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Immune Response of Sheep Vaccinated with Capripox Vaccine

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Abstract: The immune response of Sudanese sheep vaccinated with capripox vaccine was detected by IFA, Ic-ELISA test and MTT lymphocytes proliferation assays. The difference between antibodies titers before and after vaccination was found significant when tested by IFA test and the difference between the mean OD values was also significant when Ic-ELISA was used. PHA was found more effective in stimulating peripheral blood lymphocytes before vaccination compared with the virus antigen. After vaccination, the mean stimulation index of the of the virus antigen was higher

Key words: Immune response, vacenation, IFA test, Ic-ELISA

INTRODUCTION

Capripox viruses represent one of the eight genera within the Chrodopox virus subfamily of poxiviridae (George, 1986). The genera include Avipox virus, Capripoxvirus, Leporipox virus, Molluspox virus, Orthopox virus, Parapox virus, Suipox virus and Yatapox virus. The Capripox virus is currently comprised of sheep pox virus, goat pox virus and lumpy skin disease virus, causing disease in sheep, goat and cattle, respectively. These viruses are responsible for some of the most economically significant diseases of domestic ruminants in Africa and Asia (Carn, 1993).

Capripox viruses are generally considered to be host specific, because disease outbreaks of virus isolates may preferentially occur or cause disease in one host species (Munz and Dumbell, 1994). This has been especially true for Nigerian, Middle East and Indian strains of sheep pox virus, goat pox virus and lumpy skin disease virus (Kitching *et al*., 1989, 1987, Rao and Bandyopadhayay, 2000). However, the ability of sheep pox virus and goat pox virus strains to naturally or experimentally cross-infect and cause disease in both host species has been described (Kitching and Taylor, 1985, Kitching *et al*., 1987). This apparent variability in sheep pox virus and goat pox virus host range, the clinical similarity between sheep pox and goat pox and the inability to differentiate the two diseases by serological assays have led to the suggestion that sheep pox and goat pox are part of disease complex caused by a single viral species and that observable host range specificities are the result of regional virus adaptation to sheep or goat host (Davies and Otema, 1981). Capripox viruses are very resistant and can remain viable for long period on or off the animal host. Sheep pox transmitted directly by nasal secretion, saliva or dried scab and indirectly by contaminated implements vehicles and product such as litter and fodder. The virus is transmitted mechanically through insect vector (Esposito and Fenner, 2001) and by inhalation, intradermal, subcutaneous inoculation and respiratory transmucosal routes (Kitching and Taylor, 1985, Kitching and Mellor, 1986). No specific information is available on transmission of the virus through semen or embryo.

There is a close antigenic relationship between sheep pox and goat pox viruses. The result of direct and indirect fluorescent Antibody Technique (FA) and serum neutralization test demonstrated that sheep pox and goat pox strains isolated from Kenya and the Middle East were serologically identical with lumpy skin disease. There was no serological evidence that the viruses were related to camel pox virus.

The immunity acquired after infection with sheep pox virus is considered to be life long. In studies designed to determine the host mechanisms responsible for immunity to sheep pox, it was observed that passive transfer of antisera to sheep pox virus conferred partial protection on subsequent challenge by increasing incubation period and lamb hyperimmunized with sheep pox virus developed delay hypersensitivity reaction. It was therefore concluded that both humoral and cell mediated immune response were involved in immunity to sheep pox (Srivastava and Singh, 1980, Bachh *et al*., 1997). The aim of this work is to study the immune response of 0240 capripox vaccine strain in local Sudanese sheep.

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MATERIALS AND METHODS

Vaccine: The vaccine was prepared in the department of viral vaccine, Central Veterinary Research Laboratory (CVRL). Sudan It was made in a lyophilized form, from the vaccine strain 0240.

Virus titration: The virus was titrated in microtiter plate (Kitching and Taylor, 1985) and on clipped flank of two sheep using range of dilution from 10^0 to 10^6, 4 replicates intradermal inoculation were made from each dilution, results were calculated according to Karber (1931).

Experimental animals: Ten sheep purchased from the local market, they were known as non-vaccinated previously with sheep pox vaccine. The animals were divided into two groups each of 5, the first group had 1 mL of the vaccine (2.5 $TCID_{50}$), while the second group remained unvaccinated.

Screening test: This was done by immunostaining nitrocellulose dot blot test.

Sera and lymphocytes collection: The peripheral blood was collected in vacutainers containing heparin before vaccination and 21 days after. The blood was centrifuged at 1400 rpm for 10 min; sera were collected and preserved at -20°C till used. The lymphocytes were separated on Ficoll Histopaque (Sigma), fresh lymphocytes were used in proliferation test after three washes with PBS.

Indirect fluorescent antibody test (IFA): In brief, 10 μL of virus infected cells were prepared as described by Ashley *et al.* (2001), corresponding sera collected before and after vaccination were two-fold diluted then dropped onto the slides wells. The slides were incubated in moist condition at 37°C for 30 min then washed three times with PBS on an orbital shaker (Stuart Scientific). Ten microliter of diluted antisheep (Sigma) conjugate containing 0.01% Evans blue was applied onto each well and then incubated in moist condition at 37°C for 30 min. The slides were again washed 3 times as pre-mentioned. Examination of the slides was carried out in the same day with fluorescent microscope in a dark room.

Immunocaptue Enzyme Linked Immunosorbent Assay (Ic.ELISA): The test was carried out to detect antibodies raised against capripox virus (0240 vaccine strain). It was done as described by Rao *et al.* (1997) with minor modification, the conjugate was used in dilution 1:2500 instead of 1:5000 and casein (Oxoid) was used in the blocking buffer instead of Bovine Serum Albumin (BSA).

Methylthiazolyl Diphenyltetrazolium Bromide (Mtt) Lymphocytes Proliferation Assay: This test was done according to Mosmann (1983) where (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) (Sigma) was used in lymphocytes proliferation assay, 100 μL of lymphocytes suspension (5×10^5 mL^{-1}) from each animal were transferred into 3 columns (each in triplicate). The first column from each animal received 40 μL (40 μg mL^{-1}) PHA (Sigma), the second 40 μL of undiluted virus and the third remained as non-stimulated control. The plate was incubated at 37°C in CO_2 chamber for 48 hours, 20 μL of sterilized MTT (5 mg mL^{-1}) was added to each well and the plate was incubated overnight in the same previous condition. After color development, 100 μL of isopropanol with 0.04N HCL, the content of each well was mixed thoroughly by repeated pipetting to dissolve the precipitates and then read at 492 nm (Cory *et al.*, 1991, Terry and Rich, 1996)).

RESULTS

Virus titration: On microtiter plate virus titration was found 5 $TCID_{50}$ while on the sheep flank it was 3.5 $TCID_{50}$.

Screening test: Antibodies were detected in all experimental animals (Fig. 1).

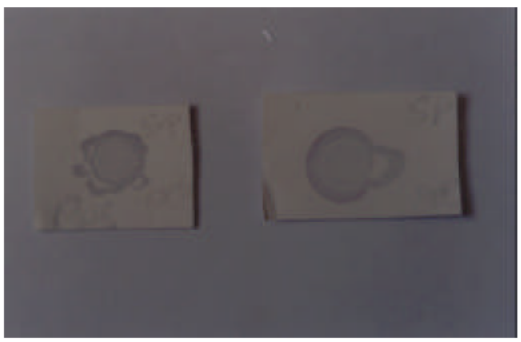

Fig. 1: Immune staining dot blot due reaction of Sheep Pox (SP) antigen and serum antibodies before (pre) and after (pos) vaccination with

Table 1: Antibodies response of sheep (vaccinated with sheep pox vaccine) to capripox virus measure by Indirect Fluorescent Antibody (IFA) technique

Sheep No	Dilution positive before vaccination	Dilution positive after vaccination	Seroconversion
1	1/2	1/16	3
2	1/2	1/32	4
3	1/4	1/164	4
4	1/4	1/64	4
5	1/8	1/256	5

Table 2: Antibodies response of sheep (vaccinated with capripox vaccine) to capripox virus using Ic- ELISA

Sheep No	Pre-vaccination sample (OD*)	Post-vaccination Sample. (OD*)
1	0.258±0.02	0.323±0.01
2	0.287±0.02	0.440±0.03
3	0.221±0.00	0.290±0.01
4	0.263±0.04	0.360±0.05
5	0.265±0.03	0.345±0.02
Mean	0.258±0.02	0.350±0.02

OD* = Optical density at 492 nm±standard deviation

Table 3: Proliferation response of non-vaccinated sheep lymphocytes stimulated with PHA and CPV antigen measured by absorbance 492 nm

Sheep No.	Mean OD of non-stimulated lymphocytes	Mean OD of lymphocytes stimulated with SP Ag	Mean OD of lymphocytes stimulated with PHA
1	0.289±0.04	0.330±0.02	0.734±0.20
2	0.133±0.03	0.211±0.07	0.558±0.00
3	0.316 ±0.00	0.787±0.10	1.016±0.20
4	0.148±0.01	0.199±0.00	0.646±0.01
5	0.296±0.03	0.405±0.10	0.442±0.01
Mean	0.236±0.02	0.386±0.06	0.679±0.08

CPV = Capripox virus, PHA = Phytohemagglutinin, OD = Optical Density±Standard Deviation

Table 4: Proliferation response of vaccinated (with CP vaccine) sheep lymphocytes stimulated with PHA and CPV antigen measured by absorbance 492 nm

Sheep No.	Mean OD of non-stimulated lymphocytes	Mean OD of lymphocytes stimulated with SP Ag	Mean OD of lymphocytes stimulated with PHA
1	0.309±0.00	0.679±0.03	0.369±0.01
2	0.481±0.00	1.150±0.10	0.520±0.03
3	0.225±0.01	0.704±0.12	0.270±0.01
4	0.220±0.02	0.690±0.07	0.245±0.03
5	0.447±0.00	1.205±0.15	0.530±0.00
Mean	0.337±0.00	0.886±0.09	0.387±0.02

CPV = Capripox virus, PHA = Phytohemagglutinin, OD = Optical Density±Standard Deviation, CP = Capripox

Indirect Fluorescent Antibody (IFA) Test: The difference between titer expressed in log 2 before and after vaccination was found significant (Table 1).

Immunocapture ELISA: The mean Optical Density value (OD) before vaccination with capripox vaccine was 0.255 and it was 0.346 after vaccination (p = 0.001) (Table 2).

MTT lymphocytes proliferation assay: Table 3 and 4 show the mean OD values of lymphocytes before and after stimulation with PHA and SPPV antigen, respectively before and after vaccination

PHA was found to be more effective in stimulating Blood Peripheral Lymphocytes (PBL) compared with the virus antigen before vaccination. The mean stimulation index due virus and PHA was 1.5 and 3.1, respectively (Table 5) Following vaccination the mean stimulation index for the virus and the PHA was 2.7 and 1.1, espectively (Table 6).

Table 5: Stimulation indices of PHA and CPV antigen-stimulated lymphocytes of sheep before vaccination with capripox vaccine

Sheep No.	SPPV	PHA
1	1.1	2.5
2	1.5	4.1
3	2.1	2.8
4	1.3	4.3
5	1.4	1.6
Mean	1.5	3.1

PHA = Phytohemaglutinin, CPV = Capripox virus

Table 6: Stimulation indices of PHA and CPV antigen-stimulated lymphocytes of sheep after vaccination with capripox vaccine

Sheep No.	SPPV	PHA
1	2.2	1.2
2	2.4	1.1
3	3.1	1.2
4	3.1	1.1
5	2.5	1.1
Mean	2.7	1.1

PHA = Phytohemaglutinin, CPV = Capripox virus

DISCUSSION

A variety of live and inactivated capripoxvirus vaccines has been used to provide protection for sheep and goats against capripox (Carn, 1993). All strains of capripoxvirus of ovine or caprine or bovine origin examined so far share a major neutralizing site, so the animals recovered from infection with one strain are resistant to infection with any other strain (Capstick, 1961). Consequently, it is possible to use a single strain of capripoxvirus to protect both sheep and goats against all field strains of viruses, regardless of whether their origin was in Asia or Africa (OIE, 2004). The strain of the virus used in this study was capripox 0240; it has been used to protect both sheep and goats (Kitching, 1986). It produced no harmful effect whether in the field or in the experimental animals group that we have used. Experimental animals revealed antibodies to the capripox virus, this result was expected because the animals were brought from the field and Sudan is endemic with sheep pox disease.

In the IFA test, there was significant difference of antibodies titers (Table 1) between the vaccinated and the non-vaccinated group. Although the indirect fluorescent antibody technique is one of the tests recommended for sheep pox diagnosis (OIE, 2004), the possibility of cross reaction with related viruses should also be considered (Hedberg *et al*., 1987) but here the virus was already known to us and beside this test immunocapture ELISA has been conducted to determine antibody titers. Different methods of ELISA are available to diagnose pox viruses, immunocapture ELISA is regarded as a relatively simple assay for detection of sheep or goat pox viruses (Rao *et al*., 1997). There were significant differences between the induced titers of antibodies in vaccinated sheep with the capripox vaccine when tested by immunocapture ELISA protocol. These results support those obtained from the IFA test.

There are evidences that cell mediated immune response plays an important role against sheep pox besides humoral immunity. Reports on the cell mediated immune response against sheep pox are conflicting and inconclusive (Bachh *et al*., 1997). In this study PHA induced significant proliferation (SI = 2.7) of prepheral blood lymphocytes of non-vaccinated sheep compared with capripox virus antigen. Interestingly, the virus antigen induced as high proliferation (SI = 2.0) in vaccinated sheep comparable with PHA. This result indicates that sheep pox virus has a role in the cell mediated immune response and it has an antimitogenic effect. The increased lymphocytes blastogenesis due specific sheep pox antigen stimulation agreed with the report of Bachh *et al.* (1997). Several independent studies have suggested that $CD8^+$ CTL are crucial for recovery from poxvirus infection (O'Neill and Bernan, 1987, Rubby and Ramshaw, 1991). However, mice deficient of T-cell subset, in addition to exhibiting severely reduced cell surface expression of class 1 Major Histocompatibilty Complex (MHC) molecules because of disruption of the B2 microglobulin gene (Koller *et al*., 1990) effectively controlled infection with Vaccinia Virus (VV) and a number of other viruses (Dohert, 1993). The recent findings of studies using gene knockout mice have questioned the importance of $CD8^+$ T cells in the control of infections caused by poxviruses and some other viruses, including influenza A virus (Doherty,1993). It is possible that the effector functions of neither CD8+ T cells are not crucial for elimination of viruses that neither replicate efficiently nor are natural pathogen of the mouse (Gunasegaran *et al*., 1996). It is also possible that these mice developed a compensatory mechanism that allowed resolution of the infection. Finally, it has been suggested that the cytolytic effector functions of $CD8^+$T cells may not be important for the elimination for the cytopathic viruses like variola virus and vesicular stomtitis viruses (Zinkernagel, 1996).

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