Immunological Study on Lumpy Skin Disease Vaccines

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ABSTRACT

Eight cattle were divided into 3 groups, the 1st group of 3 animals was inoculated intradermally (I/D) with 1 ml of alive attenuated lumpy skin disease virus (LSDV) vaccine and the 2nd group of 3 animals was inoculated subcutaneously (S/C) with 5 ml of an inactivated LSD vaccine, while, the 3rd group of 2 animals was kept as non-vaccinated control. Cell mediated immune response of the vaccinated animals was evaluated by lymphocyte transformation assay. The level of lymphocyte increased from the 1st day post vaccination for both vaccines but the live attenuated vaccine induced a higher level than that induced by the inactivated one. Serum neutralization test and enzyme linked immunosorbent assay were used for evaluation of the humoral immunity. The neutralizing antibodies appeared to be protective on the 14th day post vaccination then increased gradually reaching the maximum level by 28th day post vaccination with the live and inactivated vaccines, respectively, then decreased gradually. The protective level of the antibodies remained till the end of the experiment (180 days) for the attenuated vaccine but it remained only for 60 days in case of the use of inactivated vaccine. The results of the ELISA were parallel and related to those obtained by SNT more sensitive.

INTRODUCTION

Lumpy skin disease (LSD) is a serious disease caused by the only member of capripox (Neethling) that affects cattle *(1)*. The disease causes very devastating economic losses in milk production, hide and meat *(2)*. The mortality rate caused by LSD is low but the morbidity may reach 100%. LSD is transmitted mechanically by bitting insects *(3)* and natural transmission in the absence of insect vectors is insufficient *(4)*. Due to the serious economic losses caused by LSD, the OIE positioned the disease as one of list "A" diseases. Vaccination is the only way for controlling the disease.

The disease arrived Egypt in 1988 at local quarantine station with cattle imported from Africa, then the disease spread locally during Summer 1988. It reappeared in 1989 and spread to 22 of 26 governorates of Egypt *(5)*.

In 1998 cases of LSD were recorded among non-vaccinated cattle in El-Menia governorate *(6)*. The disease reappeared in 2006 and the virus isolated from all the outbreaks was the same strain (Neethling strain).

The aim of this study was to evaluate the immunological responses to lumpy skin disease vaccines (live attenuated and inactivated vaccines).

MATERIAL AND METHODS

Animals

Eight susceptible Balady cattle, 9 months old, were purchased from El-Wadi El-Gedid Governorate. The animals were housed inside insect proof unit and their sera were tested and proved to be free from antibodies against lumpy skin disease virus (LSDV) using SNT. These cattle were allotted into 3 groups. Group (1) of cattle vaccinated with live attenuated vaccine, group (2) of 3 cattle were vaccinated with inactivated vaccine and group (3) of 2 cattle were kept non-vaccinated control.

Vaccines

Live attenuated LSDV vaccine

The live attenuated LSDV (Neethling strain with lactalbumin and sucrose as a stabilizer) vaccine was prepared in Pox Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (SVSRI). It had a titre of $10^{6.4}$ $TCID_{50}$/ml. The minimum protective dose of LSD virus in cattle was 2 $\log_2$ ID_{50}/ml according to *(7)*.

Inactivated LSDV vaccine

It was obtained from VSVRI, Pox Department, inactivated by binary and had a titre of $10^{6.8}$ before inactivation and adjuvanted with IMS 1113 oil. It was prepared according to *(8)*.

Vaccination of animals

Vaccination of cattle with live attenuated LSDV vaccines (Group 1) was carried out through the intradermal route (I/D) and each animal received 1 ml of the vaccine. Group (2) of cattle was vaccinated with inactivated LSDV vaccines and each animal received 5 ml of the vaccine through the subcutaneous route (S/C).

Virus-antigen

It was prepared according to *(9)* from adapted tissue culture LSDV. Its titre was $10^{5.2}$ $TCID_{50}$/ml. It was used in solid phase ELISA (Indirect method) and kept at -20°C in Bijoux bottles, each of 1 ml amounts.

Cell culture

Madin Darby Bovine Kidney Cells (MDBK) were obtained from Ames, Iowa Laboratories, USA. These cells were used for virus titration as well as SNT.

Samples

Blood samples

Whole blood samples were collected from tested animals on heparin for separation of lymphocyte used in lymphocyte transformation assay on the 3rd, 5th, 7th, 10th, 14th, 21st and 28th days post vaccination. It was applied according to *(10)*.

Serum samples

Blood samples were obtained till 180 days post vaccination from all animal groups for serum separation. SNT and ELISA were applied on the separated serum samples.

Estimation of cell mediated immune response

It was done by using lymphocyte transformation test measured by MTT assay. The method was carried out as **(11)**.

Serological tests

Serum neutralization test (SNT)

This technique was done according to the method described by **(12)** using constant serum and variable virus dilutions.

Solid-phase enzyme linked immunosorbent assay (ELISA)

It was done according to *(3)*. The test was applied on the collected serum samples and S/P ratio was calculated according to *(13)*.

RESULTS

Lymphocyte transformation test

The cell-mediated immune response of cattle vaccinated individually with live attenuated LSDV vaccine and inactivated LSDV vaccine was evaluated by using lymphocyte transformation measured by MTT assay (Table 1).

Table 1: Cell mediated immune response using lymphocyte transformation assay in cattle vaccinated with LSD vaccines

Days post vaccination	OD / animal groups					
	Group (1)		Group (2)		Group (3)	
	CC	PHA	CC	PHA	CC	PHA
0 day	0.121	0.125	0.116	0.128	0.119	0.126
1st day	0.195	0.253	0.180	0.210	0.122	0.132
3rd day	0.260	0.395	0.210	0.360	0.123	0.135
5th day	0.382	0.480	0.350	0.430	0.120	0.130
7th day	0.410	0.505	0.390	0.482	0.118	0.128
10th day	0.454	0.586	0.402	0.493	0.121	0.135
14th day	0.404	0.510	0.360	0.455	0.116	0.126
21st day	0.353	0.473	0.287	0.341	0.119	0.126
28th day	0.230	0.290	0.200	0.253	0.120	0.130

Group (1): Cattle were inoculated with live attenuated LSD virus vaccine
Group (2): Cattle were inoculated with inactivated LSD virus vaccine
Group (3): Cattle were kept as non-vaccinated control
CC: Cell Control (cells without mitogen)
PHA: Cells with phytohaemagglutinin
OD: Optical Density

Serum neutralization test

The results of serum neutralization test were expressed by neutralizing index (NI). They were recorded in table (2).

Table 2: Mean LSD neutralizing index in cattle vaccinated with LSD vaccines

Days post vaccination	LSD NI (Animal groups)		
	Group (1)	Group (2)	Group (3)
0	0.6	0.4	0.4
7	0.9	0.8	0.5
14	1.9	1.2	0.4
21	3.2	1.6	0.4
28	3.5	2.0	0.4
45	3.0	1.8	0.3
60	2.9	1.6	0.4
90	2.7	1.2	0.5
120	2.4	1.2	0.6
150	2.2	1.0	0.5
180	2.2	1.0	0.4

Group (1): Cattle were inoculated with live attenuated LSD virus vaccine
Group (2): Cattle were inoculated with inactivated LSD virus vaccine
Group (3): Cattle were kept as non-vaccinated control
N.B.: Neutralizing index (NI) $\geq$ 1.5 is considered protective
LSD-NI: Lumpy skin disease neutralizing index

Solid-phase (ELISA)

The results of ELISA are calculated by S/P ratio and showed in table 3

Table 3: Results of ELISA on sera of cattle vaccinated with LSD vaccines

Days post vaccination	ELISA S/P ratio (Animal groups)		
	Group (1)	Group (2)	Group (3)
0	0.07	0.08	0.08
7	1.5	1.0	0.06
14	2.0	1.4	0.07
21	3.5	1.8	0.06
28	3.5	2.2	0.08
45	3.2	2.0	0.06
60	3.0	1.8	0.08
90	2.8	1.0	0.08
120	2.5	1.0	0.08
150	2.2	1.0	0.06
180	2.2	1.0	0.07

Group (1): Cattle were inoculated with live attenuated LSD virus vaccine
Group (2): Cattle were inoculated with inactivated LSD virus vaccine
Group (3): Cattle were kept as non-vaccinated control
S/P ratio $\geq$ 1.0 is considered positive

DISCUSSION

Viral diseases, specially those causing great economic losses, are controlled by specific potent vaccines ***(14)***. Lumpy skin disease (LSD) is an important infectious eruptive. skin disease of cattle causing mortality and great economic losses among susceptible cattle ***(15).***

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 LSDV and produce good immune response in cattle ***(16).*** Also, in Southern Africa the Neethling strain of lumpy skin disease was used for vaccine preparation and proved to be innocuous and immunogenic for cattle ***(15).*** All strains of capripox virus so far examined, whether derived from cattle, sheep or goats, share immunizing antigens and so attenuated cattle strains and strains derived from sheep and goats have been used as live vaccines ***(4).***

The results of cell mediated immune response using lymphocyte transformation assay revealed an increase in the level of lymphocytes from the 1st day post vaccination (DPV) till reaching the peak at the 10th DPV for both vaccines then decreased gradually from the 14th DPV till 28th DPV, as shown in table 1, which also showed that live attenuated LSD vaccine can induce a higher immune response in vaccinated cattle than that in cattle vaccinated with the inactivated vaccine.

These results agree with those previously recorded ***(17)*** and reported that the stimulation of the lymphocytes in cattle vaccinated with LSDV vaccine (live attenuated LSD vaccine) appeared at the 1st day post vaccination (DPV) till the 14th DPV then decreased gradually till 28th DPV.

Results of humoral immunity by SNT showed in table 2 and the results of ELISA showed in table 3; revealed that the neutralizing antibodies appeared to be protective on the 7th DPV by ELISA and 14th DPV by SNT then increased gradually till reach the maximum titre on the 28th DPV with live and inactivated vaccines respectively then

decreased gradually on the 60th DPV and still stable till 6 months PV with the live vaccine but in case of inactivated vaccine the protective level remained until 60th DPV only. These results are also correlated to those reported by several investigators ***(8, 18, 19)***, who recorded that the LSD neutralizing antibodies appeared on the 7th DPV and reached the maximum titre at 21st DPV. Results of SNT and ELISA were parallel and related to each other.

Depending on the previous results, it could be concluded that the use of the attenuated LSDV vaccine in vaccination of cattle against the LSD offers a better protection than using the inactivated vaccine as the live vaccine replicate at the site of inoculation and give long period of immunity with high level of antibodies.

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الملخص العربى

دراسة مناعية على لقاحات الجلد العقدى

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تم تقسم ٨ عجول قابلة للعدوى الى ثلاث مجاميع المجموعة الأولى تم حقنها بلقاح الجلد العقدى المستضعف بجرعة ١ سم بالجلد بينما المجموعة الثانية حقنت بلقاح الجلد العقدى الميت بجرعة ٥ سم تحت الجلد بينما تركت المجموعة الثالثة دون تحصين للتجربة كضابطة وقد تم تقييم المناعة الخلوية للحيوانات المحصنة والغير محصنة بواسطة اختبار المناعة الخلوية.

قد لوحظ زيادة فى مستوى الخلايا الليمفاوية من اليوم الثالث بعد التحصين لكل من اللقاحين ولكن اللقاح المستضعف سجل اعلى مستوى عن اللقاح الميت. كما تم دراسة المناعة المصلية باختبارى المصل المتعادل واختبار الاليزا حيث وجد ان ظهور الأجسام المضادة يكون فى اليوم السابع من التحصين باللقاح الحى والمثبط ويأخذ فى الزيادة تدريجياً حتى أعلى مستوى فى اليوم ال ٢١، ٢٨ على التوالى ثم تناقصت تدريجياً. واستمر مستوى الحماية للاجسام المضادة حتى نهاية التجربة ١٨٠ يوماً للقاح الحى المستضعف بينما استمر مستوى الحماية للاجسام المضادة لمدة ٦٠ يوماً فقط للقاح الميت. وقد كانت نتائج اختبار الاليزا فى توافق مع اختبار السيرم المتعادل.