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RESEARCH

Formulation and evaluation of inactivated lumpy skin disease virus vaccine using ISA 50 and ISA 206 adjuvants.

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ABSTRACT

Lumpy skin disease (LSD) is one of the most serious livestock diseases in Egypt since 1988 when the disease spread widely all over the country. since that attenuated sheep pox virus (SPV) used for vaccination cattle flocks against LSD due to immunological relationship between LSD and SPV. There have been numerous attempts to produce live attenuated LSD vaccine but it has stopped due to the seriousness of the disease's return to its virulence and the post vaccinal reaction of the vaccine so we prepare inactivated LSD virus(LSDV) vaccine to immunize the cattle with safe vaccine .Ismailia isolate of LSD was propagated and titrated on Madin Darby Bovine Kidney Cells (MDBK) till reach the highest titer (10 $^{5.5}$ TCID50/ml). The inactivation process was performed using Bromoethylamine hydro bromide (BEI) that did not affect the immunogenic characterization of the LSDV and the process last for 18 hours with 1% concentration of BEI at 37°c. The inactivated LSDV tested on MDBK cells and the animals free of antibodies for residual live virus and determination of minimal protective dose (MPD) of prepared inactivated LSDV. The MPD was 1.5 ml of the inactivated virus and the vaccine was prepared by adding equal volume of the adjuvants (Montanide ISA 50 and Montanide ISA 206) to the total quantity 3ml of the vaccine. The prepared vaccine tested for determination of immunological response against the vaccine with ISA 50 and ISA 206 adjuvants and the results was compared. The results of cellular and humoral immune response and the challenge test showed that using inactivated LSDV vaccine improved by Montanide ISA 206 elevate the protective immunity level and considered more safe and avoid viral reversion and transmission of the virus by the blood feeding arthropods.

Background: Objective: Methods: Results: Conclusion:

Keywords:

BACKGROUND

Lumpy skin disease (LSD) is is a viral disease of cattle caused by Neethling pox virus of genus Capri poxvirus belongs to family poxviridae (Sevik *et al.*,2016). It was introduced to Egypt during Importation of cattle from Somalia at the Suez quarantine station in May 1988 (Hayed *et al.*, 1988).

In early 2006, a lumpy skin disease (LSD) outbreak has invaded cattle in different localities of Egypt, exerting severe economic losses to livestock industry. Representative specimens (skin biopsies) were collected form nodular skin lesions of infected foreign (imported from Ethiopia, at Ismailia private quarantine) and local cattle (at Fayoum, Menofia and Sharquia governorates) (El-Kholy *et al.*, 2008). Then it reappeared again in Egypt governorates in 2012,2013 (FAO 2013). LSD is endemic in Central and Southern Africa and it is rapidly spreading throughout the Middle East, including Turkey (Charlotte and Howard 2015). The disease has become endemic in Sultanate Oman and is liable to extend to other Gulf Cooperation

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Council Countries and cause a pandemic (Tageldin, M. H. *et al.* 2014). The virus is closely related to sheep and goat poxvirus and the three diseases cannot be differentiated serologically (Abutarbush *et al.*,2015) . So sheep pox virus (SPV) vaccines have been widely used for cattle against lumpy skin disease virus (LSDV) in the Middle East and the Horn of Africa these vaccines have been associated with incomplete protection (Eva 2014).

A Live attenuated vaccines are commercially available, but problems with poor vaccine efficacy have been reported in some countries (Ayelet *et al.,* 2013; Abutarbush *et al.,* 2015). Some countries have produced vaccines by using local field strains, but these vaccines are reported by European Food Safety Authority (EFSA) to be unsafe and they do not satisfy all the OIE criteria for safe vaccine production (OIE, 2010). Some researchers report that using sheep and goat pox virus vaccine to protect cattle herds from LSD was ineffective (Brenner *et al.,* 2009 ; Ayelet *et al.,* 2013) . This lack of immunity may be a result of the vaccine strain chosen, over-attenuation of the seed virus, general lack of quality of the vaccine, the dose of vaccine used and different application methods. The Egyptian local isolate of LSDV was adapted in MDBK cell lines and was used in the trails for production of LSD vaccine and was used safely and efficiency in Egypt (Aboul Soud, 1996).

Risks of live attenuated vaccines have always been there, some of these risks are that live attenuated vaccines can produce the disease in immunosuppressed individuals by reverting it to more virulent phenotype (Amal 2003). A local reaction at the site of inoculation, as well as fever and reduction in milk yield, may follow vaccination with live, attenuated Capri pox virus (OIE 2010). From the reasons, emerged the need for inactivated vaccines, the preparation of oil adjuvanted inactivated LSD vaccine have been reported and demonstrated the potentiality of such vaccine in eliciting antibody response against LSDV (Saber *et al.,* 2000).

MATERIALS AND METHODS

Viruses and vaccines

Viruses

The local Ismailia isolate seed LSDV was kindly supplied by Pox department Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo it was propagated on (MDBK) cells according to Daoud *et al.* (1998). and titrated in cell cultures using the microtitre technique according to House (1989). The virus infectivity titers were calculated according to the method of Reed and Muench (1938).

Virulent neethling strain the virus was kindly supplied by Pox department Veterinary Serum and Vaccine Research Institute, Abbasia, used for potency test and determination of Minimal protective dose. The confluent monolayer MDBK cell line was inoculated with inactivated LSDV vaccine for determination the residual virus.

Vaccines

BEI (Sigma-Aldrich) was prepared according to Bahnemann (1975).The virus inactivation was stopped by addition of sodium thiosulphate at a final concentration of 2%.

According to Seppic manufacturing company, the vaccine was formulated using Montanide ISA 50 and ISA 206 VG . The adjuvant phase and the inactivated LSD antigen were warmed to 30°C. In a beaker, the adjuvant was agitated at 350 rpm. The antigen was added slowly at equal volume to the adjuvant with stirring at 350 rpm for 5 minutes. The agitation stopped and the emulsion was left for one hour at room temperature then stored at (20°C) to be ready for use. Samples from two vaccines were cultured on thioglycolate broth, sabouraud and nutrient agar according to (OIE 2010).

DNA extraction

DNA was extracted from virus suspensions using DNeasy Blood & Tissue Kit (Qiagen, UK) Catalogue no.51304 following the manufacturer's instructions. DNA was kept at −20 °C until further analysis.

Primers set

Primers used were supplied by Metabion (Germany) They have specific sequence and amplify a specific product acc to (Zhu *et al.,* 2013) ORF 103 gene as shown table(1)

Table (1) Sequence of forward and revese primers used for amplification of ORF 103 gene

	Sequence
Forward primer	5' ATGTCTGATAAAAAATTATCTCG3'
Reverse primer	5'ATCCATACCATCGTCGATAG3'

The temperature and time conditions of the primer during PCR are shown in Table (2) according to Emerald Amp GT PCR mastermix (Takara) kit

Table (2): Cycling conditions of the different primers during cPCR

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
ORF 103	**94˚C 5 min.**	**94˚C 30 sec.**	**52˚C 45 sec.**	**72˚C 45 sec.**	**35**	**72˚C 10 in.**

The end product of PCR amplification was transferred to Agarose gel electrophoreses according to Sambrook *et al*., (1989) using gel Pilot 100 bp ladder supplied from QIAGEN (USA) (cat. no. 239035) with Size range: 100-600 bp.

Animals free of antibodies against LSD

The animals free from antibodies were used in 3 different experiment:

Two calves were inoculated with inactivated LSDVV for virus residual determination;

Immunization of 3 groups of calves with different doses of the inactivated virus was applied. Each group consists of 4 calves, they were inoculated with 1, 1.5, and 2 ml of the inactivated LSDV and 4th group of 2 animals were left non-inoculated as a control group for detection of minimal protective dose. The 3 groups were observed for 4 weeks with weekly sera collection. After 4 weeks all the calves were challenged and sera were collected weekly for another 4 weeks and Eight calves were vaccinated by inactivated LSDV vaccine adjuvanted using ISA50 oil and 2 non vaccinated control calves were challenged 4 weeks post vaccination by inoculation with the local virulent strain of LSDV (Neethling strain). 8 calves were vaccinated by inactivated LSDV vaccine adjuvanted using ISA 206 oil and 2 non vaccinated control calves were challenged 4 weeks post vaccination by inoculation with the local virulent strain of LSDV (Neethling strain).

Evaluation for acquired immune response against prepared vaccine

Evaluation of the cell mediated immune response

It was done by using Lymphocyte transformation assay (MTT kit) (Beijing Mesochem Technology Co. ,Ltd-China) : according to kit instructions.

Evaluation of humeral immune response using Serum neutralization test (SNT):

It was carried out according to (OIE 2010)

RESULTS

Virus propagation

Appearance of Cytopathic effect (CPE) of the virus on the cells. CPE was characterized by cell rounding, cell aggregation, coalesce together to form clusters that scattered all over the monolayer within 72 hr post inoculation and gradually increased till 80-90 % of sheet was completely detached.

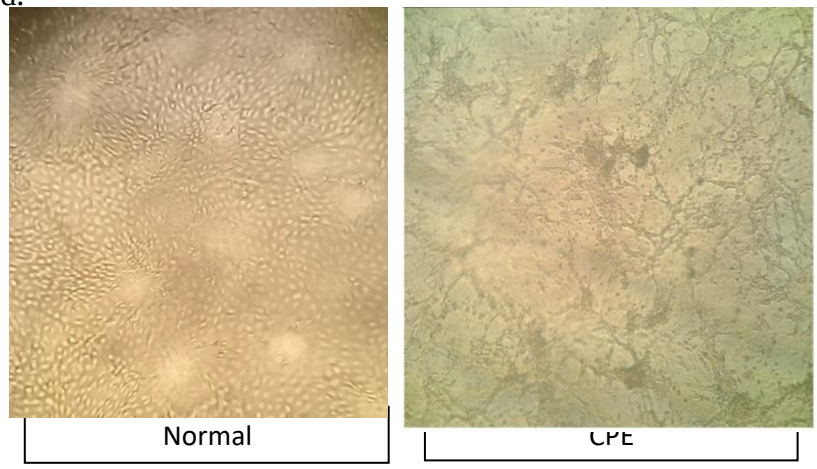

Fig. 1: The CPE of the virus appeared compared with normal cells

Virus titration

The titration of LSDV was carried out in MDBK cells and its titer was 105.5 TCID50 /ml. There are no residual virus as there no CPE on tissue culture.

Vaccine sterility

The cultivation of two vaccines, either on the thioglycolate broth, Sabouraud or neutrient agar showed no growth of any microorganism and the vaccines remained sterile .

Identification of local strain of Lumpy skin disease virus (LSDV) by PCR:

The size of PCR product of Lumpy skin disease virus (local strain) fragment of the ORF 103 gene using the specific primer was (~570bp) . As shown in Figure (2).

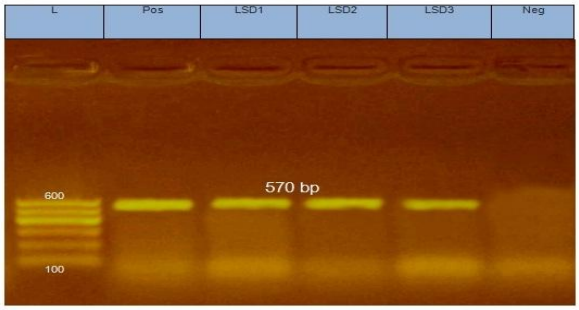

Fig. 2: Specific PCR product of lumpy skin disease at the correct expected size of the ORF 103 gene (~570 bp). Lane 1: High molecular weight nucleic acid marker. Lane 2: Positive sample. Lane 3: Sample from 80th passage. Lane 4: Sample from 50th passage. Lane 5: Sample from 30th passage. Lane 6: Negative sample.

Determination of the minimal protective dose of the inactivated LSDV:

The results of the determination of the minimal protective dose were presented in Table (3) where 50 % of animals inoculated using 1 ml of prepared inactivated virus were protected and 75 % of animals inoculated using 1.5 ml and 2 ml were protected while the non-vaccinated control group showed rise in temperature and large rounded nodules at the site of inoculation by the 14th day post inoculation and then after 21 days, skin eruption in the form of rounded nodules appeared all over the animal body with enlargement of the superficial lymph node. The minimum protective dose was 1.5 ml of the inactivated LSD virus.

Table (3) Serum neutralizing index (NI) of calves after inoculation with different doses of the inactivated virus and challenge:

Weeks	Control	1ml	1.5ml	2ml
0 time	0.1	0.2	0.1	0.2
1WPV	0.2	0.4	0.6	0.8
2WPV	0.2	0.7	0.9	1.0
3WPV	0.5	0.9	1.2	1.4
4WPV	0.4	1.2	1.7	1.8
1WPC	1.6	1.0	1.4	1.5
2WPC	1.9	1.6	1.9	2
3WPC	2.1	1.7	2.4	2.6
4WPC	2.3	1.9	2.7	2.9

WPV = weeks post vaccination WPC= weeks post challenge NI≥1.5 considered positive (Cottral,1978)

Potency test:

Challenge of all vaccinated and control animals with local virulent strain of LSD virus (neethling strain) showed that 75% of vaccinated animals with the inactivated LSD vaccine prepared by adding ISA 50 adjuvant were protected and 88% of vaccinated animals with inactivated LSD vaccine prepared by adding ISA 206 adjuvant were protected. The protected animals showed no clinical signs of the disease no local or generalized reaction while the control non vaccinated animals and the non-protected animals did not resist the challenge and showed the classical form of lumpy skin disease infection observing rise in temperature and large rounded nodules at the site of inoculation by the 14th day post inoculation and then after 21 days, Lacrimation, skin eruption in the form of rounded nodules appeared all over the animal body with enlargement of the superficial lymph nodes .

Fig. 3&4: Lacrimation , rise of body temperature and skin eruption in the form of rounded nodules appeared all over the animal body appear on non-vaccinated control animals.

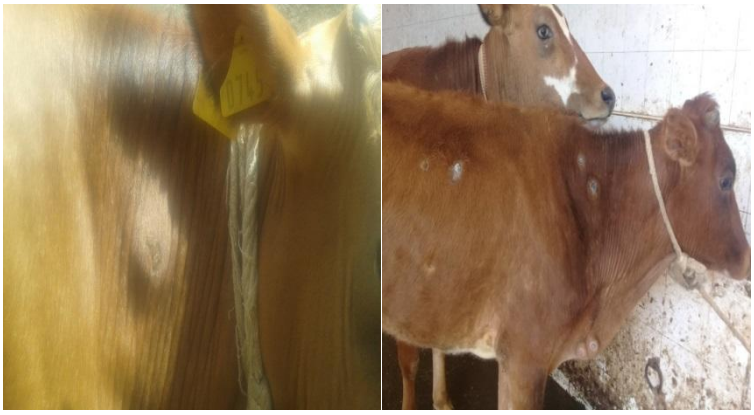

Fig. 5: Side fast appearance on non-vaccinated control animals.

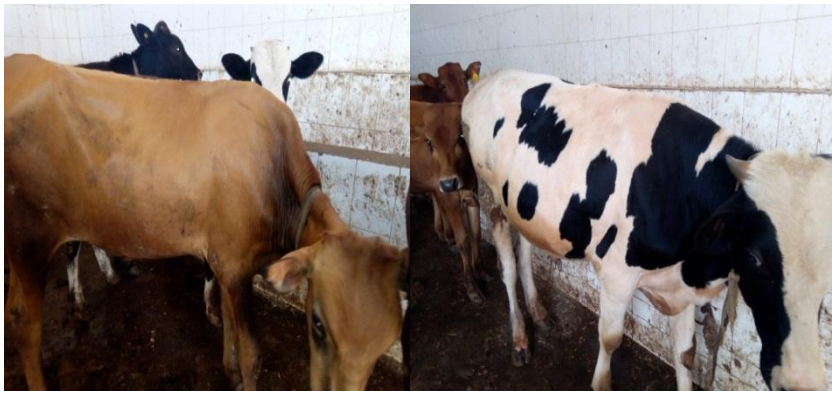

Fig. 6: Vaccinated animals appeared normal without any clinical signs.

Evaluation of cell mediated immune response of vaccinated calves with the prepared vaccines and control non vaccinated calves

The result of cell mediated immune response in vaccinated calves with inactivated LSD vaccine was illustrated in table (4)

Table (4): Cell mediated immune response of calves vaccinated with two inactivated LSDV vaccines and non-vaccinated calves expressed as OD.

Days post inoculation	Control	Montanide ISA 206	Montanide ISA 50
Pre Inoculation	0.080	0.088	0.082
1st day	0.087	0.192	0.137
3rd day	0.082	0.290	0.245
7th day	0.079	0.601	0.554
10th day	0.081	0.889	0.741
14th day	0.089	0.734	0.622
21st day	0.082	0.416	0.384
28th day	0.080	0.340	0.279

Serum neutralization test (SNT):

Table (5) showed that the neutralizing index of vaccinated and control calves all over the period of the experiment. The level of antibodies of vaccinated with Montanide ISA 206 adjuvanted vaccine recorded higher levels of antibodies than those vaccinated with Montanide ISA50 adjuvanted vaccine.

Table (5) Serum neutralizing index of calves vaccinated with two inactivated LSDVV:

Weeks	Control	Montanide ISA 206	Montanide ISA 50
0	**0.2**	**0.3**	**0.1**
1WPV	**0.3**	**1.1**	**1.0**
2WPV	**0.2**	**1.3**	**1.3**
3WPV	**0.4**	**1.9**	**1.5**
4WPV	**0.3**	**2.4**	**2.0**
1WPC	**0.6**	**2.1**	**1.7**
2WPC	**1.1**	**2.5**	**2.1**
3WPC	**1.5**	**2.7**	**2.2**
4WPC	**2.2**	**3.0**	**2.5**

WPV = weeks post vaccination WPC= weeks post challenge NI≥1.5 considered positive (Cottral,1978).

DISCUSSION

Lumpy skin disease virus (LSDV) was isolated for the first time from cattle in Egypt in 2 disease outbreaks at 1988 (House1990).Vaccination is the most effective antiviral interposition which mobilizes the host's immune system to protect animals from viral infections, and when applied properly can be remarkably effective (Eva et al., 2014). SPV vaccine has cross protection against LSD in cattle in the Middle East and the Horn of Africa(Ayelet et al. 2013). In this study we prepared inactivated LSD vaccines as the live attenuated LSD vaccines is not safe due to its reverse action. LSDV inoculated on confluent MDBK cell cultures. CPE increased daily till reaching its maximum (80-90%) on 5-6 days as showed by Aboul-Soud, (1996). The prepared LSDV was titrated on MDBK cell culture calculated by using the formula of Reed and Muench (1938). The titer of LSDV on MDBK was 105.5 TCID50/ml. Complete inactivation of the virus was achieved after 18 hours for the concentration of 1% of the BEI then immediate addition of cold sodium thiosulphate at final concentration of 2% to stop the virus inactivation by the (BEI) Blackburn,(1991).

Montanide ISA (Incomplete SEPPIC adjuvant) are generation of adjuvants, they are new oils include new immunostimulant, The Montanide ISA oils are highly efficient, fluid of low viscosity easily producable adjuvants. Also they are toxicology controlled and of high purity (Amal 2003).

The prepared inactivated LSDV vaccines were tested for sterility and the results proved that the vaccine was free from any contaminating agents (bacteria, moulds and fungi) when inoculated on specific media (code of federal Regulation 2005), The inactivated LSDV vaccines tested to be free from any residual living virus in both tissue culture (MDBK cells) and in calves. The MDBK inoculated cells showed no CPE after inoculation with inactivated LSD virus and inoculated calves showed no local reactions, no local nor generalized symptoms of the disease.

The minimal protective dose of the inactivated LSD virus was determined by inoculating 3 groups of calves (S/C) with different doses of the inactivated LSD virus. Each group was inoculated with 1, 1.5 , 2 ml respectively and all the groups including the control group were challenged 4 weeks post vaccination with the local virulent LSDV (neethling strain). Their sera samples were collected weekly post vaccination and post challenge to evaluate the immune response of the animals to each dose. The minimum protective dose was 1.5 ml of the inactivated LSD virus obtained by application the experimental design and this come in parallel with results of Amal (2003) .

The dose of 1.5 ml was the dose of choice as it protected the inoculated animals against the challenge with the virulent virus with a good level of neutralizing index (NI was 1.7 at 4th

week immediately before challenge test) where the protective NI according to (Cottral 1978) is ≥ 1.5 NI as shown in table (3) .

Eight calves were vaccinated by inactivated LSDVV adjuvanted using ISA50 oil and 2 control calves were challenged by inoculation with the local virulent strain of LSDV (Neethling strain) 4 weeks post vaccination . Another 8 calves were vaccinated by inactivated LSDVV adjuvanated using ISA 206 oil and control calves were challenged by inoculation with the local virulent strain of LSDV (Neethling strain) 4 weeks post vaccination. It was found that 75% of vaccinated animals with the inactivated LSD vaccine prepared by adding ISA 50 adjuvant were protected and 88% of vaccinated animals with inactivated LSD vaccine prepared by adding ISA 206 adjuvant were protected. The protected animals showed no clinical signs of the disease no local or generalized reaction while the control non vaccinated animals and the non-protected animals did not resist the challenge and showed the classical form of lumpy skin disease infection.

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 (Lucy 1984). Cell-mediated immune response in vaccinated calves with prepared LSDVV ranged in between 0.137 -.889 all over the experiment. There are an increase of lymphocyte activity by the 3rd day post vaccination and reached its peak on the 10th day (LSDVV with ISA 50 was 0.741 and LSDVV with ISA 206 was 0.889) then decreased till the 30th day post vaccination (LSDVV with ISA 50 was 0.279 and LSDVV with ISA206 was 0.340) Table (4) these results were in agreement with, Amira (1997).

Serum neutralization test was applied on the collected sera samples, the results showed that the neutralizing index of the vaccinated animals with the two vaccines stated to rise from the 1st week post vaccination. Table (5) showed that the NI reached to (2.0) by the 4th week in animals vaccinated with adjuvanted vaccine with montanide ISA 50 , where it reached higher levels in case of montanide ISA 206 adjuvanted vaccines (2.4) by the same time the results were in agreement with (Christine et al., 2016) .

In conclusion, the adjuvant Montanide ISA 206 used for the preparation of inactivated vaccine proved to be safe, immunostimulant, and gave results than ISA 50 and so using of such adjuvant in prepare of LSDV vaccine may be promising tool for controlling the disease in Egypt.

Further studies are needed to test the use of an additional booster dose from the vaccine to initiate the level and the period of immunity acquired from this vaccine.

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REFERENCES

Aboul Soud, E. A. (1996): Studies on the adaptation of lumpy skin disease virus (LSDV) in cell cultures. Ph. D. Thesis, Microbiol. Faculty Vet. Med. Alexandria Univ.

Abutarbush, W. M. ; Hananeh, W. ; Ramadan, O. M. ; Al Sheyab, A. R. ;Alnajjar, I. G. ;Al Zoubi, N. J.; Knowles, K. ; Bachanek, B. and Tuppurainen, E.S.M. (2015): Adverse

Reactions to Field Vaccination against Lumpy Skin Disease Article first published online: 6 AUG 2014, DOI: 10.1111/tbed.12257. Vol 4 (1): 12-13.

Ahmed, A. M.; Mukhtar, M. M.; El Hussein, A.m.; Tageldin, A. M. Nour and Fadol, M. A.(2007): Immune response of sheep vaccinated with Capripoxvaccine. Vet. Res.; 1(1): 12-16.

Amal A. F. Abdel – Fattah (2003): the role of different adjuvants on the immune response of inactivated lumpy skin disease vaccine. Ph.D. thesis .Faculty of Vet. Med. Cairo University.

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.

Ayelet, G., Abate, Y., Sisay, T., Nigussie, H., Gelaye, E., Jemberie, S., Asmare, K.,2013. Lumpy skin disease: preliminary vaccine efficacy assessment and overview on outbreak impact in dairy cattle at debre zeit, central Ethiopia. Antivir. Res. 98,261–265.

Bachh, A. S.; Ram, J.; Hopkins, J. and Bansal, M. P. (1997): Observations on cellular response in experimentally sheep pox infected lambs. Ind. J. Anim. Sci.; 64: 263-266.

Bahnemann, H. G. (1975) : Binary Ethylenimine as an Inactivant for Foot-and-Mouth Disease Virus and Its Application for Vaccine Production. Archives of Virology, 47: 47–56.

Blackburn ,N.K. (1991): A study on the effect of chemical inactivants on the epitopes of Rift Valley Fever virus glycoprotein using monoclonal antibodies. J. Vir. Method., 33:367-374.

Brenner J, Bellaiche M, Gross E, Elad D, Oved Z, Haimovitz M, Wasserman A, Friedgut O, Stram Y, Bumbarov V, Yadin H, (2009): Appearance of skin lesions in cattle populations vaccinated against lumpy skin disease: statutory challenge. Vaccine, 27(10):1500-1503.

Charlotte, B.K.; Berg, Anette, B.Y.O.; Howard, B.; Aline, D.K.; Klaus, D.; Mariano, D.; Christian, D.; Sandra, E.d.; Chihota, C.M.; Rennie, L.F.; Kitching, R.P. and Mellor, P.S. (2015): Mechanical transmission of lumpy skin disease virusby Aedesaegypti (Diptera: Culicidae).Epidemiol Infect. Vol. 126 17-321.

Christine A. Mikhael; Ibrahim, M.M; and Saad, M.A. (2016): Efficacy of alternative vaccination with attenuated sheep pox and inactivated lumpy skin disease vaccines against lumpy skin disease. SCVMJ, Vol. XXI (2) 125-142.

Code of federal Regulation (2005): Animal and animal products 9,2005. Published by the office of the federal Register National Archives and Recor Administeration. Vet. Microbial.,16(2):101-107.

Cottra1,G.E.(1978):"Pox viruses" In Manual of Stanadridized Methods for Veterinary Microbiology, pp. 273-291.

Daoud, A.M.; Michael, A.; Soad, M. Soliman; Samir, S.S. and Aboul-Soud, E.A. (1998):
Production of lumpy skin vaccine in Egypt. 4th Vet. Med. Zag. Congrress, 117-124.

El-Kholy AA, Soliman HMT, Abdelrahman KA, (2008): Polymerase chain reaction for rapid diagnosis of a recent lumpy skin disease virus incursion to Egypt. Arab Journal of Biotechnology, 11(2):293-302.

Eva 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.

FAO (2013): Emergence of lumpy skin disease in the eastern Mediterranean basin countries VOL 29 November 2013. E.

Hayed, A. A.; Saber, M. S.; Moussa, A. A. and Reda, I. M. (1988): Hourth annual report on the scientific progress of PI-480 project, Grant No.TG-EG 216, project No. PB-APHISD, October.

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

House,A.J.; Wilson, T.M.; El-Nakashly,S.; Karin1,I.A.; Ismail,l., El-Danaf,N.; Mousa,A.N. and Ayoub,N.N. (1990): "The isolation of lumpy skin disease virus and bovine herpes virus from cattle in Egypt" J.Vet.Diag.Invest., 2: 11-115.

Lucy, F. L. (1984): Proliferative response of chicken B and T lymphocytes to mitogens, Chemical regulation of immunity in vetrenary medicin, 15: 44-52.

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.

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

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

Sambrook, J.; Fritscgh, E.F.;and Mentiates (1989): Molecular coloning. A laboratory manual. Vol !., Cold spring Harbor Laboratotry press, New York.

Şevik, M. and Doğan, M. (2017): Epidemiological and Molecular Studies on Lumpy Skin Disease Outbreaks in Turkey during 2014–2015. Transbound Emerg Dis, 64:

Tageldin, M. H., Wallace, D. B., Gerdes, G. H., Putterill, J. F., Greyling, R. R., Phosiwa, M. N., Al Ismaaily, S. I. (2014): Lumpy skin disease of cattle: an emerging problem in the Sultanate of Oman. Tropical Animal Health and Production, 46(1), 241–246. http://doi.org/10.1007/s11250-013-0483-3 1268–1279. doi:10.1111/tbed.12501**.**

Zhu, X.L.; Yang, F.; Li, H.X.; Dou, Y.X.; Meng, X.L.; Li, H.; Luo, X.N. and Cai, X.P. (2013): Identification and phylogenetic analysis of a sheep pox virus isolated from the Ningxia Hui Autonomous Region of China. Genetics and Molecular Research 12 (2): 1670-1678.

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