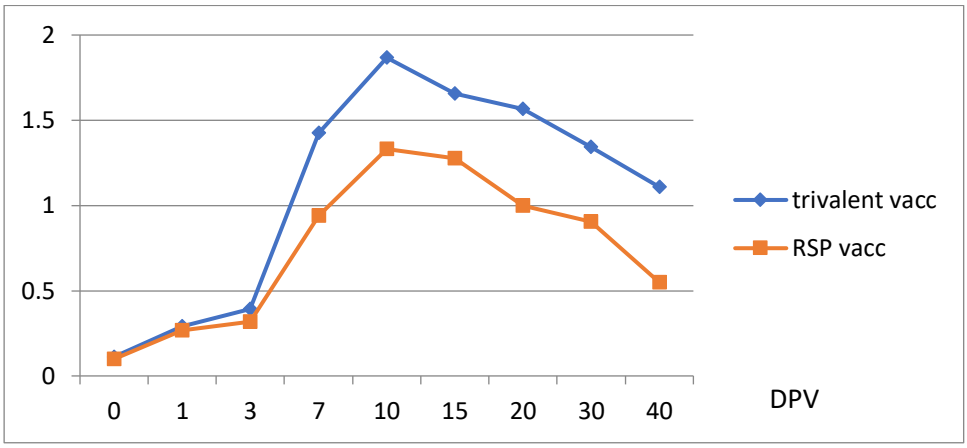

Figure (1): Cell mediated immune response of calves vaccinated with different Capripox vaccines.

2-Evaluation of humoral immune response:

Serological assays (Serum Neutralization test (SNT) and ELISA):

Serum samples were weekly collected from calves before and after vaccinated with different prepared vaccines, then were tested; the results were illustrated in table (1,2&3) clearly appeared the immunogenicity of trivalent vaccines (high level of NI and S/P)

Table (1): Comparative NI and S/P of Group 1 vaccinated calves:

VAC / WPV	Trivalent vac.		RSP vac.	
	NI	S/P	NI	S/P
0	0.50	0.39	0.50	0.38
1	1.25	0.85	1.00	0.68
2	1.25	0.96	1.25	0.77
3	1.50	1.29	1.50	1.00
4	1.75	1.75	1.75	1.28
6	2.50	1.85	2.50	1.65
8	3.00*	2.12**	2.75*	1.84
10	2.75	1.99	2.25	1.79
12	2.75	1.95	2.25	1.77
14	2.50	1.98	2.25	1.80
16	2.50	1.95	2.00	1.72
18	2.50	1.89	1.75	1.66
20	2.25	1.78	1.75	1.55
22	2.25	1.78	1.50	1.56
24***	2.00	1.70	1.50	1.50

N.B.1: Isolate and contact control calves persist negative NI till challenge time.
N.B.2:Neutralizing Index (NI) ≥ 1.5 is considered protective **(Cottral, 1978)**.
WPV = Week post vaccination. **VAC** = Vaccine.
RSP = Sheep pox vaccine (Romanian strain).
Trivalent vaccine= Sheep pox (Romanian & Kenyan) and goat pox vaccine.
NI = Neutralizing index. **S/P=** Sample to positive
*= Highest NI **= Highest S/P
***= Time of challenge.
GP1:8 calves (3 vaccinated with RSP vaccine, 3 vaccinated with trivalent vaccine & 2 control) challenged after 6 months.

Table (2): Comparative NI and S/P of Group 2 vaccinated calves:

VAC / WPV	Trivalent vac.		RSP	
	NI	S/P	NI	S/P
26	2.00	1.72	1.50	1.29
28	2.00	1.69	1.50	1.25
30	2.00	1.67	1.50	1.25
32	2.00	1.69	1.25	0.95
34	1.75	1.67	1.25	0.88
36*	1.75	1.60	1.00	0.82

N.B.1: Isolate and contact control calves persist negative NI till challenge time.
N.B.2:Neutralizing Index (NI) ≥ 1.5 is considered protective **(Cottral, 1978)**.
WPV = Week post vaccination. **VAC** = Vaccine.

RSP = Sheep pox vaccine (Romanian strain).
Trivalent vaccine= Sheep (Romanian & Kenyan) pox and goat pox vaccine
NI = Neutralizing index. **S/P=** Sample to positive

*= Time of challenge.
GP2:8 calves (3 vaccinated with RSP vaccine, 3 vaccinated with trivalent vaccine & 2 control) challenged after 9 months.

Table (3): Comparative NI and S/P of Group 3 vaccinated calves:

VAC / WPV	Trivalent vac.		RSP	
	NI	S/P	NI	S/P
38	2.00	1.72	1.25	1
40	2.00	1.69	1.25	1
42	2.00	1.69	1.20	0.95
44	1.75	1.67	1.20	0.95
46	1.75	1.67	1.00	0.88
48*	1.75	1.60	1.00	0.82

N.B.1: Isolate and contact control calves persist negative NI till challenge time.
N.B.2: Neutralizing Index (NI) ≥ 1.5 is considered protective **(Cottral, 1978)**.
WPV = Week post vaccination. **VAC** = Vaccine.
RSP = Sheep pox vaccine (Romanian strain).
Trivalent vaccine= Sheep pox (Romanian & Kenyan) and goat pox vaccine .
NI = Neutralizing index. **S/P=** Sample to positive .
*= Time of challenge.
GP3:8 calves (3 vaccinated with RSP vaccine, 3 vaccinated with trivalent vaccine & 2 control) challenged after 12 months.

3-Evaluation of the potency of the vaccine by challenge test:

Results of challenge test of vaccinated animals after injection of 0.5 ml virulent LSDV at 6 months, 9 months and 12 months post vaccination as show in the table (4); clearly appeared the potency of trivalent vaccine in protection of vaccinated animal, while monovalent RSPV showed mild protection in vaccinated.

Table(4): Results of challenge test and percent (%) of protective animals:

Vac. / Challenge time (P/V)	RSPV			Trivalent vac.		
	Animal No.	Animal protection	Protective %	Animal No.	Animal protection	Protective %
6 months	3	3/3	100%	3	3/3	100%
9 months	3	3/3	100%	3	3/3	100%
12 months	3	*1/3	33%	3	**2/3	66%

*= two animal at 12 months post RSPV vaccination showed mild fever, mild skin nodule and odema.
**= one animal at 12months post vaccination with trivalent vaccine showed few skin nodule without odema.
All control animals at all times showed high fever, sever odema and large skin nodules.

4.Discussion

Control of LSD among cattle in Egypt depends on vaccination programs, using a heterologous cross reacting sheep pox virus vaccine which is antigenically related to LSD and produce good immune response in cattle (**Michael, et al.1994).** Also, in Southern Africa the Neethling strain of lumpy skin disease was used for vaccine preparation and proved to be innocuous and immunogenic for cattle. All strains of capripox virus so far examined, whether derived from cattle, sheep or goats, share immunizing antigens so attenuated cattle strains and strains derived from sheep and goats have been used as live vaccines **(OIE, 2010).** Especially when we know luckily in this study that all the three capripox viruses were endemic in Egypt, which facilitate the usage of the trivalent against LSDV without retroactive effect. In some countries, a single-strain vaccine (mainly with Kenyan sheep and goat pox strain O 180 or O 240) is being used for immunization of both small ruminants and cattle **(Gelaye, et al., 2015).**

The type of immune response that is most efficient in stopping an infectious process depends on the site of replication of the disease agent. Antibody is effective against extracellularly multiplying infectious agents; while cell mediated immune responses are

most important for those that replicate intracellularly **(Hirsh and Zee, 1999 and Capron, et al., 1994).**Thus, it was important to estimate cell mediated immune response of calves vaccinated with different Capripox vaccines using challenge test and lymphocyte proliferation. Lymphocyte proliferation measured by XTT assay, results in fig. (1) Indicated the difference in cellular immunity between the pre vaccination and post vaccination and disclosed that the vaccinated calves with different vaccines had a variable cellular immune response according the vaccine used in different conditions appeared from the 1st day and reached to maximum assay on the 10th day post vaccination, then decreased after that time. The results also demonstrated the capacity of the trivalent vaccine to produce a good protection showing cellular immune response (=1.867) higher than SP vaccine (1.331) and long duration (all over the experiment time = 40 days). Cell mediated immune response of the contact control calves nearly did not change, all over the post vaccinal time, that meaning no horizontal transmission of the virus from the immunized to in-contact non vaccinated animals. The results of assaying the cell mediated immune response of vaccinated calves were in agreement with, **Kaaden et al. (1992), Amira (1997); Olfat et al. (2002)** who reported the increase of lymphocyte activity by the 3rd day post vaccination and reached its peak on the 10th day then decreased till the 30th day post vaccination.

The SNT results indicated that the vaccinated animals antibodies appear at 7 day post vaccination and increase from 30 days later **Kitching (1996) and Hunter and Wallace (2001).**

The SNT antibody reached the peak (NI= 3.00) at the 8th week post vaccination with trivalent vaccine and remained protective (NI = 1.75) till the 36th week PV while for RSP vaccine SNT antibody reached peak (NI = 2.75) at the 8th weeks PV and remained protective till the 36th week PV (NI = 1.50).

ELISA antibody reached the peak at the 8th week PV (S/P = 2.12) and remained protective at the week 36 PV (S/P = 1.6) for trivalent vaccine, while for RSP vaccine antibody reached peak at 8th week PV (S/P = 1.84) and remained protective at the 36th week PV (S/P=1). Results of serological tests in vaccinated and non vaccinated calves sera agreed with those obtained by **Agag et al. (1992)** who mentioned that a significant

rise of serum neutralizing antibodies titre was recorded from the 21th to 42th day post inoculation and with **Aboul Soud (1995),** who recorded that studies on the collected sera by SNT, AGPT and solid phase ELISA revealed that antibodies appeared by the 10th day P.I. and increased gradually till reaching the maximum by 40th and 50th day P.I. and remained stable after 90 day P.I. till the end of study at 120th day P.I. and also with **Amal et al. (2007)** who said that the neutralizing antibodies appeared to be protective on the 14th DPV (NI= 1.9 & S/P= 2.0) then increased gradually reaching the maximum level by 28th DPV (NI= 3.5 & S/P = 3.5) with the live LSD vaccine then decreased gradually and the protective level remained till the end of the experiment (180 days DPV NI= 2.2 & S/P= 2.2).

The antibodies production were started before 7th day PV and the mean antibodies of all the 3 vaccines (KSGP , SAN & RSP) were increased across each day of the followed up. The high antibody titres were observed in the monitored cattle at day 35 of post vaccination and remained peak until the follow up ends at day 63. These results come in agreement with **Kaaden et al. (1992), Amira (1997) and Olfat et al. (2002),** who reported that the neutralizing antibodies of the vaccinated animals appeared at the decreasing time of the cellular immunity on the 14th day P.V. and reached the peak 21day P.V., then decline but persist within the protective levels.

The NI coincided with the ELISA antibody results and corroborated the results of cell mediated immunity that proved the superiority of attenuated LSD and dual capripox vaccines on the other vaccines in protection of cattle against lumpy skin disease infection.

Selection of the vaccine should always be based on demonstrated efficacy of the vaccine against LSD by challenge trial carried out in a controlled environment, if acceptable efficacy of sheep and goat pox viruses vaccines is demonstrated. So it can be used provided that full vaccination coverage, and it is agree with our challenge test result which estimate cell mediated immune response of calves vaccinated with different Capripox vaccines and the result appeared the potency of trivalent vaccine in protection of vaccinated animal, while monovalent RSPV showed mild protection in vaccinated when table (4) illustrate that the protection percent at 9 months post RSPV vaccination was 100% but 33% at 12 months post RSPV vaccination showed mild to fever skin nodule and odema,While in

vaccination with trivalent vaccine protection percent was 100% at 9 months post vaccination, 66% at 12 months post vaccination showed few skin nodules without odema and all control animals at all times showed high fever, sever odema and large skin nodules. These results agree with **Gari et al (2015)**, who say that the challenge study performed recently in Ethiopia showed that an attenuated Gorgon strain GTPV vaccine is effective in preventing LSD after challenge.

In conclusion this study proved that the trivalent vaccine come better than the Romanian sheep pox vaccine in the control of lumpy skin disease. Field application of trivalent vaccine should be applied in further studies to ensure its valuability and significance for using on large scales.

5. References

Aboul Soud, E. A. (1995): Studies on the adaptation of lumpy skin disease virus (LSDV) in cell cultures. Ph. D. Rhesis, Microbiol. Faculty Vet. Med. Alexandria Univ.

Agag, B.I.; Mousa, S.; Hassan, H.B.; Saber, M.S.; El-Deghidy, N.S. and Abdel Aziz, A.M. (1992): Clinical, Serological and biochemical studies on LSD. J. appl. Anim. Res.,1:13-2.

Amal, A. Fatouh, Amira, A. El-Said, Soad M. Soliman and Michael, A. (2007): Immunological study on Lumpy skin diseas vaccines. Zag. Vet. J. Issn.1110-1458. Vol. 35, No.1 PP.14-19.

Amira, A. El-Said (1997): Evaluation of lumpy skin disease virus vaccine using cell-mediated immune parameters. M.V.Sc. thesis Virology, Fac. Vet. Med., Cairo Univ.

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., (2009): Yemen and Vietnam capripoxviruses demonstrate a distinct host preference for goats compared with sheep. J. Gen. Virol. 90, 105–114.

Bowden, T. R.; Babiuk, S. l.; Parkyn, G. R.; Copps, J. S. and Boyle, D. B. (2008): Cappripox virus tissue tropism and shedding :A quantitative study in experimentally infeted sheep and goats. Virology. 371: 380-393.

Brenner, J., M. Bellaiche, E. Gross, D. Elad, Z. Oved, M. Haimovitz, A. Wasserman, O. Friedgut, Y. Stram, V. Bumbarov, and H. Yadin, (2009): Appearance of skin lesions in cattle populations vaccinated against lumpy skin disease: statutory challenge. Vaccine 27, 1500–1503.

Buller, R.M.; Arif, B.M.; Black, D.N.; Dumbell, K.R.; Esposito, J.J.; lefkowits, E.J.; McFadden, G.; Moss., B.; Skinner, M.A. and Tripathy, D.N. (2005): Family Poxviridae In: Virus Taxonomy. 117-133.

Capron, A.; Locht, C. and Fracchia, G.N. (1994): Safety and efficacy of new generation vaccines. Vaccine(12):667.
Coackely, W. and Capstick, P.B. (1961): Isolation and titration of sheep and goat pox viruses in sheep thyroid cell cultures. Res. Vet. Sci.; 2: 369.

Davies, F. G. (1991): Lumpy skin disease of cattle: a growing problem in Africa and the near east. World Anim. Rev. 68, 37–42.

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.

Eeva S.M. T, Caroline R., Katarzyna B-B, Nick J. K, Shadi A, Lorraine F, Mark R. H, Charles E. L and Peter P.C. M. (2014): Characterization of sheep pox virus vaccine for cattle against lumpy skin disease virus. Antiviral Research 109 (2014) 1–6.

El-Kenawy, A. A., and El-Tholoth, M. S. (2010): Sequence analysis of attachment gene of lumpy skin disease and sheep poxviruses. Journal Virologica Sinica, Vol. 25, Iss. 6, pp 409-416.

Gari, G, Abie G, Gizaw, D, Wubete A, Kidane, M, Asgedom, H, Bayissa B, Ayelet G, Oura CA, Roger F, Tuppurainen ES (2015): Evaluation of the safety, immunogenicity and efficacy of three capripoxvirus vaccine strains against lumpy skin disease virus. Vaccine. 22;33(28):3256-61. doi: 10.1016/j.vaccine. 01.035.
Gelaye, E., Belay, A., Ayelet, G., Jenberie, S. and yami, M (2015): Capripox disease in Ethiopia. Antiviral research 119,28-35.

Gera, B., Klement, E. Khinich, E., Stram, Y., and Shpigel, N. (2015): Comparison of the efficacy of Neethling Lumpy Skin didease virus and x10RM65 sheep pox live attenuated vaccine for the prevention of LSD. Vaccine 34, 3317-3323.

Hirsh D.C. and Zee Y.C. (1999): Veterinary Microbiology and immunology. Ed.2 , Amazon.

House, J.A.; Terrance, M.; Wilson, T.M.; El-Nakashly, N.; Abdel Karim, I.; Ismail, I.; El-Danaf, N.; Mousa, M.A. and Ayoub, N.N. (1990): The isolation of lumpy skin disease virus and bovine herpes virus 4 from cattle in Egypt. J. Vet. Diag. Invest., 2: 111-115.

Hu SL, Klaniecki ,J., Dykers ,T., Sridhar, P. and Travis, B.M. (1991): Neutralizing antibodies against HIV-1 BRU and SF2 isolates generated in mice immunized with recombinant vaccinia virus expressing HIV-1(BRU) envelope glycoproteins and boosted with homologous gp160. AIDS Res Hum Retroviruses.1991; 7:615–620. [PubMed: 1768463].

Kaaden, O.R., Walz, A., Czerny, C.P. and Wernery, U. (1992): Progress in the development of camel pox vaccine. In proceeding of the first International Camel Conf., Daubai.

Kitching, R.P. (1986): Passive protection of sheep against capripox virus. Res. Vet. Sci., 41(2): 247-250.

Le Goff, C., Lamien, C.E., Fakhfakh, E., Chadeyras, A., Aba-Adulugba, E., Libeau, G., Tuppurainen, E., Wallace, D.B, Adam, T., Silber, R., Gulyaz, V., Madani, H., Caufour, P., Hammami, S., Diallo, A. and Albina, E. (2009): Capripoxvirus G-protein-coupled chemokine receptor: a host-range gene suitable for virus animal origin discrimination. J Gen Virol vol. 90 no. 8 1967-1977.

Michael A., Soad ,M. S., Saber, M., Fayed, A. A., Hosein, N.A. and Moussa A.A.(1994): Vaccination of cattle against lumpy skin disease with the tissue culture sheep pox vaccine. 6th SC. Con., 20-22 nov., 1994.Fac. Vet. Med., Assuit, Egypt, 536-541.

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. And Chapter 2.4.1 4. LUMPY SKIN DISEASE

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

Olfat, E. Nakhla., Samir, S.S., Manal, Awad, Soad, M. Soliman and Daoud, A.M. (2002): Studies on cell mediated immune response of Goats vaccinated with Goat pox vaccine. 6th Vet. Med. Zag. Conference., 139-151.

Reed, L.I. and 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.

Sabban, M.S. (1960): Sheep pox and its control in Egypt. *Bull. Off. Int. Epiz.*, 53: 1527-1539.

Somasundaram, M.K., (2011): An outbreak of lumpy skin disease in a holstein dairy herd in Oman: a clinical report. Asian J. Anim. Vet. Adv. 6, 851–859.

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

Tulman, E.R., Afonso, C.L., Lu Z., Zsak L., Sur, J.H., Sandybaev, N.T., Kerembekova, U.Z., Zaitsev V.L., Kutish, G.F.and Rock, D.L.(2002): The genomes of sheeppox and goatpox viruses. J. Virol. ;76:6054–6061.

الملخص العربي

تحضير لقاح ثلاثى ضد مرض الجلد العقدى باستخدام عترات مختلفه من فيروسات الكابرى

*د. محمد هاشم كفافى *** د.شريف عبد الغفار *اد .عماد عبد السلام **د. مصطفى زغلول ** د. كريستسن عزيز

*معهد بحوث الامصال والقاحات البيطريه

**المعمل المركزى للرقابه على المستحضرات الحيويه البيطريه

***المستشفى البيطرى العسكرى بالقاهرة

تهدف هذه الدراسه لتحضير لقاح ثلاثى العتره من جدرى الاغنام والماعز لتحصين الابقا ر ضد مرض الجلد العقدى بالمقارنه بالعتره الاحاديه لجدرى الاغنام.

تم تحضير ومعايره اللقاح الثلاثى بتمرير العترتين الرومانييه والكينيه لفيروس جدرى الاغنام وعتره الهيلد الخاصه بفيروس جدرى الماعز على خلايا الفيرو حيث بلغت العياريه النهائيه للقاح $4.5\log_{10}$ $TCID_{50}$ وقد تم تتبع رده الفعل والمناعه المكتسبه السائله باستخدام التعادل المصلى والاليزا والمناعه الخلويه باستخدام اختبار (XTT) فى الابقارالمحصنه باللقاح الثلاثى واللقاح الاحادى واشارت النتائج الى ارتفاع مستوى الاجسام المناعيه والخلويه التى تم قياسها بدرجه اعلى ولفتره اطول (12 شهر) فى الابقار المحصنه باللقاح الثلاثى عن مثيلاتها المحصنه باللقاح الاحادى .

وباجراء اختبار التحدى بالعتره الضاريه من فيروس الجلد العقدى على مجموعتى الابقار المحصنه ومجموعه الابقار الغير محصنه الضوابط اشارت النتائج الى ان نسبه الوقايه بلغت 0% فى الابقار الغير محصنه الضوابط بينما بلغت نسبه الوقايه 100% فى مجموعه الابقار المحصنه باللقاح الثلاثى وتراجعت الى 33 %فى اللقاح الاحادى وذلك بعد مرور 12 شهر من التحصين.