Evaluation of Humoral and Cell-mediated Immunity of Lumpy Skin Disease Vaccine Prepared from Local strainin calves and Its Related to Maternal Immunity

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Abstract: In this study, Lumpy skin disease virus(LSDV) (Local Ismailia strain) was identified by using PCR, Live attenuated local Ismailia lumpy skin disease vaccine (LSD) was prepared, its titer on MDBK cell was log $10^{3.0}$ $TCID_{50}$/dose. It was sterile, safe. Ten susceptible calves 6-8 months old were used. Two calves of them were used for safety so it was vaccinated I/D with 20xof field dose of LSD vaccine (log $10^{3.0}$ $TCID_{50}$), while five calves were vaccinated with 0.5 ml of prepared vaccine intra dermally (I/D) and three of them were kept as control. Also five pregnant cattle at the last 4thmonths of gestation were used. Three pregnant cattle were vaccinated with 0.5 ml I/D of prepared vaccine (log $10^{3.0}$ $TCID_{50}$) and two of them were kept as control. Cell-mediated immunity was evaluated. Lymphocyte proliferation began to increase till reach to its peak (1.84) at 10th day then decrease after that, While Interferon gamma(IFN-γ)detected in 1stday (25%) till 7thday (17%) post vaccination then decrease after that. The humoral immunity was evaluated by SNT and ELISA, Protective serum neutralizing antibody titer started at two weeks (1.5), (1.19) post vaccination then reach to its peak at 12th weeks (3.1),(2.34) respectively and persisted till 40 weeks, So LSD vaccine was highly immunogenic, inducing higher lymphocyte and interferon γ levelanda higher level of antibody titer with prolongation of the duration of immunity, so it considered the best choice of vaccine to control LSD in cattle. The maternal immunity was detected in newly born calves from vaccinated dams by SNT and ELISA, the titer started higher from 1st day (2.33), (1.956) till 4th month (1.57),(1.058)respectively then decrease after that, So newly born calves should be vaccinated before the end of the four months of age.
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1. Introduction:

Lumpy skin disease (LSD) is a viral disease of cattle caused by Neethling pox virus of genus Capri poxvirus belongs to family poxviridae(***Sevik et al., 2016*)**. LSD was first reported in Central and southern Africa and it is rapidly spreading throughout the Middle East, including Turkey**(Charlotte and Howard, 2015).** It was introduced to Egypt during Importation of cattle from Somalia at the Suez quarantine station in May 1988 **(Hayed *et al.*, 1988)** and reappearance again during the out- break in EL-Mania governorate outbreak, then it reappeared again in Egypt governorates in 2005,2006,2012,2013 and **(EL-Bagoury *et al.*,1995, Fayed *et al.*, 2006and FAO 2013)**. It mechanically transmitted by biting insects **(Gari *et al.*, 2012)**. It is characterized by fever, nodules on the skin, lesions in the mouth, pharynx and respiratory tract, emaciation, enlargment of lymphnodes **(Tuppurainen and Oura 2012**) The disease cause economic losses due to sever damage of hide, loss of milk production, mastitis, infertility and death **(Weis, 1968 and OIE, 2000).**

Control of the disease without vaccination is extremely difficult in endemic areas. Recently developed live attenuated LSD vaccine provides good, lifelong protection which is dependent on stimulating cell- mediated and humoralimmunity, Therefore, the purpose of this research was to evaluated the efficacy (Safety and Potency) of prepared live attenuated lumpy skin disease (Local Ismailia strain) vaccine under Egyptian condition, in protecting indigenous cattle against LSD and identify the optimal age at which newly born calves from vaccinated dams with LSD vaccine should be vaccinated.

2. Material and methods:-

2.1. Animals:

a) Calves:

Ten susceptible calves 6-8 months old apparently health were tested to be free from antibody against LSD. Two calves were used for safety test, while five calves were inoculated with 0.5 ml I/D in tail folds by prepared vaccine (log $10^{3.0}$ $TCID_{50}$) and three of them were kept as control.

b) pregnant cattle:

Five pregnant cattle at the last 4thmonths of gestation were tested to be free from antibody against LSD. Three pregnant cattle were vaccinated with 0.5

ml I/D of prepared vaccine (log $10^{3.0}$ $TCID_{50}$) and two of them were kept as control.

2.2Virus:

Lumpy skin disease virus (LSDV):

It was supplied from Pox Department, VSVRI, Egypt. Ismailia strain was isolated from Egypt (**House *et al*., 1990)**. The virus was adapted in(MDBK) according to(**Daoud *et al*., 1998).**

2.3. Primers:

LSDV sequences within the gene for viral attachment proteinaccording to (**Ireland and Binepal 1998**).

Forward primer: 5'-AAATTATATACGTAAATAAC-3' andReverse primer: 5'- ATAGTAAGAAAAATCAGGAAA -3'. It was obtained from Jena Bioscience company, Germany.

2.4. Titration of local Ismailia lumpy skin disease virus:

Titration of LSDV on MDBK cell according to (**Rao and Malik 1982)**. The titer of virus was expressed as log $_{10}$ $TCID_{50}$/ml of the original inoculation using the formula of **(Reed and Muench 1938).**

2.5 Preparation of live attenuated local Ismailia lumpy skin disease vaccine:

Live attenuated Ismailia lumpy skin disease vaccine was 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 according to (**OIE, 2004).**

2.6. Titration of the prepared vaccine:

Titration of prepared LSD vaccine according to (**Raoand Malik, 1982) and (Tiwariand Negi 1995)**

2.7. Sterility test:

The prepared vaccine was tested for its sterility and purity to be free from bacterial, fungal and mycoplasmal contamination according to **(OIE,2004).**

2.8. Safety of prepared vaccine:

The prepared vaccine was tested for its safety by inoculation two calves I/D with 20xof the protective dose of LSD vaccine to check its safety as described by (**Mahmood et al., 1988).**

2.9 Vaccination of animals:

As described by (**Wang and Jiang 1988a) and (Daoud et al., 1998)** five calves and three pregnant cattle were vaccinated I/D in the tail fold with 0.5 ml of the prepared vaccine (log $10^{3.0}$ $TCID_{50}$), while three calves and two cattle were kept as control. Animals were observed daily for one month for clinical signs, including body temperature, swelling at the site of inoculation, or generalized skin lesions.

2.10. Evaluation of the cell mediated response:

2.10. A. by using lymphocyte proliferation assay

Whole blood was collected 1, 3, 5, 7, 10, 14, 21 and 28 days post vaccination for estimation of the cellular immunity according to **(Charles et al., 1978) and (Lucy 1984),** kits were purchased from Promokine, Catalog Number: PK-CR-20-300-1000, Lot No.:743182, Germany.

2.10. B. by using interferon gamma bioassay using Sandwich ELISA:

It measured quantitatively by using ELISA according to **(Lin Fan *et al*., 2012**). Kits were purchased from ID Vet, Catalog Number: IFNG-2P-0912, Lot No 540, France.

2.11. Evaluation of humoral Immunity by using serum neutralizing test (SNT) and Enzyme linked immune sorbent assay (ELISA).

Serum samples were collected from vaccinated calves and newly born calves from vaccinated dams at the time of vaccination then weekly at1st month, each two weeks at 2nd month then monthly andit was stored at -20^{o}c until examined by SNT and ELISA according to **(Martin et al., 1975) and (OIE 2010).**

3. Results:

3.1. Identification of local Ismailia lumpy skin disease virus by PCR.

The size of PCR product of the fragment of the attachment protein gene using the specific primer was (~192pb) as shown (**Figure.1)**

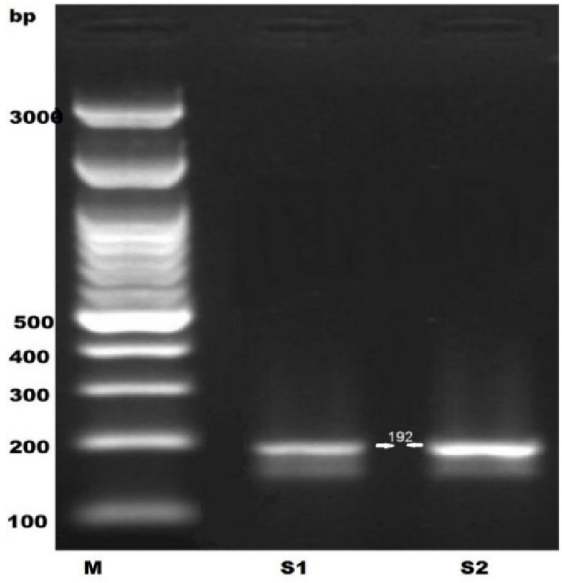

Figure (1). Specific PCR product of the fragment of the attachment protein gene at the correct expected size (~192pb), **Lane M:** High molecular weight nucleic acid marker. **Lane 1and Lan2:** PCR product (~192pb).

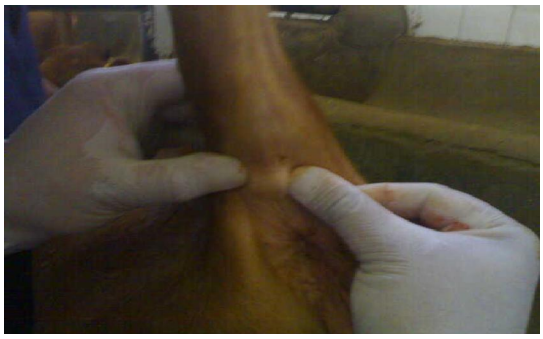

Photo (5): Post vaccinal reaction in calves vaccinated with LSD vaccine

3.2. Titration of LSD (local Ismailia strain) virus on MDBK cells.

LSDV was titrated carried out for several successive passages each was titrated in MDBK in cells and the highest titer among them was the passage Number 10 where its titer was $10^{6.8}$ log 10 $TCID_{50}$/ml. As shown in **(Table.1)**

Table.1: Titration of different passages of the propagated LSD virus on MDBK cells.

	Passages number												
	origin	1	2	3	4	5	6	7	8	9	10	11	12
Titer	10^4	$10^{4.4}$	$10^{4.6}$	10^5	$10^{5.4}$	$10^{5.6}$	$10^{5.6}$	$10^{5.8}$	10^6	$10^{6.4}$	$10^{6.8}$	$10^{6.4}$	$10^{6.6}$

3.3. Titration of prepared vaccine.

The titer of prepared lumpy skin disease vaccine on MDBK was $10^{3.0}$ $TCID_{50}$/ml.

3.4. Sterility and Safety test.

The prepared vaccine was free from aerobic, anaerobic bacteria, fungi and mycoplasma. The vaccine was safe with accepted rise in body temperature as it remained within the normal temperature range (38.3-39.1C°) for 15 days post vaccination with accepted local reaction at site of inoculation.

3.5. Clinical signs in vaccinated calves.

Body temperature of vaccinated calves showed rise in body temperature started on the 7thday 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, Also local reaction appeared at the inoculation site on the 8th day as a small size round firm nodule 2 to 4 cm in diameter and persist for 12-15 days before hiding.

3.6. Evaluation of cell mediated immune response of vaccinated and Non-vaccinated calves.

It began to increase till reach to Its peak at 10th day (1.84) then decreased until the end of the experiment at 28th day post vaccination and were tested by using lymphocyte proliferation assay as clarified in **(Table.2).**

Table.2: Cell mediated immune response of calves vaccinated with prepared vaccine.

Calves No.	Days post vaccination								
	0	1	3	5	7	10	14	21	28
Calf 1	0.080	0.290	0.416	0.601	0.741	1.880	1.642	0.730	0.340
Calf 2	0.072	0.262	0.397	0.611	0.822	1.632	1.618	0.684	0.321
Calf 3	0.077	0.206	0.401	0.715	0.909	1.998	1.621	0.893	0.364
Calf 4	0.082	0.215	0.364	0.622	0.856	1.779	1.633	0.691	0.354
Calf 5	0.079	0.201	0.422	0.734	0.917	1.895	1.611	0.760	0.265
Average	0.078	0.235	0.401	0.656	0.849	1.84	1.625	0.752	0.329
Control	0.077	0.082	0.085	0.087	0.087	0.088	0.090	0.80	0.077

0 day: before vaccination (on the time of vaccination). **OD:** optical density.

3.7. Evaluation of Immune response of calves vaccinated with the prepared vaccine by Interferon gamma Bioassay

Interferon gamma (IFN-γ) was detected in the first day (25%) till seven days (17%) post vaccination then decrease after that. as clarified in **(Table.3)**.

3.8. Evaluation of neutralizing antibody titer in calves vaccinated with LSD vaccine by SNT.

Serum samples were collected from calves vaccinated with LSD reached protective level (1.5) at the second week post vaccination and increased gradually till reach (3.1) at 12 weeks post vaccination

then reached to (1.5) at 40 weeks from vaccination. The result was showed in (**Table.4).**

Table.3: Cell mediated immune response of calves vaccinated with LSD vaccine by Interferon gamma Bioassay (IFN-γ).

Calve No. AndStatus	Days post vaccination								
	0	1	3	5	7	10	14	21	28
Calf 1	8%	25%	28%	19%	17%	14%	11%	9%	7%
Status	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Calf 2	7%	23%	26%	22%	19%	13%	10%	8%	7%
Status	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Calf 3	7%	20%	22%	18%	16%	14%	12%	9%	6%
Status	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Calf 4	6%	19%	23%	20%	17%	13%	9%	6%	4%
Status	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Calf 5	9%	21%	26%	23%	18%	14%	11%	9%	6%
Status	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Control	0%	0%	0%	0%	0%	0%	0%	0%	0%
Status	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Positive: S/P% more than 15% Negative: S/P% less than 15% S/ p: mean sample positive.

Table 4: Mean of neutralization index invaccinatedcalves with LSD vaccine.

NO. of vaccinated calves	SNT Titer*																
	Weeks post vaccination																
	0	1	2	3	4	6	8	12	16	20	24	28	32	36	40	44	48
Calf 1	0.3	0.6	0.6	0.9	1.2	2.1	2.4	2.7	2.7	2.1	2.1	1.8	1.8	0.9	0.6	0.6	0.6
Calf 2	0.6	0.9	0.9	1.8	2.1	2.4	2.7	3.0	2.7	2.4	2.1	1.8	1.8	1.8	0.9	0.9	0.6
Calf 3	0.6	0.9	1.8	1.8	2.1	2.4	2.7	3.2	3.0	2.4	2.4	2.1	2.1	1.8	1.8	0.9	0.6
Calf 4	0.6	0.9	2.1	2.1	2.4	2.7	3.0	3.2	3.0	2.7	2.4	2.4	2.1	2.1	2.1	0.9	0.6
Calf 5	0.5	0.9	2.1	2.4	2.4	2.7	3.0	3.2	3.0	2.7	2.7	2.4	2.4	2.4	2.1	0.9	0.6
Mean	0.5	0.9	1.5	1.8	2.1	2.5	2.8	3.1	2.8	2.5	2.3	2.1	2.0	1.8	1.5	0.9	0.6
Control	0.23	0.21	0.23	0.3	0.36	0.36	0.3	0.36	0.3	0.3	0.2	0.3	0.36	0.33	0.19	0.12	0.10

* Serum neutralizing antibody titer expressed as log 10.

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

3.9. Evaluation of immune response in calves vaccinated with prepared LSD vaccine measured by ELISA.

Serum samples were collected from calves vaccinated with LSD reached protective level (1.19) at the second week post vaccination and increased gradually till reach (2.34) at 12 weeks post vaccination then reached to (1.13) at 40 weeks from vaccination. The result was showed in**(Table.5).**

Table 5: Immune response in calves vaccinated with prepared LSD vaccine by ELISA.

NO. of Calves	Optical density																
	Weeks post vaccination																
	0	1	2	3	4	6	8	12	16	20	24	28	32	36	40	44	48
Calf 1	0.76	0.97	1.18	1.42	2.19	2.26	2.29	2.30	1.91	1.70	1.62	1.42	1.31	1.20	1.13	0.84	0.64
Calf 2	0.69	0.88	1.14	1.49	2.18	2.28	2.28	2.39	1.93	1.71	1.65	1.46	1.34	1.26	1.11	0.88	0.67
Calf 3	0.67	0.98	1.25	1.31	2.19	2.26	2.27	2.38	1.97	1.73	1.66	1.47	1.37	1.23	1.10	0.81	0.61
Calf 4	0.72	0.84	1.22	1.40	2.17	2.20	2.26	2.31	1.90	1.71	1.63	1.40	1.33	1.28	1.14	0.85	0.63
Calf 5	0.79	0.79	1.19	1.46	2.18	2.23	2.27	2.32	1.95	1.72	1.62	1.43	1.30	1.21	1.18	0.89	0.62
Mean	0.73	0.89	1.20	1.42	2.18	2.24	2.27	2.34	1.93	1.71	1.64	1.44	1.33	1.24	1.13	0.85	0.63
Control	0.33	0.29	0.26	0.25	0.23	0.21	0.19	0.12	0.11	0.09	0.08	0.06	0.06	0.05	0.05	0.04	0.03

+ Ve: equal or more than 1 -Ve: less than 1

3.10. Evaluation of maternal immunity in newly born calves from pregnant cattle vaccinated with prepared LSD vaccine by using SNT and ELISA

Serum samples were collected from newly born calves from vaccinated dams with prepared LSD vaccine at during six months showed that highly antibody titer from the first week after partulation and

persisted in protective level until 16 weeks then decrease than protective level. The results were showed in**(Tables.6.7.)**

Table 6: Mean of neutralization index in newly born calves from vaccinated dams by SNT.

newly born calves	SNT Titer*										
	Weeks post parturition										
	1	2	3	4	6	8	12	16	20	24	28
Calf 1	2.25	2.4	2.4	2.1	2.1	1.8	1.76	1.5	1.43	1.22	0.96
Calf 2	2.33	2.33	2.29	2.1	1.8	1.8	1.77	1.64	1.47	0.92	0.68
Calf 3	2.40	2.22	2.13	2.08	1.95	1.8	1.79	1.59	1.33	0.95	0.69
Mean	2.33	2.31	2.27	2.09	1.95	1.8	1.77	1.57	1.41	1.03	0.77
Control	0.34	0.36	0.38	0.32	0.33	0.33	0.33	0.34	0.33	0.33	0.31

Protective antibody titer = 1.5 *Log10 serum neutralizing antibody titer.

Table 7: Mean of Immune response in newlyborn calves by using ELISA.

Newly born calves	Optical Density										
	Weeks post parturition										
	1	2	3	4	6	8	12	16	20	24	28
Calf 1	1.934	1.878	1.610	1.534	1.414	1.365	1.198	1.050	0.988	0.681	0.516
Calf 2	1.944	1.883	1.603	1.544	1.401	1.334	1.201	1.102	0.862	0.695	0.528
Calf 3	1.990	1.877	1.613	1.590	1.459	1.380	1.197	1.021	0.871	0.650	0.599
Mean	1.956	1.879	1.609	1.556	1.425	1.359	1.199	1.058	0.907	0.675	0.548
Control	0.211	0.221	0.132	0.133	0.123	0.022	0.013	0.018	0.099	0.098	0.077

+Ve: equal or more than 1- Ve: less than 1

4. Discussion

In enzootic situation vaccination has been considered to be the cheapest and suitable means of Capripox diseases control as it stimulate both humoral and cell mediated immune response **(Kallesh *et al.*, 2009).**

In our study we tried to control LSD through preparation of live attenuated LSD vaccine (Local Ismailia strain) to improve the health and welfare of animals as well as age of newly born calves from vaccinated dams by prepared vaccine.

PCR was the test of choice for rapid identification and confirmation of LSD virus, PCR product of attachment protein gene of LSD virus was (~ 192bp) this results agreed with (**Alaa *et al.* 2008)** who indicated that agarose gel electrophoresis of the PCR amplicons (~ 192 bp) of genomic DNA of LSD virus.

LSD virus inoculated on confluent MDBK cell cultures, cytopathic effect (CPE) 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. as showed **(Singh and Rai 1991), (Rizkallah 1994), (Aboul-Soud, 1996), (Olfat 2000) and (Christine, 2008)** who found that the highest titre obtained from the bottles harvested 5-6 days post inoculation**.**

The prepared LSD vaccine was titrated on MDBK cell culture calculated by using the formula of **(Reed and Muench 1938**). The titer of LSDV on MDBK$10^{3.0}$ TCID50/ml**.** as found (**Wang and Jiang 1988a)** and **(OIE 2012).** The prepared attenuated vaccine was tested for sterility and the results proved that the vaccine was negative to any contaminating agents as bacteria, moulds and fungi when inoculated on specific media. As found **(Wassel*et al.* 1996) and (code of federal Regulation 2005),** who reported that the final product should be free from bacteria, fungi and mycoplasma. Safety of the prepared vaccine was also detected in calves (as 20x of vaccine dose) gave satisfactory results of safety with no rise in body temperature. It remained within the normal temperature range (38.3 -39.1)for 15 days post vaccination and no clinical abnormalities. As recorded **(Mahmoud *et al.* 1988), (Code of federal Regulation 2005) and (OIE 2012)** which reported that the final product should gave good satisfactory result of safety with no rise in body temperature of animals and it remained healthy for period of 15 days post vaccination which indicated that the vaccine is safe.

Clinical examination in calves vaccinated with prepared LSD vaccine showed a pronounced local reaction (2-4 cm in diameter) at the point of inoculation of two calves and a mild reaction (10mm) and disappeared within 12-15 days. as recorded by **(Woods 1988), (OIE, 1992), (Carn 1993) and (Coetzer 2004).** The recorded clinical signs were also in agreed 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.

Cell- mediated immune response plays an important role against capripox beside humoral immunity **(Bachh *et al.* 1997**). The increased lymphocyte proliferation due to specific LSD antigen stimulation was found (**Ahmed *et al.* 2007**). Lymphocyte MTT proliferation assay was chosen for estimation of cell mediated immune response (**Charles *et al*. 1978) and (Lucy 1984).** Cell-mediated immune response in vaccinated calves with prepared LSD vaccine ranged in between 0.078 -1.837 all over the experiment. The results 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.

Gamma interferon Bioassay (IFN-γ) is an important cytokine in the host defense against infection by viral and microbial pathogens **(Strichman and Samuel 2001),** and it activates pathways that can directly inhibit virus **(Biron and Brossay 2001).** It was detected in the first day (25%) till seven days (17%) post vaccination then decrease after that**.** the results revealed that the prepared lumpy skin disease vaccine induced higher level of interferon γ gene expression and this result was found **(Charles *et al.* 2012)** who detected that experimentally infected calves produced serum IFN-γ, IL-12 and other pro inflammatory cytokines but not IFN-α. Despite the lack of IFN-α, innate immunity via the IL-12 to IFN-γ circuit possibly contributed to early protection against LSD since neutralizing antibodies were detected after viremia had cleared.

Evaluation of humoral immune response depending mainly on the antibody titers in sera of vaccinated calves by using SNT and ELISA. LSD reached protective level (1.5) at the second week post vaccination and increased gradually till reach (3.1) at 12 weeks post vaccination then reached to (1.5) at 40 weeks from vaccination. These results of seroconversion were recorded by (**Agag *et al.* 1992)** where they mentioned that serum neutralizing antibodies develop on the 2nd day and a significant rise of antibody titer was detected from the 21th to 42th day post inoculation. Neutralization is very specific for almost all viruses **(OIE 2000**). Our results were in contrast with **(Kitching *et al.* 1989) and (Rao and Negi, 1997),** who reported that the immune status of a previously infected or vaccinated animal cannot be related to serum level of neutralizing antibody, 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.

ELISA result indicated that vaccinated calves reached protective level (1.19) at the second week post vaccination and increased gradually till reach (2.34) at 12 weeks post vaccination then reached to (1.13) at 40 weeks from vaccination, this result was mentioned by (**Suri *et al*. 1984), (Wassel *et al*.1996)** who conculated that caperipox vaccines is the most effective immunogen available and provide both cellular and humeral immunity.

By SNT and ELISA antibody titers in newly born calves from vaccinated dams started from the first week(2.33),(1.095) after parturition and persisted in protective level until 16 weeks (1.57), (1.058) respectivley then decrease than protective level

From above result showed that maternal immunity maintained in calves till 16 weeks of age then disappeared before six months so newly born calves should be vaccinated at four month of age. this results were reported by **(Fahmy 2000)** who stated that LSDV maternal antibodies via colostrum maintained in calves till 4.5 month of age then disappeared, On the other hand, this result contrasted with (**Woods 1988)** that mentioned that passive immunity from vaccinated dams might interfere with the efficient vaccination of calves less than 6 months old. The result supported by**(Kitching *et al.* 1989)** and (**Fahmy 2000)** who confirmed transfer of maternal lymphocytes from dams vaccinated with LSDV vaccine to newly born calves via colostrum.

We conculated that LSD vaccine was, highly lymphocyte and interferon γ level, highly immunogenic, inducing a higher level of antibody titer with prolongation of the duration of immunity so it considered the best choice of vaccine to control the disease in the field, so It recommended to be used to control the disease and newly born calves from vaccinated dams should be vaccinated at 4thmonths of age.

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