Vet.Med.J.,Giza. Vol.50, No.4. (2002):963-975.

EFFICACY OF VACCINATION STRATEGY AGAINST LUMPY SKIN DISEASE WITH THE USE OF NEW GENERATION OF INACTIVATED VACCINE

H. M. MADBOULY*, H. A. HUSSEIN**, FATEHIA M. MOHAMED***, OMYMA M EL-DESAWY***M. S. SABER** and M. A. SHALABY**

* Department of Virology, Faculty of Veterinary Medicine , Beni--Suef Branch, Cairo University.
**Department of Virology, Faculty of Veterinary Medicine , Cairo University, Giza-Egypt.
*** Department of Virology, Animal Health Research institute, Dokki Giza-Egypt.

Received: 30. 6. 2002
Accepted: 17. 10. 2002

SUMMARY

Lumpy skin disease (LSD) local strain (Ismalia 88) was propagated in MDBK cell line for six serial passages to obtain a titer of 107.5 /ml TCID50. Harvestation of the adapted virus from inoculated cell culture was based on the time at which a clear CPE was observed (5 days post inoculation). The virus was inactivated using binary ethyleneimine (BEI) at concentration of 2% for 12 hours. The inactivated virus was used with new generation of adjuvant (Nigella sativa oil) in the preparation of inactivated LSD local vaccine. The prepared vaccine was characterized using different techniques including the drop test, emulsion viscosity and stability. In addition, it was tested for the sterility and safety. Twenty Friesian calves (7 month-old) divided into 4 groups were inoculated with the different vaccination programmes as follow: group 1 was vaccinated with 2 doses of sheep pox vaccine with 6 months interval; group 2 was vaccinated with sheep pox vaccine and boostered after 6 months by the prepared LSD inactivated vaccine; group 3 was vaccinated with 2 doses of prepared inactivated LSD vaccine with 6 months interval and group 4 was left non vaccinated calves as negative control. Sera were collected monthly post vaccination for 8 months. The sera were tested by virus neutralization test and ELISA to evaluate the immune response of different vaccination strategies. The results of the in Vivo experiment revealed that the inactivated LSDV adjuvated with Nigella sativa oil was sterile, safe and elicited hight antibody response against LSD either when used for priming or boostering of animals alone or in combination with sheep pox vaccine.

INTRODUCTION

Lumpy skin disease virus (LSDV), also known as "Neethling virus", is a capripoxvirus that cause a serious viral disease of cattle. Genus capripox of family Poxviridae contains LSDV of cattle and the very closely related viruses of sheep and goat pox (Woods, 1988). In Vivo capripoxviruses demonstrate cross protection and most of the strains do not posses absolute host specificity (Capstick, 1959; Capstick and Coackley, 1961; Davies and Mbugwa, 1985., Kitching and Taylor, 1985; Yeruham et al.,1994). Sequence analysis of capripox viruses (field and vaccine isolates) of cattle, sheep and goat revealed a high degree of homology (Black et al., 1986).

LSD was first recognized in Egypt in 1988 through cattle imported from Somalia (Fayed et al., 1988; Anonymous, 1988). In 1989 the disease was spread rapidly and caused several outbreaks in different governorates (Suez, El-Tal El-Kabir in Ismalia governorate [highest incidence of the disease] and El- sharkia). The disease was diagnosed clinically and pathologically (Ali et al., 1990) and the virus was isolated form samples obtained from the first outbreak (Ismalia governorate) which was investigated by Ali et al, (1990). Several local isolates of LSD were identified later during outbreaks of the disease in Nag-Hamadi, New Valley, Mousha El- Badary, Menia, Assuitt and Upper Egypt (El-Allawy et al., 1992).

Due to the serious economic losses caused by LSD, Office International des Epizooties (OIE) positioned the disease one of list A diseases. Control strategy of LSD based on the use of the live attenuated strains of capripoxviruses has been adopted and demonstrated its efficiency (Capstick and Coackley,1961; Carn, 1993). Also, sheep poxvirus (Romanian strain) vaccination strategy was applied to control LSD outbreaks in Egypt (Michael et al., 1996). Several published studies have convincingly suggested the need of development of an attenuated cell culture vaccine from LSD to control the disease (Nawathe et al., 1982; Asagba, 1984; Sewell and Brocklesby, 1990). Recently the Egyptian local isolate of LSD was adapted in MDBK cell line and was used in the production of LSD vaccine in Egypt with great safety and efficiency (Aboul Soud, 1996; Daoud et al., 1998).

Risks of live attenuated vaccines are threaten because of many aspects including: such vaccines depend on the passage levels in cell culture which could harmly affect both immunogenicity and virulence moreover live attenuated vaccines can produce the disease in immunosupressed individual by reverting it to a more virulent phenotype (Singh and OíHagan, 1999). In addition, the used live modified LSD virus vaccines reside in their pathogenicity that produce a large local reaction at the site of the injection which some stockowners find it unacceptable (OIE, 1996). In addition event of recombination between a vaccine and a

naturally occurring poxvirus increases the chance of evolving a virus with increased pathogenicity and could be highly transmissable (Gershon et al., 1989). Saber et al., (2000) report the preparation of oil inactivated LSDV and demonstrate the potentiality of such vaccine in eliciting antibody response against LSDV. They propose that the use of inactivated LSD vaccine with a new generation of adjuvants would be the base and favorite vaccination policy to eradicate the disease in Egypt. Hence, we believe that inactivated LSDV vaccine alone or in combination with sheep poxvirus vaccine would be a good vaccination policy to control LSD in Egypt. In the present study, we investigate the efficiency of different vaccination programms with the use of new generation of adjuvant (Nigella stiva) in the preparation of inactivated LSD vaccine.

MATERIAL AND METHODS

1-Propagation of lumpy skin disease virus on MDBK cell line:

Local isolate of LSD (Ismalia 88 strain) adapted on MDBK cell line [105 TCID50 /ml original titer at the department of virology, Fac. Vet. Med, Cairo University] was used in the study. The virus was propagated on MDBK cell line for six successive passages.

2-Titration of LSDV on MDBK cell line:

Virus infectivety assay was carried out as previously described (Frey and Liess, 1971) uing 96 well microtiter tissue culture plate. The virus infectivety titer was determined according to method of Reed and Muench (1938).

3- Sheep Pox vaccine

Sheep poxvirus vaccine Kenyan strain was obtained from Serum and Vaccine Research Institute, Abbasia, Cairo-Egypt.

4-Virus inactivation:

Binary ethyleneimine (BEI) was used for virus inactivation. BEI was prepared through cyclization of 0.1 M of 2-bromo-ethylamine hydrobromide in 0.2N NaoH in a water bath (37°C) for 1-2 hours. 2% concentrations of BEI was mixed with LSDV and incubated for 24 hours. Samples were taken after zero,1,3, 6,9,12,18,24 hours and assayed for virus infectivety to determine the concentration and time at which complete inactivation was obtained. Virus inactivation was stopped by immediate addition of cold sodium thiosulphate at a final concentration of 20%. To evaluate the BEI inactivation virus preparations were inoculated in cell culture and examined daily for the presence of CPE , in mean time three 7 month-old calves were injected intradermal with the inactivated virus and observed daily to detect any clinical symptoms.

5- vaccine Preparation:

5-1-Preparation of Nigella sativa adjuvant:

Nigella sativa oil was prepared as adjuvant according to the method described by Madbouly et al., (2000). Nigella sativa oil was mixed with Arlacel A in a ratio of 9 parts oil to 1 part of Arlacel A (W/V). After through mixing, It was sterilized through seitz filter and the mixture was stored at room temprature and used within few weeks of preparation to be mixed with the inactivated LSDV.

5-2- Preparation of Nigella sativa oil adjuvant inactivated LSD vaccine:

Inactivated LSDV with a titer of 107.5 suspension (96% inactivated LSDV and 4% Tween 80) was emulsified with Nigella sativa oil in a ratio of 1:1. Continuous mixing was applied by the aid of sterile syringe until the production of a stable emulsion.

6- Characterization of the inactivated LSD vaccine:

The LSD oil emulsion vaccine preparation was evaluated for its emulsification process using [a] the drop test as previously described (Roshdy, 1996) [b] emulsion viscosity (Cunliffe and Graves, 1963) and [c] emulsion stability (Cunliffe and Graves, 1963) .

7- Vaccine sterility and safety:

The vaccine preparation was cultured on thioglycolate broth, Sabouraud nutrient agar, blood agar and phenol dextrose media to test the sterility of the vaccine. Safety test was conducted by S/C inoculation of 10 ml of the vaccine preparation in a susceptible cattle . Postvaccinal was observed for one week to record the presence of fever, local or general lesions had been applied.

8- In vivo experimental studies on the effect of different vaccination strategy against LSD using the prepared inactivated LSD vaccine alone or in combination with sheep poxvirus vaccine:

Twenty calves 7 month-old were sero-negative from LSD antibodies (by SNT and ELISA) were classified into four groups (five each). Group 1 was vaccinated with 2 doses of sheep pox vaccine with 6 months interval; group 2 was vaccinated S/C with sheep pox vaccine and boostered after 6 months by the prepared inactivated LSD vaccine; group 3 was vaccinated with 2 doses of prepared inactivated LSD vaccine with 6 months interval and group 4 was left non vaccinated calves as negative control. Sera were collected monthly post vaccination for 8 months and tested by virus neutralization test and ELISA to evaluate the immune response of different vaccination strategies (House et al.,1990).

RESULTS

LSDV (Ismalia strain) propagated on MDBK cells for 6 successive passages showed an increase in virus titer with a log difference form 105.5 to 107.5 TCID50/ml (Fig 1). The CPE induced by the virus was characterized by cell rounding and shrinking resulting in empty patches in the cell monolayer start to appear 3-4 days post inoculation and ended by cellular degeneration like those of tissue sections. The sequential microscopical changes developed by Ismalia strain of LSDV were similar in each passage in MDBK cells.

As shown in Fig (2), BEI was able to inactivate the virus completely after at least 12 hrs of incubation at a concentration of 2%. When inoculating the calves with the inactivated virus preparation no clinical symptoms have been observed confirming the process of inactivation used in the study. Also no CPE was developed when the inactivated virus was inoculated in cell culture. Vaccine preparation using Nigella sativa oil adjuvant was examined for a period 6 months for its stability, viscosity and sterility. The vaccine preparation remained stable and quite emulsified at 4°C. Beside its sterility along the 6 months period of examination. Moreover, when inoculating in cell culture or 10x dose S/C in 3 calves, the inactivated LSD vaccine found to be safe with no CPE in MDBK cells and no clinical symptoms including fever, local or general lesions or death have been recorded. All animals used in experimental studies were sero-negative to LSDV before being vaccinated. The vaccinated animals did not reveal or manifest any thermal reactivity after vaccination with the different vaccines along the period of observation (one month) as there was no change in body temperature recorded daily after vaccination. In the first group of vaccinated calves, all animals developed antibodies where the highest neutralization titer (NT) observed at one month post vaccination with sheep pox (21.6 NT) and started its decline phase of antibodies which was sharper 6 months post vaccination (Fig 3). Boostering of those animals elvated the antibody titers to its peak after one month where it started to decrease at 2 months post boostering, however, the neutralizing titer considers high (8 NT). The NT of antibodies found to decrease approximately one log2 monthly after active immunization with sheep pox vaccine and the results were in parallel with such obtained by ELISA. In the seconed group, animals primed with sheep pox vaccine revealed high NT of antibodies which reached its peak 1 month post vaccination (NT 17.6) and decreased gradually till 6 months Fig (4). Boostering of those animals by inactivated LSD vaccine which adjuvated with Nigella sativa oil induced high NT (44.8 and 38.4, after one and 2 months post boostering, respectively) indicating the higher stimulation of the immune response than those animals of group 1 (NT of 16 and 8). Fig (5) il-

lustrates the obtained results in group 3 where 2 doses of the prepared inactivated LSD were used. As expected, vaccination of calves with the Nigella sativa adjuvated LSD vaccine elicited the greatest NT that reached its peak after 2 months post vaccination (41.6) and continually to be high in comparison with the results obtained in groups 1 and 2 till 4 months post vaccination. Boostering of those animals with the same inactivated vaccine elicited high antibody titers (NT of 17.6 and 35.2, one and 2 months post boostering, respectively) which were higher than such obtained in group 1 which primed with sheep pox vaccine. Assessment of the obtained results of the three groups showed clearly the great immunogenic effect of inactivated LSD adjuvated with Nigella sativa oil when it used either for priming or boostering of animals (Table 1). Fig (6) presentes the obtained results of group 4 which were non vaccinated calves. There was no detectable antibody titer observed either by neutralization test or ELISA. These animals had a normal temperature of 38 oC during the course of the experiment. Testing the same sera of vaccinated animals by ELISA , It was also observed that all the animals had antibodies to LSD in parallel with the obtained results of virus neutralization test. Moreover, analysis of the obtained data revealed a correlation between the NT detected in the examined sera and the ELISA optical densities (Table 2).

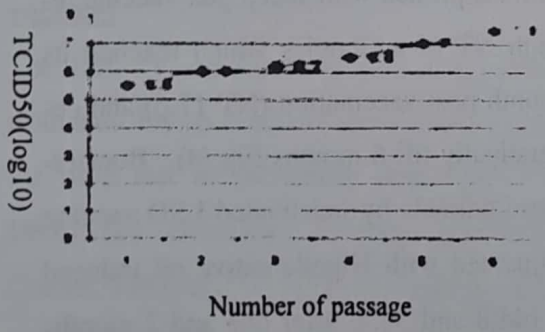

Fig. (1): Infectivity titers of propagated LSDV on MDBK cell line.

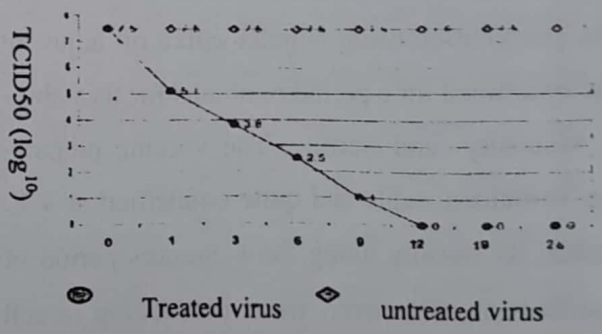

Fig. (2): Hours of BEI inactivation for LSDV Ismailia 88 strain

Table (1): Comparison of the mean neutralizing antibody titers to LSDV in the animals of groups 1, 2 and 3 which received different vaccination strategies

Group No.	Mean neutralizing antibody titers								
	Months Post vaccination								
	Pre-vaccination	1	2	3	4	5	6*	7	8
1	-	21.6	12.4	8	5.6	3.2	2	16	8
2	-	17.6	10.4	5.6	5.2	3.6	1.6	44.8	38.4
3	-	16	91.6	35.2	25.6	12.8	7.2	17.6	35.2

* All animals were boostered at 6 months post first dose of vaccination.

Table (2): Corelation between the obtained optical densities (O.D) by ELISA and Virus Neutralizing antibody titers by VNT against LSDV in sera of different groups of vaccinated and control calves.

Mean O.D measured by ELISA	Antibody titers measured by VNT
Less than 0.2	-
0.21-0.31	2
0.31-0.41	4
0.41-0.51	8
0.51-0.71	16
0.71-0.91	32
0.91-1.41	64
1.51-1.91	128 or more than 64

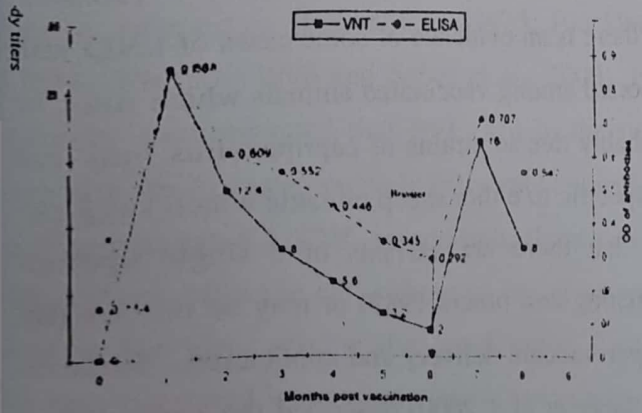

Fig. (3): The anitbody titers to LSDV in animals vaccinated with 2 doses of live sheep pox vaccine as measured by virus neutralization test and ELISA.

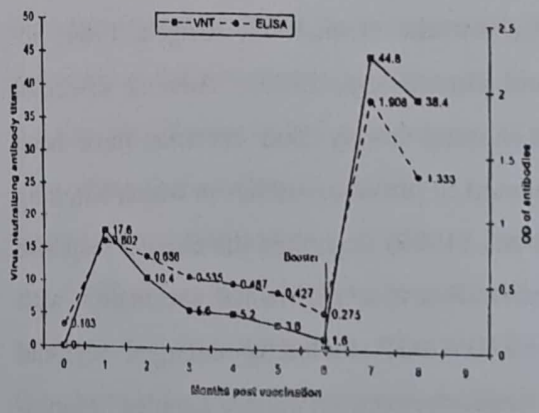

Fig. (4): The antibody titers to LSDV in animals vaccinated with live sheep pox vaccine and boostered with inactivated LSD vaccine adjuvated with Nigella sativa oil as measured by virus neutralization test and ELISA

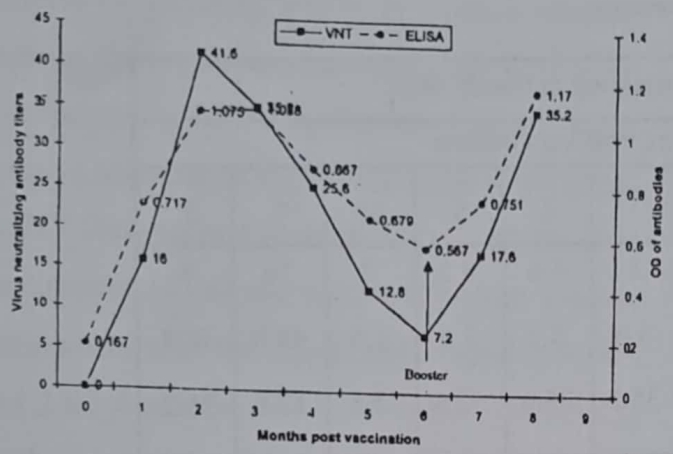

Fig. (5): The anitbody titers to LSDV in animals vaccinated with 2 doses of inactivated LSD vaccineadjuvated with Nigella sativa oil as measured by virus neutralization test and ELISA.

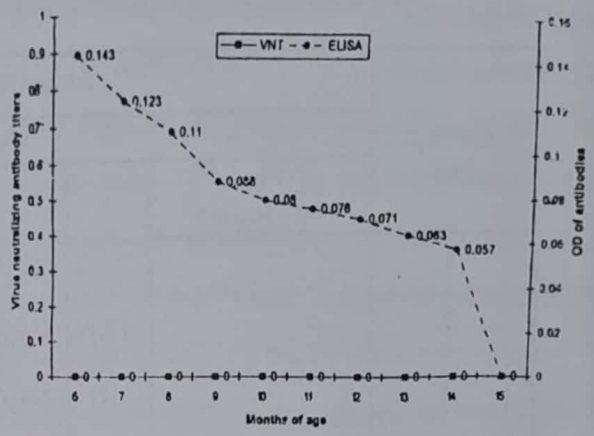

Fig. (6): The antibody titers to LSDV in non vaccinated animals as measured by virus neutralization test and ELISA

DISCUSSION

The use of the live attenuated strains of capripoxviruses proposed to be the base for control of LSD (Capstick and Conackley, 1961, Carn, 1993). Sheep pox virus vaccines were sufficiently related to the LSDV to induce cross protection (Davis, 1976; and Capstick,1959). Despite the success of this stategy in some countries, several researches have convincingly suggested the need for development of a LSD vaccine derived form LSDV (Nawathe et al.,1982; Asagba, 1984; Sewell and Brocklesby, 1990). Adverse effect of live attenuated lumpy skin vaccines have been documented in previous studies in which Kitching and others, (1989) described the clinical response of some heifers to experimental vaccination with strain 0240/KSGP including enlarged regional lymph node, pyrexia, secondary papules. Moreover, recently Yeruham and others (1994) reported the incidence of generalized reactions with LSD-like lesion in lactating dairy cattle and stressed animals vaccinated with capripox strain 0240. Conversely, the Romanian strain of sheep pox was also used as vaccine during the outbreaks of LSD in Israel and no post vaccinal reaction has been observed besides the vaccinated animals did not serve as a potential source of infection by horizontal transmission (Yeruham and others , 1994). In Egypt, Micheal et al., (1996) have also used the Romanian strain of sheep pox vaccines. However, there is an evidence of some cases of LSD still reported among vaccinated animals which may be probably due to strains of capripoxvirus may not be specific to either sheep or cattle which suggesting that there are variants of a single species (Kitching and others 1989) or may be due to improper vaccine delivery and application. Recently, Saber et al.,(2000) described the preparation of an inactivated LSDV vaccine. They proposed

that development of such vaccine from a locally isolated LSDV is of need specially after the appearance of the disease again in upper Egypt (El-Allaway et al.,1992). In addition, they recommend the application of inactivated LSD vaccine containing a new generation of adjuvatns for long term control and eradication of the LSD.

In the present study, we describe the efficacy of vaccination strategy against LSD using different alternatives including sheep pox and new generation of inactivated LSD vaccine. LSDV (Ismalia strain) was propagated on MDBK cell line to increase its titer and the CPE induced by the virus was similar to those observed by others (Alexander et al., 1957; De Lange, 1959; Prydie and Coackley, 1959). The best time of harvestaion of the virus with high titer was obtained after 120 hrs post inoculation. This observation is previously reported by others who propagated LSDV (Ibrahim, 1993, Yousif, 1995, Saber et al., 2000). BEI has been previously used to inactivate LSD propagated in MDBK without alteration on its immunogenic properties (Bahnemann, 1975, Blackburn, 1991, Shahin, 1998 and Saber et al., 2000). It is clearly demonstrated that BEI used in the study has the ability to completely inactivate compeletly the propagated LSDV. In addition, there was no live virus effect in cell culture or in calves when tested for virus residue after inactivation. Testing of the prepared inactivated Nigella sativa adjuvated LSD vaccine revealed marked stability, viscosity, and sterility of the oil adjuvated vaccine preparation over a period of 6 months. Besides the good safety profile of the vaccine which was clearly demonstrated in cell culture or in calves with no record of CPE or post vaccinal reactions, respecively.

Inspection of the composite results of in vivo experiments indicated that the prepared LSD vaccine clearly demonstrated its ability to develop a protective levels of neutralizing antibodies with no adverse reactions post vaccination confirming the similar results of Saber et al., (2000). Moreover, the inactivated LSD vaccine presented here has several advantages over the live attenuated vaccine. First, it showed the potential ability to induce a protective level of neutralizing antibodies against LSD when compared to the group one in which animals received sheep pox vaccine or even when compared with the results obtained by others who used sheep pox and LSD live attenuated vaccines (Micheal et al., 1996 and Daoud et al., 1998 [b]); second, BEI concentration used in the study to inactivate the virus assure that the antigenicity of the virus was not affected and the virus titer steadily decreased in direct relation with time of incubation (Fig 2) similar to those previously reported (Bahnemann, 1975 and 1991; Shahin, 1998, Saber et al., 2000); third, It is well documented that live attenuated vaccines can cause the disease in immunosupressed individuals or stressed animals (Kitching and others, 1989; Ye-

ruham and others, 1994) or can revert to a more virulent phenotype (Sing and OfHagan, 1999). In addition, in some countries it was reported that the used live modified LSDV vaccines resides their pathogenicity and produce a large local reaction at the site of inoculation which come stock-owner find unacceptable (OIE, 1996). On the other hand, in the event of recombination between a vaccine and a naturally occurring poxvirus, any hazard from increased pathogenicity would be exacerbated if the new virus was highly transmissble. Nature occurring of genetic recombination of poxviruses is likelihood specially in the absence of laboratory screening or selection of the vaccine and field viruses (Gershon et al., 1989). Given all together, the safety and the immunogenicity of inactivated vaccines are considered the favorite vaccine to apply policy to eradicate the disease.

The selection of an effective and potent adjuvant which will serves as antigen reservoir preventing rapid elimination and promoting sustained release of the antigen will be of added interest. A new trend in vaccine production is the use of immunomodulators that influence both the magnitude of immune response which might reveal Th1/Th2 balance and hence the isotope of the produced antibody and DTH (Cox and Coulter, 1997). Moreover, there are some adjuvants which are capable of cytolytic delivery (stimulate CTL response) and targeting the antigen which increase the efficiency of delivary of antigen to APCs (Singh and OfHagan,1999).

In the present study, we used Nagilla sativa oil to be the vehicle and the biological response modifier with immunostimulatory effect. Nagilla sativa oil has been demonstrated several record of safety and effectiveness when used alone or in combination with veterinary vaccines (Hailat et al., 1995, Agarwal et al., 1979, El-Kadi et al., 1990, Madbouly et al., 1999a ,1999b, 2000). The mechansim of immunostimulation of Nigella sativa is through its effect on the antigen presenting cells (APCs) (Haq et al., 1995). Activation status of APCs could stimulated either by the expression of the major histocombitability antigens or secretion of cytokines. The type of immune response is greatly depend on both the viral antigen and the type of adjuvant included in the inacticvated vaccine. Thus, Nigella sativa alone could direct the type of immune response induced by the vaccination. In the present study, it was clearly demonstrated the effect of Nigella sativa oil used in the prepared vaccine. Its effect was potent in two of the three vaccinated groups (Fig 4 and 5). The use of inactivated LSD vaccine adjuvated with Nigella sativa oil in priming the animals demonstrates comparable antibody titers to those primed with the live attenuated sheep pox vaccine. Moreover, priming and boostering of animals with such inactivated vaccine elicited higher immune response than those received 2 doses of sheep pox. As expected superior increase in NT in the group

primed with Nigella sativa oil vaccine in the primary immune response was observed compared to the animals primed with sheep pox vaccine. The explanation of that is the dose of the antigen presented to the immune system which was superior in case of inactivated vaccine . Also, the costimulatory effect of Nigella sativa oil on the APCs which directly stimulate the T helper cells to induce such superior response. Such response could compansate the role played by the live attenuated vaccine. Several investigators have demonstrated the role of Nigella sativa in inducing cell mediated immune response (Haq et al., 1995, El-Kadi et al., 1990, Basel and Erwa, 1993, Madbouly et al., 1999 a, b and 2000). However, the exact role of cell mediated immune response in such stimulation by inactivated LSD vaccine need to be addressed.

Alternatively, boostering of animals with Nigella sativa adjuvated vaccine revealed the greatest response compared with sheep pox vaccine. Selection of an adjuvant that stimulate both cellular and humoral immune response consider the gold standard of adjuvanted vaccines. In conclusion, the the use of a vaccination policy depends on the utilization of 2 doses of the inactivated LSD adjuvated with Nigella sativa oil represent the most efficient strategy for control and eradication of LSD.

REFERENCES

Aboul Soud, E.A.(1996). Studies on the adaptation of LSD in cell cultures. Ph.D.Thesis (Microbilogy). Fac. Vet. Med ., Alexandria University.

Alexander, R.A; Plowright, W. and Hiag, D.A. (1957). Cytopathogenic agents associated with LSD of cattle. Bull. Epiz. Dis. Afr, 5: 489-492.

Agarwal, R; Kharya, M. and Shrivastava, R. (1957). Antimicrobial and anthelmintic activities of the essential oil of Nigella sativa Linn. Ind. J. Exp. Biol, 17: 1264-1265.

Ali, A.A,; Esmat, M.; Attia, H.; Selim, A. & Abd El ñHamid , Y.M. (1990) Clinical and pathological studies on lumpy skin disease in Egypt. Vet Rec. 127: 549-550.

Anonymous, (1988). Lumpy skin disease. OIE. Disease information. Paris, France. Vol. 1 no.1.

Asagba, M.O. (1984). Assessment of virulence in cattle inoculated with Lumpy Skin disease virus attenuated in cell culture. Abstracts of the 10th international congress of virology, 1-7 September 1984. 49; Abstract p. 30-38. Sendai, Japan .

Bahnemann, H.G. (1975). Binary Ethylenimine as an inactivant for FMDV and its application for virus vaccines. Archives of virology. 47:47-56.

Basil, A.A. and Arwa, H.H. (1993). Effect of Nigella sativa on ingestion ability of mice peritoneal macrophages. Saudi Pharmaceut. J. 1: 18.

Black, D.N.; Hammond, J.M. and kitching, R.P. (1986).Genomic relationship between capripox viruses. Virus research. 5: 277-292.

Blackburn, N.K. (1991) . A study on the effect of the chemical inactivants on the epitopes of Rift vally fever virus

glycoprotein using monoclonal antibodies. J. Virol. Methods. 33:367-374.

Capstick, P.B.& Conackley, W. (1961). Protection of cattle against Lumpy skin disease. 1.Trials with vaccine against Neethling type infection. Res Vet. Sci., 2: 361-365.

Capstick, P.B. (1959). Bull. Epizoot. Dis. Afr. 7: 51-62.

Carn, V.m.(1993). Control of capripoxvirus infections . Vaccine. 11:1275-1279.

Cox,C. & Coulter, R. (1997) . Adjuvants- a classification and review of their modes of action. Vaccine. 15.No. 3: 248-256.

Cunliffe, H.R.& Graves, J.H. (1963). Formlin treated FMDV comparison of two adjuvants in cattle. J. Comp.Med Vet. Sci. 27:193-197.

Daoud, A.M.; Michael, A. ;Soad, M.; Samir, S.S.& Aboul Soud , E.A.(1998) . Production of lumpy skin vaccine in Egypt. Fourth Vet. Med. Conference, 26-28-August 1998, 117-124.

Davies, F.G. & Mbugwa, G. (1985). The alteration in pathogenicity of a Kenya sheep and goat pox virus on serial passage in bovine fetal muscle cell culture. J.Comp. Path., 95, 565 ñ 572.

Davies, F.G. (1976). Characteristics of a virus causing a pox disease in sheep and goat in Kenya with observation on epidemiology.

De Lange, M. (1959). The histology of the cytopathogenic changes produced in monolayer epithelial cultures by viruses associated with LSD. Onderstepoort J. Vet. Res, 28:2, 245-252.

El-Alawy, T.A.; El-Tarabili, M.A.; Morad, M.I. and S dek, S.R. (1992). Isolation and identification of Lumpy skin disease from upper Egypt. Assuit Vet. Med. 28 (55): 279- 289.

El-Kadi, A; Kandil, O. and Tabuni, A. (1990). Nigella sativa and cell mediated immunity. Arch of AIDS Res, 1: 232.

Fayed, A.A.; Saber, M.S.; Moussa, A.A. and Reda, I.M. (1988) Epidemiology of Lumpy skin disease in Egypt. Fourth annual report on the scientific progress of PI-480 project, Grant no. TG-EG 216, project no. PB-APHISD, October, 1988 - September, 1989.

Frey, H.R. and Liess , B. (1971). Growth curve studies and applicability of a highly cytopathogenic BVD/MD virus strain for diagnostic purposes using the micro-titer method . Zbi. Vet. Med. B18:16-71.

Gershon, P.D; Kitching, P.R; Hammond, JEF.M and Black, D. N. (1989). Poxvirus genetic recombination during natural virus transmission. J. Gen. Virol. 70: 485-489.

Hailat, N; Bataineh, Z; Lafi, S; Raweily, E; Al Khatib,A; and Hanash, S. (1995). Effect of Nigella sativa volatile oil on Jurkat T-cell leukemia polypeptides. Int. J. Pharmacog. 33: 16-20.

Haq, A; Abdullatif, M; Lobo, P.I; Khabar, K.S; Sheth, K.V and Al-Sedairy, S.T. (1995). Nigella sativa: effect on human lymphocyte and polymorphonuclear phagocytic activity. Immunopharmacol. 30: 147-155.

House, A.J; Wilson, T.M.; El-Nakashly, S.; Karim , 1.A; Ismail, 1; El-Danaf, N; Moussa , A.M. & Ayoub, N.N. (1990). The isolation of LSDV and bovine herpes virus from cattle in Egypt. J.Vet. Diag. Invest. 2:111:-115.

Ibrahim, M.I. (1993). Studies on growth cycle of Lumpy skin disease virus. MVSc thesis . Cairo University.

Kitching, R.p. & Taylor , W.P. (1985). Tropical animal

health and production 17: 64-74.

Gitching, R.P.; Bhat, P.H.& Black, D.N. (1989). The characterization of African strains of capripoxvirus. Epidemiology and infection. 102:335.

Madbouly, H. M.; El-Kady, M.F. and Abd El-Moneim, A.S. (1999a). The effect of Nigella sativa (seed and oil) on humoral and celluler immune response of chickens. J. Egypt. Vet. Med. Assoc. 59: 5, 1497-1511.

Madbouly, H. M.; El-Kady, M.F. and Abd El-Moneim, A.S. (1999b). The effect of Nigella sativa on chicken: II. The effect of N.sativa seeds on IBDV challenge in chickens. J. Egypt. Vet. Med. Assoc. 59: 5, 1513-1523

Madbouly, H. M.; El-Kady, M.F. and Tamam, S.H. (2000). Preparation of infectious laryngeotracheitis inactivated viral vaccine from a locally isolated strain adjuvated with Nigella sativa oil as adjuvant. Seuz Canal. Vet. Med. J, III, 1, 281-290.

Michael, A.;Saber, M.S.; Soad, M.; Soliman, M.; Moussa, A.A.; Salama, A.A.; Fayed, A.A.; Monira, I.Nasser & House , A.J.(1996) . Control of LSD outbreak in Egypt with Romanian sheep pox vaccine. Assuit Vet. Med. J. 36(71). 173-180.

Nawathe, D.R.; Asgaba, M.O.; Abegunde, A.; Ajayi, S.A. & Durkwa, L. (1982). Some observation of the occurrence of Lumpy skin disease in Nigeria. Zbi-Vet. Med B., 29:31-36.

OIE (Office International Dis . Epizzotic) (1996). Manual of standards for diagnostic tests and vaccines. Third edition. P.p. 93-101-. OIE, Rue de prony, 75017 Paris - France.

Prydie, J. and Coackley, W. (1959). Lumpy skin disease-tissue culture studies. Bull. Epiz. Dis. Afr, 7: 37-50.

Reed, L.J.& Muench, H. (1938). A simple method of estimating 50% end points. Am. J.Hyg. 27.493-497.

Roshdy, O.H.(1996).Studies on inactivated oil FMD vaccine.Ph.D. (Virology), Fac.Vet.medicine Cairo University.

Saber, M.S; Daoud, A.M; Fatchia. M. Mohammed; Hussein, H.A; Micheal, A.M; Mervat, A.M; Yousif, A.A. and Amin, A.S. (2000). Preparation of inactivated lumpy skin virus vaccine. Vet. Med. J. Giza, 40: 4, 633-646.

Sewell, M.H. & Brocklesby, D.W (1990). Hand book on animal disease in the tropics. Fourth edition. Bailliere Tindall, London. Philadellphia, Toronto, Tokyo. 326-329.

Shahin, M.A.A. (1998). The effect of physical and chemical agents on the stability of LSDV . Ph D thesis. Cairo University.

Singh, M.& OfHagan, D. (1999). Advances in adjuvants. Nature Biotechnology. Vol. 17. 1075-1081.

Woods, J.A. (1988). Lumpy skin disease ñ A Review. Trop. Anim. Hlth. Prod. 20: 11-17.

Yeruham,I.; Perl, S. ; Nyska, A.; Abraham , A.; Davidson, M.; Haymovitch, M.; Zamir, O.& Grinsten, H. (1994). Adverse reactions in cattle to a capripox vaccine. 135: 330-332.

Yousif, A.A. (1995). Immunobilogical studies on Lumpy skin disease virus. MVSc thesis. Cairo University.