

Comparative study on the efficiency of some Capripox vaccines in protection of cattle against Lumpy skin disease
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Abstract

In this study two vaccines were prepared, Romanian sheep pox (RSP) and Ismailia lumpy skin disease (LSD) vaccines, they were evaluated and proved sterile and potent , and were used for vaccination of susceptible calves against lumpy skin disease for selection of the most appropriate one , depending on their protectively effect .Twelve susceptible calves 6-8 months old were equally divided into two groups ,1st one was divided into two subgroups (each of 3 calves) ,the 1st one (G1) was vaccinated with RSP vaccine and the 2nd one (G2) was vaccinated with LSD vaccine, vaccination was applied intra dermally with 0.5 ml contain $10^3 \log_{10}$ TCID₅₀ the 2nd group (6 calves) was divided into three equal non vaccinated control subgroups (Sg) (each of two calves), one left in contact with the 1st vaccinated animal (G1) and the 2nd Sg left in contact with the 2nd vaccinated calves (G2) while the 3rd Sg calves were left as an isolated control Sg. The clinical changes and post vaccinal reactions recorded .The acquired humoral and cellular acquired immunity were evaluated by neutralization test and lymphocyte blastogenesis assay. The results proved that the second vaccinated subgroup (with LS vaccine) acquired a slight prominent immunity more than (SP vaccine) .generally both of Romanian SP and Ismailia LS vaccines can protect the cattle from infection with lumpy skin disease.

Keywords: Lumpy skin disease, sheep pox, acquired humoral and cellular acquired immunity, efficiency, capripox vaccines.

Introduction:

The capripoxviruses cause the most severe pox diseases of animals. Epidemiologically the diseases of sheep pox , goat pox and lumpy skin disease differ, but all three viruses may be mechanically

transmitted by biting insects, and control without vaccination is extremely difficult in endemic areas. Recently developed live attenuated vaccines provide good, virtually lifelong, protection, which is dependent on stimulating cell-

mediated immunity. Lumpy skin disease currently threatens to extend beyond its existing boundaries, causing concern and renewed interest in vaccine development (*Carn, 1993*).

Lumpy skin disease was first reported in Africa, where it crippled the production potential of cattle and compromised vulnerable livelihoods on the continents (*FAO-RNE, 2013*). The virus mainly affects cattle. LSD can cause devastating economic impacts (*Gari et al, 2011*).

In *May 1988*, LSD was recognized clinically in the Suez Governorate of Egypt, where it was thought to have arrived at the local quarantine station with cattle imported from Africa (Somalia). The disease spread locally in the summer of 1988 and apparently overwintered with little or no manifestation of clinical disease. It reappeared in the summer of 1989 and, in a period of five to six months, spread to 22 governorates of 26 governorates of Egypt. A rapid reaction to the problem led to the vaccination of nearly two million cattle with a sheep pox vaccine (*FAO, 2000*).

The disease also reappeared in 2006, 2012, 2013 (*FAO-RNE, 2013*).

Vaccines used presently in Middle East include:

- 1- Homologous live attenuated LSD vaccines containing Neethling strain (in South Africa and Namibia)
- 2- Hetrologous live attenuated Sheep pox vaccines produced in the

region (Romanian in Egypt, Jordan and some Middle East and African countries) and other countries.

The purpose of this research was to assess the efficacy of each of sheep pox (Romanian strain) and lumpy skin disease (Local Ismailia strain) virus vaccines, under Egyptian condition, in protecting indigenous cattle against LSD. For identifying the safe and more potent attenuated live vaccine.

Material and methods:-

1. Viruses:

a- Romanian sheep pox virus (RSPV):

It was supplied from pox department, Veterinary Serum and Vaccine Research Institute (VSVRI), Egypt. The virus was cultivated on African green monkey kidney cells (Vero cells) according to *Singh and Rai (1991) and Rizkallah (1994)*.

b- Lumpy skin disease virus (LSDV):

It was supplied from pox department, VSVRI, Egypt. Ismailia strain was isolated from Egypt during the outbreak of 1988 (*House et al., 1990*). The virus was adapted in Madin-Darby bovine kidney cells (MDBK) according to *Daoud et al. (1998)*.

Activation of the inoculum on the MDBK cells was performed in this study.

2. Cell cultures:

a- African green monkey kidney cells (Vero):

Vero cells were supplied by Dr. J. House, FADDL, Plum Island, USA. The cells were subcultured and maintained in pox department, VSVRI. These cells were used for preparation and titration of sheep pox vaccine and for serological test.

b- Madin-Darby bovine kidney cells (MDBK):

MDBK cells were obtained from Ames, Iowa Laboratories, USA. The cells were used for preparation, titration and neutralization test.

3- Media: Minimum Essential Medium (MEM):

The media used for cell cultures was (MEM) with Earle's salts and L-glutamine without sodium bicarbonate. It was supplied by Sigma Chemical Company USA. 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.

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 Manual*

volume 1989), the mixture was sterilized by filtration.

5- Animals (cattle):

Twelve susceptible calves 6-8 months old were used. They were divided into 2 equal groups and were housed in insect proof isolated units and observed for 2 weeks before starting the experiment. Their sera were tested for its freedom from antibodies against sheep pox and lumpy skin disease viruses using neutralization test. These animals were fed a complete balanced diet and water ad libitum. The calves were examined daily before and after vaccination with the different vaccines.

6- Biological reagents:

a- Bovine Serum: Produced by Gibco Laboratories, New Zealand lot 6834217 D sterile A. It was used for media preparation.

b- Fetal calf serum: It was obtained from Gibco LTD, UK. To be used in lymphocyte blastogenesis assay.

7- Preparation of sheep pox and lumpy skin disease vaccines:

According to *OIE Manual volume I (2004)* the two different Romanian sheep pox and Ismailia lumpy skin disease vaccines were prepared by mixing of stabilizer solution with the virus fluid of attenuated virus at the ratio 1:1 (v:v). Each 100 ml vaccine mixed with 100IU/ml penicillin and 100µg/ml streptomycin sulfate.

8- a) Titration of caprine pox vaccines before and after lyophilization:

Titration of RSP and LSD virus vaccines were applied according to *Rao and Malik (1982) and Tiwari and Negi (1995)*. The titre of the two virus vaccines was expressed as TCID₅₀ and calculated according to the method of *Read and Muench (1938)*.

b) Sterility test:

Sterility test of the prepared vaccines was carried out according to *OIE Manual volume 1 (2004)*.

c) Safety and potency of the vaccines:

Were performed as described by *Mahmood et al. (1988)*.

9- Vaccination of calves:

As described by *Wang and Jiang (1988a) and Daoud et al.(1998)* the calves in Group 1 (number 1-3) were vaccinated with the field dose (10^3) of tissue culture Romanian sheep pox vaccine, and the calves in Group 2 (number 4-6) were similarly vaccinated with the field dose (10^3) of Lumpy skin disease vaccine. The 6 animals were vaccinated intradermally in the tail fold. Each vaccinated calve received 0.5 ml of the vaccine of choice by I/D inoculation. 6 calves in group 3 (number 7-12) were kept as unvaccinated controls; two non vaccinated calves in contact with vaccinated G1; two non vaccinated calves in contact with vaccinated G2 and two isolated non vaccinated calves.

Animals were observed daily for one month for clinical signs, including body temperature,

swelling at the site of inoculation, or generalized skin lesions.

10- Serological assays:

Serum samples were collected from calves just before and after vaccination in a weekly interval for one month. Samples were stored at -20°C until examined by micro neutralization test (NT). According to the method described by *Martin et al. (1975) and OIE (2010)*.

11- Evaluation of the cell mediated response: Assay of lymphocyte blastogenesis.

Whole blood was collected 1, 3, 5, 7, 10, 14, 21 and 28 days post vaccination for estimation of the cellular immunity. It was applied according to the method adopted by *Charles et al. (1978) and Lucy (1984)*, For determination of live cell number, kits were purchased from Promokine, cat. No.: PK-CR-20-300-1000, Lat No.:743182. .

Results:

-Propagation of sheep pox virus "Romanian strain" and cytopathic changes (CPE) in vero cells:

The CPE changes from the vaccinal seed virus began to appear 2 day post inoculation and reach the maximum (70-80%) on the 5th day, and its titre reached log $10^{6.0}$ TCID₅₀.

- Activation and propagation of LSDV in MDBK cells:

The previously adapted LSDV(Passage 60) was propagated in MDBK cell culture for activation for 6 successive passages, The cells

showed no CPE in the first 2 passages and the cellular changes appear by the 3rd passage (passage 63) five days post inoculation. Results of titration for six successive passages of LSD in MDBK cells were illustrated in *table (1)*.

The 4th passage which accomplishes 66 passages was assumed for vaccination.

An immunizing dose of both Romanian SP or LSD vaccine of $10^{3.0}$ TCID₅₀ is described for cattle vaccination.

-Clinical effect of vaccination:

The post vaccinal reaction in calves vaccinated with Romanian sheep pox vaccine started by slight increase in body temperature (0.4°C) in average 3 days post vaccination, mild skin lesions appeared as redness and hotness at the inoculation site 5 to 7 days post vaccination. These reactions diminished within one week and disappeared later (*Photo 1*).

On the other hand, calves inoculated with Lumpy skin disease vaccine showed rise in body temperature started on the 7th day with 39°C and reached to 39.5°C on the 9th day, it is began to decrease and returned to the normal temperature (38.3 or 38.5°C) 11 day post inoculation. Local reaction appeared at the inoculation site on the 8th day as a small size round firm nodule, 2 to 4 cm in diameter (*Photo 2*), and persist for 12-15 days before hiding.

The non vaccinated contact and isolated control calves showed no thermal or clinical reaction and remained apparently clinically normal.

Evaluation of neutralizing antibody titer in calves vaccinated with RSP and LSD vaccine:

Serum samples were weekly collected from calves vaccinated with RSP vaccine, LSD vaccine and the control non vaccinated animals for four successive weeks. They were used for measuring neutralizing index according to the methods previously described by *Martin et al. (1975) and OIE (2010)*.

The neutralizing index (NI) of calves vaccinated with any of two vaccines appeared from the second week post vaccination. The results proved that both of RSP vaccine and LSD vaccine create a protective effect in the inoculated animals with a mild variation, (*table 2 and figure 1*).

Evaluation of cell mediated immune response of vaccinated and control non vaccinated calves:

The whole blood samples were collected from vaccinated and non vaccinated control calves on heparin sodium, and were tested by using lymphocyte proliferation assay according *Charles et al (1978)*.

The results were clarified in *table 3 and figure 2*.

Table (1): Titration of six successive passages of LSDV on MDBK cells:

<i>Virus passages No.</i>	<i>Titre log₁₀ TCID₅₀ / ml</i>
1 (3 rd passage)	3-9
2 (4 th passage)	4-5
3 (5 th passage)	5-2
4 (6 th passage)	5-5
5 (7 th passage)	5-5
6 (8 th passage)	5-5

TCID₅₀ = Tissue culture infective dose 50.

**Photo 1:** Post vaccinal reaction in calves vaccinated with Romanian sheep pox vaccine.**Photo 2:** Post vaccinal reaction in calves vaccinated with Lumpy skin disease vaccine.**Table (2):** Neutralizing index of calves vaccinated with RSP or with LSD vaccine:

<i>Vaccinated calves</i>	<i>Calve No.</i>	<i>Days post vaccination</i>				
		<i>0</i>	<i>7</i>	<i>14</i>	<i>21</i>	<i>28</i>
<i>G1</i>	<i>1</i>	<i>0.5</i>	<i>1.0</i>	<i>1.7</i>	<i>2.4</i>	<i>2.2</i>

<i>(with RSP vaccine)</i>	2	0.2	1.2	1.9	2.6	2.4
	3	0.3	1.1	1.8	2.5	2.5
	Average	0.33	1.1	1.8	2.5	2.36
G2 <i>(with LSD vaccine)</i>	4	0.3	1.4	2.1	2.7	2.5
	5	0.4	1.3	2.4	2.9	2.9
	6	0.1	1.5	2.5	2.8	2.6
	Average	0.26	1.4	2.3	2.8	2.66
Control (1)	7	0.3	0.2	0.4	0.3	0.3
	8	0.2	0.4	0.3	0.1	0.2
	Average	0.25	0.30	0.35	0.2	0.25
Control (2)	9	0.2	0.2	0.3	0.4	0.2
	10	0.4	0.5	0.3	0.4	0.4
	Average	0.30	0.35	0.30	0.4	0.3
Control (3)	11	0.1	0.2	0.1	0.5	0.3
	12	0.5	0.4	0.4	0.5	0.5
	Average	0.30	0.30	0.25	0.50	0.40

Positive NI appeared in vaccinated calves 2 weeks post inoculation
 RSP vaccine = Romanian sheep pox vaccine.

LSD vaccine = Lumpy skin disease vaccine.

0 day = before vaccination (on the time of vaccination).

G1 = calves vaccinated with RSP vaccine.

G2 = calves vaccinated with LSD vaccine.

Control (1) = two non vaccinated calves in contact with vaccinated G1.

Control (2) = two non vaccinated calves in contact with vaccinated G2.

Control (3) = two isolated non vaccinated calves.

NB: Neutralizing Index (NI) ≥ 1.5 considered protective mean against capripox viruses (*Cotral, 1978*).

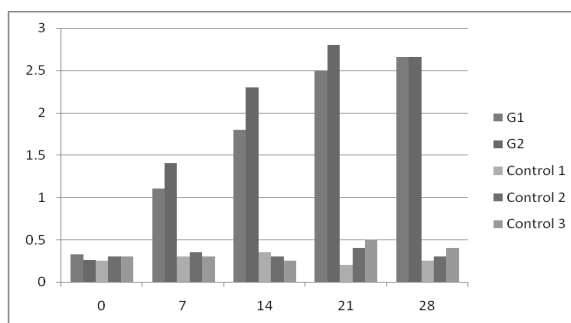


Fig (1): Neutralizing index of calves vaccinated with RSP or with LSD vaccine

Table (3): Cell mediated immune response of calves vaccinated with RSP and LSD vaccines and control non vaccinated calves (registered as optical density):

Vaccinated calves	Calve No.	Days post vaccination									
		0	1	3	5	7	10	14	21	28	
G1 (with RSP vaccine)	1	0.075	0.191	0.250	0.362	0.680	1.125	1.000	0.784	0.375	
	2	0.079	0.218	0.282	0.440	0.714	1.200	1.055	0.692	0.267	
	3	0.090	0.245	0.316	0.511	0.800	2.250	1.048	0.717	0.320	
	Average	0.081	0.218	0.282	0.478	0.731	1.525	1.034	0.731	0.321	
G2 (with LSD vaccine)	4	0.080	0.290	0.416	0.600	0.741	1.880	1.642	0.730	0.340	
	5	0.072	0.262	0.397	0.611	0.822	1.632	1.618	0.684	0.321	
	6	0.077	0.306	0.401	0.715	0.909	1.998	1.621	0.893	0.364	
	Average	0.076	0.286	0.405	0.642	0.824	1.836	1.621	0.769	0.342	
Control (1)	7	0.081	0.080	0.084	0.081	0.078	0.076	0.082	0.078	0.079	
	8	0.091	0.091	0.091	0.095	0.094	0.086	0.085	0.074	0.080	
	Average	0.086	0.085	0.087	0.088	0.086	0.081	0.084	0.076	0.080	
Control (2)	9	0.070	0.074	0.077	0.080	0.086	0.080	0.082	0.085	0.085	
	10	0.073	0.083	0.080	0.077	0.075	0.075	0.080	0.086	0.074	
	Average	0.072	0.079	0.079	0.079	0.081	0.078	0.081	0.086	0.080	
Control (3)	11	0.081	0.075	0.079	0.080	0.074	0.075	0.086	0.081	0.070	
	12	0.083	0.079	0.072	0.080	0.080	0.080	0.079	0.075	0.072	
	Average	0.082	0.077	0.076	0.080	0.077	0.078	0.083	0.078	0.071	

RSP vaccine = Romanian sheep pox vaccine.

LSD vaccine = Lumpy skin disease vaccine.

0 day = before vaccination (on the time of vaccination).

G1 = calves vaccinated with RSP vaccine.

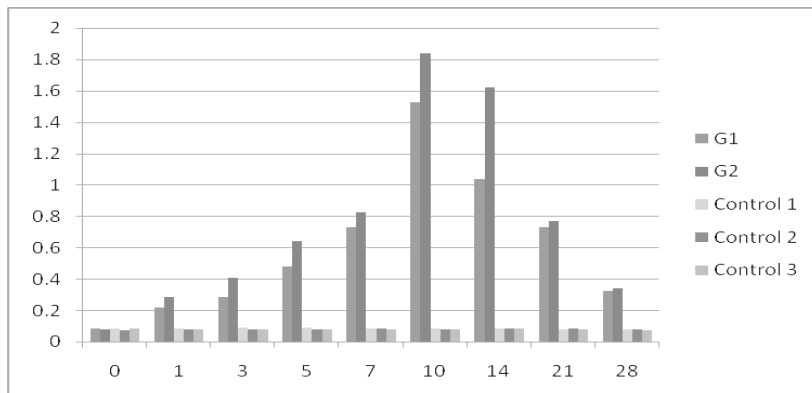
G2 = calves vaccinated with LSD vaccine.

Control (1) = two non vaccinated calves in contact with vaccinated G1.

Control (2) = two non vaccinated calves in contact with vaccinated G2.

Control (3) = two isolated non vaccinated calves.

NB: cell mediated immune response in contact and isolated control calves optical density between 0.070-0.095 all over the experiment.



(Fig 2): Cell mediated immune response of calves vaccinated with RSP and LSD vaccines and control non vaccinated calves (registered as optical density)

Discussion:

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 sheep pox or LSD vaccine was practiced in different countries but the comparison between their protectivity of cattle from infection with LSDV has not yet been tried. Here we tried to control LSD through selection of the most effective vaccine that could when used optimally improve the health and welfare of animals.

In this research Romanian sheep pox virus was inoculated on confluent Vero cell cultures according to *Singh and Rai (1991) and Rizkallah (1994)*. Cytopathic change began to appear early, 2 days post inoculation, because it was previously activated by propagation on the vero cells for different passages after activation through the primary cell cultures. On the contrary when LSD virus inoculated on confluent MDBK cell cultures, cytopathic changes began to appear late, because it was not previously activated since 1989 (*Daoud et al, 1998*). CPE increased daily till reaching its maximum (80-90%) for both viruses on 5-6 days. Our results agreed with *Singh and Rai (1991); Rizkallah (1994); Aboul Soud (1995); Olfat (2000)*

and *Christine (2008)* who found that the highest titre obtained from the bottles harvested 5-6 days post inoculation. The prepared attenuated vaccines were tested for sterility and the results proved that the two vaccines were negative to any contaminating agents as bacteria, moulds and fungi when inoculated on specific media.

For detection of the enduring effect of the prepared vaccines different experiments were applied, the first was vaccination of susceptible calves then the detection of humoral and cellular immunity. The usage of animal experimentation for testing of the prepared vaccines is inevitable, as no in vitro model can predict a candidate vaccines ability to induce protection in the target species.

According to *OIE (2004)*, the two prepared vaccines should have a minimum titre $\log_{10} 4.5$ TCID₅₀/ ml equivalent to a field dose $\log_{10} 2.5$ TCID₅₀. According to *Wang and Jiang (1988a)* twelve apparently healthy susceptible calves 6-8 months old were divided into two groups, each group of 6 animals, the first group was subdivided into two equal group of 3 calves (G1&G2) in which G1 individually vaccinated intradermally (I/D) with 0.5 ml from RSP vaccine and G2 with LSD vaccine. The second group also divided into three equal

subgroups each of two calves and used as control unvaccinated animals in which the 1st one used as contact control for vaccinated G1 and the 2nd subgroup also as a contact control with vaccinated G2 while the 3rd subgroup left as isolated control.

The post vaccinal responses, showed that there was no generalized of infection among any of the vaccinated animals, but other reactions differ between the two groups (G1&G2). In G1 calves vaccinated with heterologous RSP vaccine only mild local reaction appeared in the form of redness and mild swelling; On the other hand G2 calves vaccinated with homologous LSD vaccine a pronounced local reaction (2-4 cm in diameter) at the point of inoculation of two calves and a mild reaction (10mm) in the 3rd calve and disappeared within 12-15 days which agree with these recorded by **Woods (1988)**, **OIE (1992)**; **Carn (1993)** and **Coetzer (2004)**. The recorded clinical signs was also in harmonization with **Diallo and Viljoen (2007)**, who stated that the clinical signs caused by different Capripox viruses are very variable, depending not on individual host susceptibility but also on the virus strain.

Slight reaction and slight rise in temperature could be explained that the vaccine stimulated the immune system in the susceptible animal.

Vaccination or infection with Pox viruses evokes both humoral and

cell mediated immune response (**Pandey et al, 1969b** and **Deshmukh and Gujar, 1992**).

Results of neutralization test in vaccinated and non vaccinated calves sera are present in table (2) and figure (1) that show the appearance of neutralizing antibodies in the vaccinated groups (G1&G2) from the 7th day post vaccination and increased on the 3rd week post vaccination. The contact and isolated non vaccinated control calves still negative all over the experiment time (4 weeks). Variation between the neutralizing index obtained P.V. with RSP and LSD vaccines, in which the LSD vaccine overcome the RSP vaccine. Results of seroconversion agree with those obtained by **Agag et al (1992)** where they mentioned that serum neutralizing antibodies develop on the 2nd day and a significant rise of antibody titre was recorded from the 21th to 42th day post inoculation.

Neutralization is very specific for almost all viruses, and the use of Vero cells has been reported to give more consistent results than the 1ry cell cultures (LT or LK cells) (**Manual OIE, 2000**).

Davies and Otema, (1981) and **Fassi-Fehri et al (1984)** employed SNT to study the antigenic relationship between SPV, GPV and LSD, to confirm the disease or to assess the post vaccinal immune status. Our results were in contrast with **Kitching (1986)** who reported that the immune status of a

previously infected or vaccinated animal cannot be related to serum level of neutralizing antibody. **Rao and Negi (1997)** was also in contradistinction with our results they concluded that, although the virus neutralization test is the most specific serological test, but because immunity to capripox infection is predominantly cell mediated, the test is not sufficient.

The neutralizing antibodies are produced as early as a month post-vaccination and these limits only the spread of the virus but do not prevent the replication of the virus, since most of the virus remains naked intracellular. In addition immunity to capripox viruses is predominantly cell mediated (**Carn, 1993**).

The lymphocyte proliferation assay was chosen for estimation of cell mediated immune response.

Table (3) declared the results of cell mediated immune response of calves vaccinated with different Capripox vaccine using lymphocyte proliferation measured by XTT assay which 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 3rd day and reached to maximum assay on the 10th day post vaccination, then decreased after that time. The results also demonstrated the

difference in capacity of the homologous vaccines that the heterologous RSP vaccine cause a good protection, in which the homologous LSD vaccine overcome the heterologous vaccine, and the result appear the lower acquired cellular immunity after vaccination with the heterologous sheep pox vaccine. Cell mediated immune response of the contact and isolated calves nearly did not changed, all over the post vaccinal time, that meaning no horizontal transmission of the virus from the immunized to in-contact non vaccinated animals.

Our results were corresponded to (**Ramyar et al, 1974 and Domenech et al, 2006**) who illustrated that usually homologous vaccinations incorporating locally prevalent strains of capripox virus are quite successful in protecting animals against infection.

There are evidences that cell mediated immune response play an important role against capripox beside humoral immunity (**Bachh et al, 1997**). The increased lymphocyte proliferation due specific lumpy skin disease or RSP antigens stimulation agreed with the reports of (**Ahmed et al, 2007**).

Our results of assaying the cell mediated immune response of vaccinated calves were in agreement with, **Kaaden et al (1992); Amal (1995); Amira (1997) and 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, also 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.

In Egypt we prefer RSP vaccine to avoid the transmission of LSD specially when we know that LSD is prevalent mainly in wet seasons and during times when insects are abundant, strongly suggest a major role for biting arthropods in mechanical transmission of the causal agent between animals, (e.g) mosquito *Aedes aegypti* and *Stomoxys calcitrans*. (*Kitching and Mellor, 1986 and Chihota et al, 2003*)

Neither sheep pox nor goat pox has been reported in sheep or goats in South Africa where LSD is endemic, and this might indicate that LSDV cannot cause serious disease in these species. This is strengthened by experimental findings that inoculation of different LSDV strains into sheep and goat only induce granulomatous reaction at the site of inoculation (*Kitching et al, 1989*).

In spite of the usage of LSD vaccine in S. Africa, there are outbreaks of LSD in cattle in S. Africa every few years (*Weiss, 1968 and Hunter and Wallace, 2001*).

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الملخص

دراسه مقارنه علي فاعليه بعض لقاحات الكابري الجدرية في حمايه الابقار ضد مرض الجلد العقدي

كرستين عزيز ميخائيل - ماجد منير ابراهيم - منال عوض ميخائيل - سعاد محمد سليمان - عزيز ميخائيل اسحق

في هذه الدراسة تم تحضير لقاحي ، جذري الأغنام العترة الرومانيه ، و الجلد العقدي عترة الإسماعيلية ، و تم تقييمها وأثبتت نفاوتها و قوتها العياريه و إستخدمت لتحصين العجول القابله للعدوي ضد مرض الجلد العقدي لإختيار أكثرها ملائمه إعتماًداً علي تأثيرها الوقائي . إستخدم إثناعشر عجلاً قابلاً للعدوي عمرها 6-8 شهور قسمت إلي مجموعتين متساويتين - المجموعه الأولى (6 عجول) قسمت إلي تحت مجموعتين متساويتين (3 عجول في كل منها) - الأولى حصنت بلقاح جذري الأغنام المستضعف العترة الرومانيه و الأخرى بلقاح الجلد العقدي المستضعف عترة الأسماعيلية - تم التحصين في أوديم الجلد بمقدار 0,5 ملل من محلول اللقاح تحتوي علي $10^3 \log_{10} \text{TCID}_{50} / \text{ml}$ من الفيروس. و قسمت المجموعه الثانيه (6 عجول) التي تركت بدون تحصين كضوابط إلي 3 أقسام كل منها من عجلين - وضع عجلين مخالطين للمجموعه الأولى و عجلين مع المجموعه الثانيه و العجلين الباقيين تركوا معزولين ضوابط . سجلت التغييرات الإكلينيكيه و ردود الأفعال و تم قياس المناعه السيرولوجيه (المصليه و الخلويه) بإختبار التعادل المصلي وإختبار تحور الخلايا الليمفاوية. أوضحت النتائج أن المجموعه الثانيه المحصنه بلقاح الجلد العقدي إكتسبت مستوي مناعي أعلا قليلاً عن المجموعه المحصنه بلقاح جذري الأغنام. عموماً فإن كلاً من لقاحي جذري الأغنام و الجلد العقدي قادرين علي حمايه الماشيه من الإصابه بالجلد العقدي .