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Original Research Article

Study on the capability of a dual capripox vaccine in protection of cattle against LSD infection

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ABSTRACT

The experiment applied on four groups of calves, each of four calves. Three calves from each group were vaccinated with one of the following attenuated vaccines: Lumpy skin disease vaccine (LSD), Romanian sheep pox (RSP) vaccine, Held goat pox (HGP) vaccine and dual (bivalent) vaccine of SPV and GPV. All vaccines were evaluated by estimating the cellular immunity using lymphocyte blastogenesis measured by XTT assay, and humeral immunity using serum neutralization and ELISA tests of vaccinated calves. The NI coincided with the ELISA antibody results and corroborated the results of cell mediated immunity which demonstrated the capacity of LSD and dual vaccines to induce immune response higher than SP vaccine and GP vaccines. In conclusion, the current study proved that the LSD and dual vaccines were highly immunogenic than the RSP and HGP vaccines, and dual vaccine could be safely used for vaccination of cattle against lumpy skin disease.

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1. Introduction

Lumpy skin disease (LSD) is an insect born infectious seldom fatal disease of cattle clinically characterized by skin eruption. Transmission is thought to be primarily by biting insects or ticks. The incidence of LSD is high during wet seasons when biting insect populations are abundant, and decreases during the dry season (Gari et al. 2010). The disease is caused by a double stranded DNA virus of the family Poxviridae and genus Capripox which is also termed as Neethling virus (OIE, 2010; Salib and Osman, 2011). The disease was first described in Northern Rhodesia (currently Zambia) in 1929 and then rapidly spread in cattle over most of the African countries (Davis, 1991). It is a viral, enzootic infectious, eruptive disease cause significant economic losses to cattle industry due to chronic debility in infected cattle, reduction in milk production, abortion, temporary or permanent sterility, damaged hides and deaths (Tuppurainen and Oura, 2012).

Lumpy skin disease is an OIE list: A disease, which shows its serious socio-economic status. The disease presents itself as an acute, sub-acute or inapparent disease with variable severity depending upon Capripox virus strain and the affected breed. It is less contagious with low mortality (less than 10%) but has been as high as (20-75%) in some outbreaks and varying (1-90%) morbidity rate, even sometimes reach as high as 100% during some outbreaks

(Tilahun et al., 2014). It is widely agreed that vaccination is the only effective way to control the spread of LSDV in endemic countries like Egypt (Ayelet et al., 2013).

Many live attenuated strains of Capripox virus have been used as vaccines for the control of LSD, like Kenyan sheep pox virus and Romanian sheep pox strains in Egypt and Neethling lumpy skin disease virus strain in south Africa. It is thought that strains of Capripox virus share a major neutralizing site (Carn and Kitching, 1995; Kitching, 2003; Brenner et al., 2006).

The increase of vaccination failure in the last years and the appearance of new outbreaks gave us the idea to test another Capripox member (Goat pox virus) in vaccination against LSD compared to the currently used SP vaccine in Egypt.

The aim of this work is to evaluate the capability of dual capripox vaccine (Romanian sheep pox and Held goat pox) for protection of cattle against LSD infection in Egypt compared to the attenuated LSD virus vaccine, sheep pox virus vaccine and goat pox virus vaccine by detecting both the cellular and humoral immunity of cattle.

2. Materials and methods

2.1. Animals

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

2.2. Cells culture

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

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

2.3. Viruses and vaccines

a- Romanian sheep pox virus and vaccine (RSPV): The virus was cultivated and propagated on African green monkey kidney cells (Vero cells) and the vaccine is produced according to Rizkallah (1994).

b- Held goat pox virus (HGP): Reference goat pox virus "Held strain" (HGP), originated in Turkey, had been supplied from Foreign Animal Disease Diagnostic laboratory (FADDL), plum Island – N.Y.–USA. It was passaged two times on lamb testicle cells and for one passage on sheep choroid plexus cells. In Egypt, the virus adaptation was completed by Nackhla (2000), for another sixteen passages on lamb testicle cells and for fourteen passages in Vero cells.

c- Lumpy skin disease virus (LSDV): LSDV, Ismailia strain was isolated from Egypt during the outbreak of 1988 (House et al. 1990). The virus was adapted in MDBK cells according to Aboul Soud (1995) and Daoud et al. (1998).

d-Preparation of dual Romanian SP and Held GP experimental vaccine: The attenuated RSP and HGP viruses strains fluids were mixed with each others at the ratio of 1:1 (v:v) of equal titres (104.5 and 104.5 TCID50/ml).

2.4. Stabilizer

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

2.5. Chemical and biological reagents

Heparin, foetal calf serum, trypan blue stain are used.

2.6. Kits

-XTT Cell Viability Assay Kit: The kit was used in the lymphocyte blastogenesis assay.

2.7. Conjugate

The anti-bovine conjugate was used in ELISA.

2.8. Titration of capripox vaccines before and after lyophilization

It was applied according to Rao and Malik (1982) and Tiwari and Negi (1995). The titre of each of vaccines was expressed by TCID50 and calculated according to the method of Reed and Muench (1938).

2.9. Sterility test

It was carried out according to OIE (2010).

2.10. Animal vaccination

As described by Sabban (1960) and Wang and Jiang (1988), 16 apparently healthy susceptible calves of about 6-12 months old were divided into 4 groups, each of 4 animals. Three calves in each group 1-4 were vaccinated intradermally in the tail fold with 1ml of the field dose (102.5 TCID50) of the attenuated LSD, RSP, HGP, bivalent (RSP& HGP) respectively; while one calf in each group was kept as unvaccinated in contact with other vaccinated three calves. Another 2 calves were kept isolated in a separate pen (non-vaccinated).

2.11. Sample collection

Serum samples were collected from calves just before and weekly after vaccination interval for twenty weeks. Samples were stored at -20°C until examined by serological test. Whole blood samples were collected on heparin (heparin sodium) containing syringe then directly tested for estimation of the cellular immunity at day 0, 1, 3, 5, 7, 10, 15, 21, 28 and 35 post vaccination.

2.12. Evaluation of the cell mediated immune response

Assay of lymphocyte blastogenesis (XTT) was applied according to the method adopted and modified by El Watany et al. (1999).

2.13. Evaluation of humeral immune response

A. Serum Neutralization Test (SNT): It was applied according to the method described by House et al. (1990).

The neutralizing index (NI) was calculated according to Reed and Muench (1938).

B. Indirect ELISA: It was applied according to Babiuk et al. (2009).

3. Results

3.1. The post-vaccinal reaction

The post-vaccinal reactions were recorded in calves vaccinated with LSD and dual vaccines and not visually detected in calves vaccinated with goat pox vaccine while sheep pox vaccine immunized calves showed just mild swelling at the site of injection (Figs. 1-3). Calves in both contact and isolated control showed no post-vaccinal reaction.

3.2. Evaluation of the cell mediated immune response

Assay of lymphocyte blastogenesis (XTT)

All vaccinated calves with different capripox vaccines had variable cellular immune responses depending on the vaccine used and they reached to the maximum nearly on the 10th day post-vaccination, then decreased (Table 1 and Fig. 4).

Fig. 1. Post-vaccination reaction in calves vaccinated with LSD vaccine. It appeared as cutaneous swelling in the tail fold. Its diameter measured about 2-3 cm and disappeared within 2-3 weeks.

Fig. 2. Post-vaccination reaction in calves vaccinated with bivalent (dual) vaccine. It appeared as a mild swelling and disappeared within few days.

3.3. Evaluation of humoral immune response Serological assays (Serum neutralization test and ELISA)

Serum samples were weekly collected from calves before and after vaccinated with different prepared vaccines, then were tested. Results (Table 2 and Figs. 5,6) clearly appeared the potency of homologous vaccines and bivalent sheep pox and goat pox vaccine in protection of vaccinated animals while the heterologous Romanian sheep pox vaccine and Held goat pox vaccine cause a mild protection in vaccinated calves respectively.

Fig. 3. Post-vaccination reaction in calves vaccinated with Romanian SP vaccine. Post vaccinal reaction appeared as sensitization which disappears within a few days.

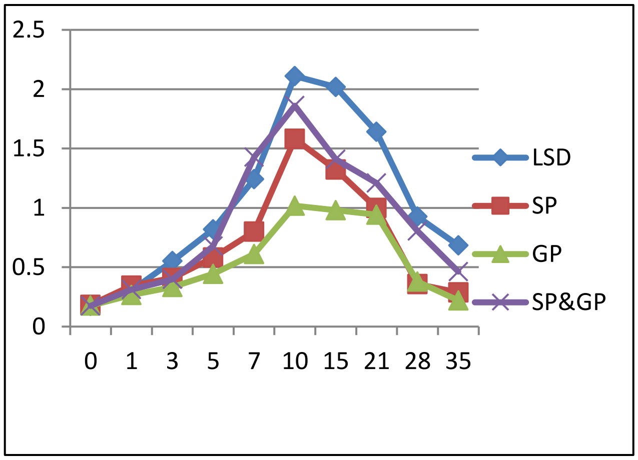

Fig. 4. Cell mediated immune response of calves vaccinated with different Capripox vaccines.

Table 1. Cell mediated immune response of calves vaccinated with different Capripox vaccines (recorded as absorbance).

Vac. / DPV	LSD	Dual vac.	RSP	HGP
	Absorbance value of lymphocyte proliferation			
0	0.176	0.175	0.183	0.177
1	0.303	0.313	0.345	0.265
3	0.551	0.398	0.409	0.334
5	0.818	0.680	0.581	0.445
7	1.241	1.428	0.801	0.611
10	2.110	1.861	1.581	1.018
15	2.018	1.408	1.322	0.981
21	1.641	1.211	1.000	0.942
28	0.928	0.805	0.358	0.380
35	0.683	0.462	0.288	0.220

Vac. =Vaccine. DPV =Day post vaccination. LSD =Lumpy skin disease vaccine. RSP =Sheep pox vaccine (Romanian strain). HGP = Goat pox vaccine (Held strain). Dual vaccine (RSP&HGP) = Sheep pox and goat pox vaccine.
Note 1: Cell mediate immune response in contact and isolated calves (absorbance) not exceeded 0.085-0.094 allover the time of study. Note 2: Lymphocyte activity reached its peak on the 10th days**.**

Table 2. Cell mediated immune response of calves vaccinated with different Capripox vaccines (recorded as absorbance)

VAC / WPV	LSD		Dual vac.		RSP		HGP	
	NI	**S/P**	**NI**	**S/P**	**NI**	**S/P**	**NI**	**S/P**
0	0.25	0.28	0.50	0.37	0.25	0.41	0.25	0.31
1	1.25	1.03	1.00	0.89	1.25	0.97	1.00	0.81
2	1.50	1.21	1.25	1.06	1.25	0.99	1.25	1.11
3	1.75	1.35	1.50	1.35	1.50	1.21	1.25	1.21
4	2.00	1.56	1.75	1.38	1.75	1.35	1.50	1.38
5	2.50	1.81	2.00	1.66	2.00	1.31	1.75	1.49
6	2.75	1.76	2.50	1.65	2.50	1.58	2.25	1.68
7	3.25	1.91	2.75	1.97	2.50	1.71	2.50*	1.76
8	3.50	2.36**	2.75	1.92	2.75*	1.62	2.25	1.80**
9	3.75*	2.25	2.75	1.96	2.50	1.88**	2.25	1.80
10	3.75	2.16	3.00*	2.20**	2.25	1.66	2.25	1.70
12	3.50	2.05	3.00	1.89	2.25	1.68	2.50	1.66
14	3.50	2.12	3.00	1.85	2.00	1.79	2.00	1.70
16	3.25	1.86	2.75	1.92	2.25	1.51	2.00	1.70
18	3.25	1.95	2.50	1.87	2.00	1.48	1.75	1.46
20	3.25	1.77	2.50	1.76	2.00	1.38	1.75	1.39

Note 1: Isolate and contact control CALVES persist negative NI till (20 weeks post vaccination).
Note 2: Neutralizing Index (NI) ≥ 1.5 is considered protective (Cottral, 1978).
Note 3: S/P >1.0 is considered protective Babiuk et al. (2009).
WPV= Week post vaccination. VAC = Vaccine. LSD = Lumpy skin disease vaccine. RSP = Sheep pox vaccine (Romanian strain). HGP = Goat pox vaccine (Held strain). Dual vaccine= Sheep pox and goat pox vaccine.
NI = Neutralizing index. S/P= Sample to positive ratio. *= highest NI **= highest S/P ratio.

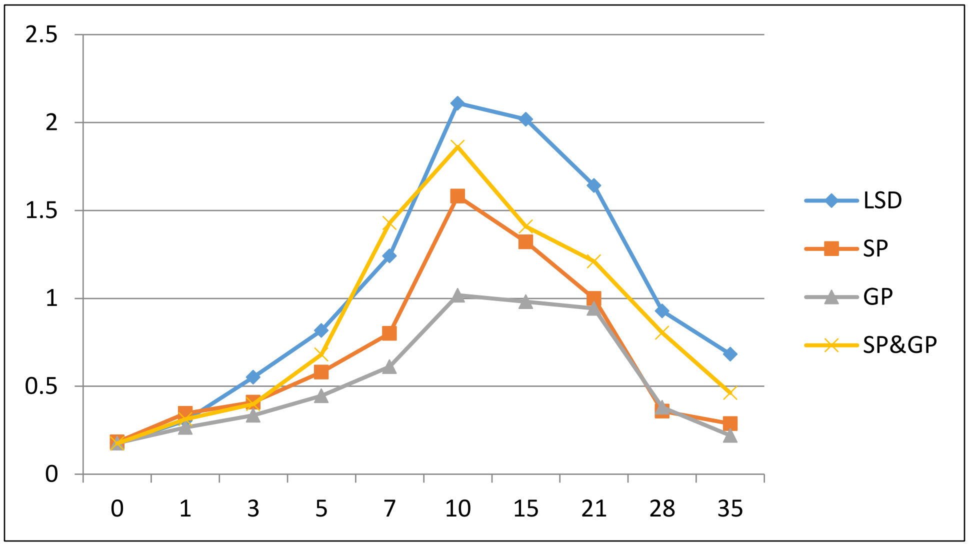

Fig. 5. Cell mediated immune response of calves vaccinated with different Capripox vaccines.

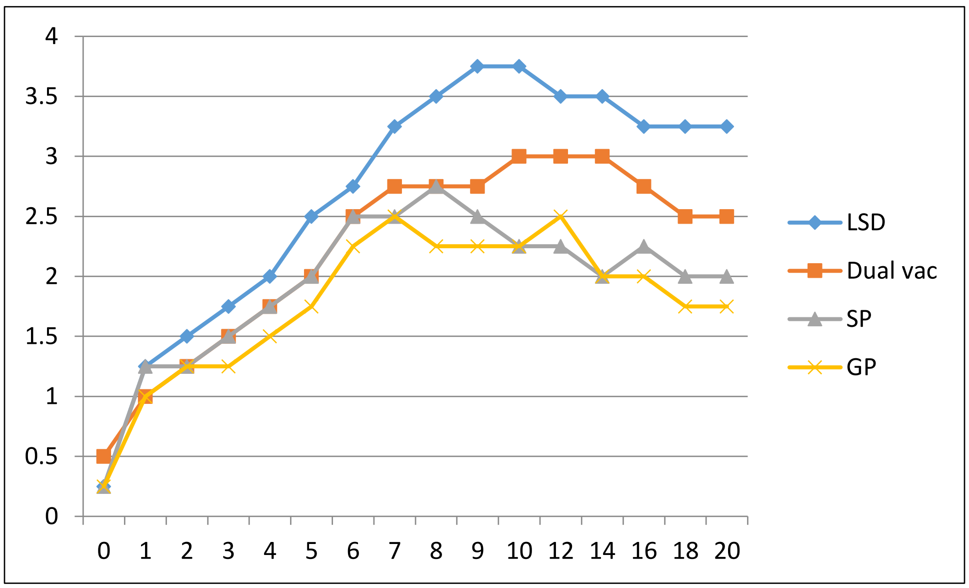

Fig. 6. Comparative NI of different Capripox vaccines in calves.

4. Discussion

Control of LSD among cattle in Egypt depends on vaccination programs, using a heterologous cross reacting sheep pox virus vaccine which is antigenically related to LSD and produce good immune response in cattle (Michael et al., 1994). In Southern Africa, the Neethling strain of lumpy skin disease was used for vaccine preparation and proved to be innocuous and immunogenic for cattle. All strains of capripox virus so far examined, whether derived from cattle, sheep or goats, share immunizing antigens so attenuated cattle strains and strains derived from sheep and goats have been used as live vaccines (OIE, 2010).

However, little is known about the immunological response and immune dynamics against this disease. Therefore, the objectives of this trial on the four capripox vaccine strains were to give an insight to the comparative SNT and ELISA antibody response (humeral immunity) and cellular immunity resulting from these vaccines in calves.

The post vaccinal reaction appeared from homologous LSD vaccines is a pronounced local reaction (2-4 cm in diameter) at the point of inoculation of two calves and a mild reaction (10 mm) in the 3rd calf and disappeared within 12-16 days which in agreement with those recorded by OIE (1992), Carn (1993), Coetzer (2004). Heterological vaccine (Romanian sheep pox vaccine and the bivalent vaccine) showed only mild local reaction appeared in the form of redness and mild swelling; and no reaction with Held goat pox vaccine (Figs. 1-3). The recorded clinical signs were also in harmonize with those of Diallo and Viljoen (2007), who stated that the clinical signs caused by different capripox viruses are variable, depending not only on individual host susceptibility but also on the virus strain.

The type of immune response that is most efficient in stopping an infectious process depends on the site of replication of the disease agent. Antibody is effective against extracellularly multiplying infectious agents, while cell mediated immune responses are most important for those that replicate intracellularly (Capron et al., 1994; Hirsh and Zee, 1999).

Thus, it was important to estimate cell mediated immune response of calves vaccinated with different Capripox vaccines using lymphocyte proliferation measured by XTT assay. Table 1 and Fig. 4 indicated the difference in cellular immunity between the pre vaccination and post vaccination and disclosed that the vaccinated calves with different vaccines had a variable cellular immune response according the vaccine used in different conditions appeared from the 1st day and reached to maximum assay on the 10th day post vaccination, then decreased after that time. The results also demonstrated the capacity of the homologus LSD vaccine to produce a good protection showing highest cellular immunity, while the bivalent sheep pox and goat pox vaccine cause the cellular immune response higher than SP vaccine and GP vaccine and long duration (all over the experiment time). Cell mediated immune response of the contact and isolated calves nearly did not change, all over the post vaccinal time, that meaning no horizontal transmission of the virus from the immunized to in-contact non vaccinated animals.

Assaying the cell mediated immune response of vaccinated calves was in agreement with those given by Kaaden et al. (1992), Fatouh (1995), El-Said (1997), Nackhla et al. (2002) and Ahmed et al. (2007) who reported the increase of lymphocyte activity by the 3rd day post vaccination and reached its peak on the 10th day then decreased till the 30th day post vaccination.

The cellular immune response, not only lyses host cells in which infectious agents are present (cytotoxic T-cells), but also facilitates production of different types of antibodies (Hirish and Zee, 1999) (Table 2 and Figs. 5, 6).

The SNT results indicated that vaccinated animals antibodies appear at 7 day post vaccination and reaches a peak 30 days later (Kithing and Hammond, 1992; Kitching, 1996; Hunter and Wallace, 2001).

The SNT antibody reached peak (NI= 3.75) at the 9th week post vaccination with LSD vaccine and remained protective (NI = 3.25) until the 20th week PV (time of study) for LSD vaccine, while for bivalent SP & GP vaccine SNT antibody reached peak (NI = 3.0) at the 10th week PV and remained protective until the ends at the week 20 PV(NI = 2.5); for SP vaccine SNT antibody reached peak (NI = 2.75) at 8th weeks PV and remained protective until the end at the 20th week PV (NI = 2.0), for GP vaccine antibody reached peak (NI = 2.50) at the week 7 post vaccination and remained protective until the week 20 PV (NI = 1.75).

ELISA antibody reached peak at the 8th week PV (S/P = 2.36) and remained protective until the ends at the week 20 PV (S/P = 1.77) for LSD vaccine, while for bivalent SP & GP vaccine antibody reached peak at the 10th week PV (S/P = 2.2) and remained protective until the follow up ends at the week 20 PV (S/P = 1.76); for SP vaccine antibody reached peak at the week 9 PV (S/P = 1.88) and remained protective until the end at the week 20 PV (S/P = 1.38), for GP vaccine antibody reached peak at the week 8 PV (S/P = 1.80) and remained protective until the follow up ends at the week 20 PV (S/P = 1.39).

Results of serological tests in vaccinated and non vaccinated calves sera agreed with those obtained by Agag et al. (1992) who mentioned that a significant rise of serum neutralizing antibodies titre was recorded from the 21th to 42th day post inoculation and with Aboul Soud (1995) who recorded that studies on the collected sera by SNT, AGPT and solid phase ELISA revealed that antibodies appeared by the 10th day P.I. and increased gradually till reaching the maximum by 40th and 50th day P.I. and remained stable after 90 day P.I. till the end of study at 120th day P.I. and also with Fatouh et al. (2007) who said that the neutralizing antibodies appeared to be protective on the 14th DPV (NI= 1.9 & S/P= 2.0) then increased gradually reaching the maximum level by 28th DPV (NI= 3.5 & S/P = 3.5) with the live LSD vaccine then decreased gradually and the protective level remained till the end of the experiment (180 days DPV NI= 2.2 & S/P= 2.2).

Meanwhile, Tilahun et al. (2014) antibodies production started before the day 7 PV and the mean antibodies of all the 3 vaccines (KSGP , SAN & RSP) were increased across each day of the followed up. The peak antibody titres were observed in the monitored cattle at day 35 of post vaccination and remained peak until the follow up ends at day 63. After vaccination all animals had antibody titres of > log5 starting from day 21 and remain within protective range at day 63 too.

The current findings came in accordance with Kaaden et al. (1992), Fatouh (1995), El-Said (1997), and Nackhla et al. (2002) who reported that the neutralizing antibodies of the vaccinated animals appeared at the decreasing time of the cellular immunity on the 14th day P.V. and reached the peak 21day P.V., then decline but persist within the protective levels. As well, Barmana et al. (2010) showed that antibodies are increased above 1:16 titres at day 21 and reached peak (1:32) at 3 months and remain peak for 1 year post vaccination.

On the other hand, Kitching (1986) 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) disagreed with our results concluding 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 NI coincided with the ELISA antibody results and corroborated the results of cell mediated immunity that proved the superiority of attenuated LSD and dual capripox vaccines on the other vaccines in protection of cattle against lumpy skin disease infection.

In conclusion, the present study proved that the dual vaccine come just after the homologus LSD vaccine in control of lumpy skin disease and gave much better results compared to sheep pox or goat pox vaccines when used solely. Field application of dual vaccine should be applied in further studies to evaluate the use on large scales.

5. Conclusion

The use of homolgous LSD vaccine is much recommended for control of lumpy skin disease in calves.

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