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A COMPARATIVE STUDY ON THE IMMUNE RESPONSE OF CATTLE TO SHEEP POX AND LUMPY SKIN DISEASE VIRAL VACCINES.

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SUMMARY

An experiment was conducted to determine the differences in the humoral and cell mediated immune responses of cattle to tissue culture adapted-lumpy skin disease (LSD) and sheep pox (SP) viral vaccines.

Concerning humoral immune response, both viruses induced high antibody titres as measured by serum neutralization test and ELISA . No significant differences in the ability of sera of cattle vaccinated with SPV or LSDV vaccines to neutralize both viruses were detected . This indicated that epitopes responsible for humoral immune response are cross-reactive to a higher degree in both viruses.

Concerning the cell-mediated immune response, peripheral blood lymphocytes from animals vaccinated with LSDV and stimulated with inactivated LSDV (homologous mitogen) showed higher stimulation percentage than those stimulated with inactivated SPV (heterologous mitogen). This indicated that LSDV as a vaccine produces better cell mediated immune response specific against LSDV which has a significant role in protection of animals against virulent field LSDV infection.

INTRODUCTION

Lumpy skin disease (LSD) is an acute, subacute or chronic generalized eruptive viral skin disease affecting cattle of all ages and sexes (Ayre-Smith, 1960). The disease is caused by a single strain of Capri-pox virus-prototype Neethling virus (Fenner and Burnett, 1957). The causative virus is antigenically related to African sheep and goat pox viruses (Mattews, 1982).

The disease was first reported in Northern Rhodesia (Zambia) in 1929 among cattle (MacDonald, 1931). Between 1971 and 1982,

continuous epizootics of LSD appeared in African countries (Nawathae et al., 1978 Anon, 1988). In Egypt, the disease first appeared in June 1988 among cattle in Suez and Ismailia regions (House et al., 1990b). Other outbreaks occurred in Egypt in October 1988 (Youssef et al., 1990a), August 1989 (Youssef et al., 1990b), and in 1992 (El-Allawy et al., 1992).

In Egypt , to protect cattle against the disease a locally produced tissue culture sheep pox vaccine was used (Michail et al., 1994.,) Although tissue culture sheep pox vaccine was recommended to control LSD in Egypt, yet the development of an efficient vaccine prepared from the locally isolated strain is still required (Youssef et al., 1990a), especially after appearance of the disease in Upper Egypt during 1992 (El-Allawy et al., 1992). LSDV adapted in MDBK cell culture proved to be safe and potent and capable of producing a good immune response protecting cattle against challenge with virulent LSDV (Aboul-Soud, 1996).

The present study was conducted to compare between pox viral vaccine and tissue culture adapted LSDV in producing humoral and cell mediated immune response in cattle.

MATERIAL AND METHODS

Material:

1- Vaccines:

The live attenuated LSDV adapted in MDBK cell culture for 80 passage (Aboul-Soud, 1996) and the locally produced SPV vaccine were kindly obtained from Pox virus Dept. (Vet. Vaccine & Serum Research Institute, Abbasia, Cairo). They were used for vaccination of cattle at a dose of 0.5ml containing $100ID_{50}$).

2. Animals:

35 Friezian bulls 15-20 months old kept in insect proof building were used. Before starting the experiment, cattle sera were tested for its freedom from antibodies against SP and LSD viruses using serum neutralization test with reference SP and LSD viruses.

The animals were divided into 3 groups. The first group (14 animals) was vaccinated intradermally (LD) with dose of the tissue culture adapted LSDV (0.5 ml containing $100ID_{50}$). The second group (14 animals) was vaccinated ID with one dose of SPV vaccine (0.5 ml containing $100ID_{50}$). The third group (7 animals) was left without vaccination as a control.

3- Blood samples:

Two sets of blood samples were collected from the experimental animals at 5 days and 2,4, and 6 weeks post-vaccination. One set of blood samples was collected without anticoagulant for separation of sera to detect antibodies by serum neutralization test and ELISA. The other set was collected with anticoagulant for separation of peripheral blood lymphocytes used for lymphocyte blastogenesis assay.

4- Viruses:

Vaccinal strain of SPV (Romanian strain) and LSDV (Kenyan strain) obtained from Pox

vaccine Department (Vet.. Serum, and Vaccine Research Institute, Abbasia, Cairo) were used. SPV and LSDV were propagated in Vero and MDBK cells, respectively. The virus stocks were titrated in microtitre plate (Michael et al., 1994), and frozen at -70°C till used .

5- Cells:

Madin-Darby Bovine Kidney (MDBK) and Vero cells were cultured in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum . They were used for propagation of LSDV and SPV, respectively.

6. Other material:

Eagle's minimum essential medium (EMEM) (Sigma, USA) was used for propagation of Vero and MDBK cell lines.

RPMI 1640 medium (Sigma, USA) was used for culturing peripheral blood lymphocytes (PBL).

Lymphocyte separation medium (Ficoll-hypaque) (Sigma, USA) was used for separation of PBL. It has a density of 1.077g/ml.

MTT [3-(4,5) dimethyle hiazol 2-YL -2,5 diphenyl tetrazolium bromide], (SIGMA,USA) was used for lymphocyte blastogenesis assay.

Methods

1- Serum neutralization test:

The neutralizating titres of cattle sera against SPV and LSDV were determined as previously described (House et al., 1990a). Sera were heat inactivated at 56°C for 30 minutes and serially diluted in microtitre plales. An equal volume of LSDV or SPV (50ul containing 100 $TCID_{50}$) were added to each well and incubated for 1 hour at 37°C. 100ul of MDBK or Vero cell suspension containing 10,000 cells per well added to all plates and incubated at 37°C in CO_2 incubator. The plates were examined microscopically for 5 days post inoculation for characteristic cytopathic effect (CPE). The reciprocal of the dilution of serum which completely inhibitis CPE was expressed as the neutralizing titre.

2-Enzyme linked immunosorbent assay (ELISA)

ELISA was used for quantification of antibodies against LSDV and SPV in cattle sera. Each well of microtitre plate was coated overnight with 100ul of LSDV or SPV ultrapure soluble antigen (House et al., 1990a) diluted in 0.05% carbonate/ bicarbonate buffer (pH 9.6) .The plates were washed 6 times with phosphate buffer saline-tween (PPST) and serial dilution of cattle sera were added to the plate in PBST. After 2 hours incubation at room temperature , the plates were washed 6 times with PBST before adding a peroxidase conjugated antibovine IgG in PBST. After incubation at room temperature for one hour, the plates were washed 6 times with PBST before the addition of the substrate, Ortho-phenylen-diamine (Sigma Company, USA). The reaction was stopped by adding 2.5M H2SO4. The absorbance values were read at 450 nm wave length on Behring ELISA reader..

3- Lymphocyte transformation assay:

The test was carried out according to Ral et al.

(1985). Briefly, equal volumes of heparinized bovine blood were layered onto Ficoll-hypaque in sterile plastic tubes. The cells were centrifuged at 2000 rpm for 30 minutes and the band of the cells at the interface was aspirated, pelleted and washed three times in RPMI. Viability as assessed by trypan blue exclusion should be greater than 95%. The cells were distributed in sterile microtitre plates ($5X10^6$ cell per well) and the specific mitogens (LSDV and SPV) were added. After incubation of the plates for 24 hours at 37°C, the MTT [3-(4,5 dimethyle thiazol-2-YL), 2.5 diphenyl tetrazolium bromide]. was added. After 4 hours, the lysing buffer (Lauryl sulfate) was added, then the plate was incubated overnight, then read by ELISA reader at 570 nm filter.

RESULTS

SNT and ELISA were done to determine the antibody response to SPV and LSDV in cattle vaccinated with SPV vaccine and tissue culture adapted LSDV. Results of neutralization test of cattle sera over a period of 6 weeks are presented in table (1). Titration of sera of cattle vaccinated with LSDV against homologous (LSD) and heterologous (SP) viruses showed that there was no significiant difference in titres between the homologous and heterologous viruses, at 5 days, 2,4,6, weeks post-vaccination. In the mean time animals vaccinated with SPV vaccine did reveal significant differences in neutralizing antibody titres detected between homologous (SP) and heterologous (LSD) viruses.

In ELISA , sera of cattle vaccinated with LSDV or SPV showed an appreciable seroconversion. On titration of cattle sera against homologous and heterologous viral antigens by ELISA, no significant differences were detected between the viruses (Table 2).

To measure the cell mediated immune response, lymphocyte transformation assay was done, using LSDV or SPV as mitogen . Significantly higher stimulation percentages of peripheral blood lymphocytes were observed when using homologous mitogen than in case of using heterologous mitogen (Table 3).

DISCUSSION

Control of lumpy skin disease in Egypt depends on vaccination programs, using a heterologous cross-reacting SPV which can replicate in the host but does not induce clinical disease. Successful control of the disease by this vaccine was achieved in Egypt (Michail et al., 1994). However, Brenner et al. (1992) reported that 40% of calves vaccinated against LSD using SPV showed lesions when challenged using virulent LSDV. After the appearance of the disease again in Upper Egypt during 1992 (Al-Allawy et al., 1992), the question aroused for the need for vaccine for control of LSD prepared from the locally isolated strain of LSDV.

In this study we compared the immune response of cattle to SP and tissue culture adapted LSD viral vaccines and their interrelationship by measuring the respective cell mediated and humoral responses against homologous and heterologous viruses.

Table (1): Average neutralizing antibody titres for sera of cattle vaccinated with tissue culture adapted LSDV and SPV vaccines.

Period post-vaccination	SPV vaccinated animals		SPV vaccinated animals		Control animals	
	LSDV*	SPV**	LSDV	SPV	LSDV	SPV
5 days	008.00±01.02^{A}#	009.71±01.61^{A}	008.57±00.92^{A}	008.56±00.92^{A}	<4*	<4
2 weeks	019.43±01.61^{B}	023.99±02.21^{B}	018.28±01.55^{B}	021.71±02.34^{B}	<4	<4
4 weeks	113.13±04.32^{C}	096.00±03.89^{C}	048.00±04.20^{C}	068.85±08.23^{C}	<4	<4
6 weeks	627.49±12.12^{D}	427.14±19.12^{D}	257.82±11.12^{D}	374.35±34.13^{D}	<4	<4

* Sera were titrated against LSDV — The titre was less then starting dilution (4)

** Sera were titrated against SPV

The average titre of 14 animals.

Means with different superscript within the same row are significantly different (P<0.01).

Table (2): Average ELISA titres for sera of cattle vaccinated with tissue culture adapted LSDV and SPV vaccines.

Period post-vaccination	LSDV vaccinated animals		SPV vaccinated animals		Control animals	
	LSDV*	SPV*	LSDV	SPV	LSDV	SPV
5 days	008.00±05.13^{A}#	0083.23±03.10^{A}	084.28±02.27^{A}	008.0±02.12^{A}	<60	<60
2 weeks	0210.00±11.80^{B}	0193.26±12.90^{B}	178.57±05.32^{B}	0212.13±13.03^{B}	*	<60
4 weeks	0737.14±36.61^{C}	0695.02±30.51^{C}	642.85±18.97^{C}	0640.34±20.64^{C}	<60	<60
6 weeks	1017.14±31.80^{D}	987.14±40.12^{D}	987.14±34.74^{D}	1017.76±43.23^{D}	<60	<60

* Sera were titrated against LSDVantigen — The titre was less then starting dilution (60)

** Sera were titrated against SPV antigen

The average ELISA titre of 14 animals.

Means with different superscripts within the same row are significantly different (P<0.01).

Table (3): Results of cell mediated immune response of cattle vaccinated with SPV and LSDV vaccines as measured by lymphocytes blastogenesis assay using MTT.

Period post-vaccination	LSDV vaccinated animals		SPV vaccinated animals		Control animals	
	LSDVM*	SPVM**	LSDVM	SPVM	LSDVM	SPVM
5 days	52.00 ± 1.84^{A}#	34.66 ± 1.14^{B}	27.00 ± 0.67^{B}	49.33 ± 1.10^{A}	17.34 ± 0.12^{C}	19.23 ± 1.02^{C}
2 weeks	66.66 ± 1.64^{A}	45.66 ± 1.22^{B}	44.66 ± 1.62^{B}	65.16 ± 0.97^{A}	15.23 ± 0.67^{C}	18.34 ± 1.23^{C}
4 weeks	50.16 ± 1.01^{A}	30.83 ± 1.63^{B}	32.33 ± 0.68^{B}	54.33 ± 2.61^{A}	15.25 ± 0.34^{C}	16.00 ± 1.20^{C}
6 weeks	34.00 ± 1.64^{A}	21.83 ± 0.56^{B}	18.50 ± 0.49^{B}	39.1 ± 0.78^{A}	19.23 ± 1.40^{B}	18.16 ± 0.89^{B}

* Lymphocyte transformation assay was carried against LSDV as a mitogen.

** Lymphocyte transformation assay was carried against SPV as a mitogen.

values expressed are the average stimulation percentage.

Means with different superscripts within the same row are significantly different ($P<0.01$).

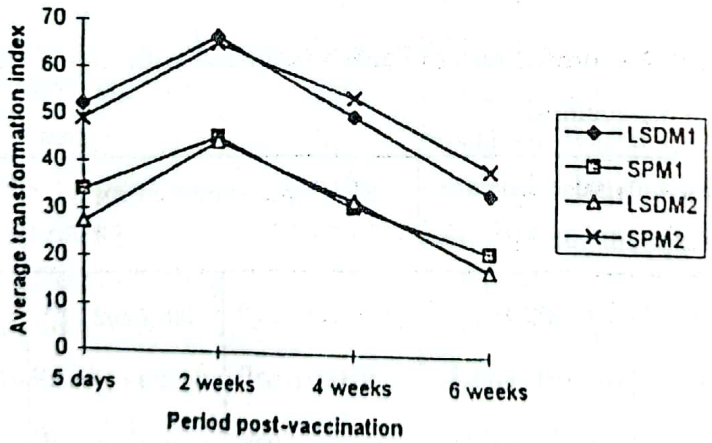

Fig.(1): Results of cell mediated immune responsene of cattle vaccinated with SPV and LSDV vaccines as measured by lymphocytes blastogenesis assay using MTT.

LSDMI = Animals vaccinated with LSDV and lymphocytes stimulated with LSDV moitogen

SPMI = Animals vaccinated with LSDV and lymphocytes stimulated with SPV mitogen.

LSDM2 = Animals vaccinated with SPV and lymphocytes stimulated with LSDV mitogen.

SPM2 = Animals vaccinated with SPV and lymphocytes stimulated with SPV mitrogen.

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