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Studies on the Humoural Immune Response of Sheep to Sheep Pox Thermostable Vaccine

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ملخص البحث

قيست الاستجابة المناعية للضأن السوداني بعد تحصينه تجريبياً بواسطة اللقاح الثابت حرارياً لفيروس جدري الضأن و المنتج محلياً ، باستخدام المقايسة المناعية المرتبطة بالانزيم و اختبار معادلة المصل.معمليا تم تقسيم الضأن الي مجموعتين تحتوي كل منهما علي 5 خراف.حصنت الأولي باللقاح الثابت حراريا بـ$10^{2.5}$جرعة مخمجة لنصف النسيج المستزرع, بينما تركت الا خرى دون تحصين أي مجموعة تحكم .في اليوم الخامس و أربعين من التحصين أجري **اختبار** الاسقامية لكل الحيوانات بفيروس عترة سوبا الحقلية الضارية ب 10^4 جرعة مخمجة لنصف النسيج المستزرع. بلغت نسبة الحماية 80 %. أحدث اللقاح الثابت حراريا استجابة مناعية جيدة و ذلك بحسب قياس اختبار معادلة المصل ، المقايسة المناعية المرتبطة بالانزيم و الاسقامية.حقليا حصنت 250 رأس من الضأن ذات أعمار مختلفة لم تظهر عليها أى أعراض مرضية أو نفوق. عندما تعرضت للعدوي مع 2045 رأس أخري غير محصنة تختلط معها في المرعي و مواقع الشرب .حيث أظهرت الأخيرة **18.9 %**, **1.4. %** كمعدلات امراضية و نفوق علي التوالي . دلت التجربة الحقلية أن التحصين السنوي يؤدي الي التحكم في المرض .

Summary

The immune response of Sudanese sheep vaccinated with an experimental sheep pox thermostable vaccine, produced in the Viral Vaccines Compound, Veterinary Research Instatute, Khartoum, Sudan; was measured using Serum Neutralization Test (SNT) and Immuno-capture Enzyme-linked Immunosorbent Assay (Ic-ELISA). One group of 5 sheep was inoculated with 10 $^{2.5}$ tissue culture infective dose 50 (TCID50) and another group of five sheep was kept as a control. On day 45 post vaccination (P.V)the two groups were challenged with 10^4 $TCID_{50}$ of a hot strain (Soba Field strain). Protection of the challenged sheep was 80 %. The thermostable vaccine induced an excellent immune response as assessed by SNT, Ic–ELISA and challenge. All vaccinated 250 sheep did not experience any morbidities or mortalities, while un-vaccinated flocks (2045 sheep of different age groups) showed a morbidity rate of 18.9% and a mortality rate of 1.4%. It was obvious from the field trial that annual vaccination will lead to disease control.

Introduction

Capripoxviruses are among list A diseases of the Office Internationale d'Epizooties (OIE); they cause sheep pox , goat pox, and lumpy skin disease in cattle. These diseases are characterized by pyrexia, generalized skin and internal pox lesions and adenopathy, (Kitching and Taylor, 1985; Carn, 1993). The diseases are restricted to parts of Asia and Africa and are a cause of major economic losses, associated with trade restrictions following out-breaks and mortalities. The incubation period following natural infection is 6-12 days. Infection is usually caused by aerosol following close contact in the acute stage of the disease. The virus persists in the scabs, developed from the papules following recovery, for over 3 months (Kitching and Taylor, 1985).

The presence of sheep pox in the Sudan was confirmed in 1940 by Bennet *et al* (1944) who gave a brief note on sheep pox in the Sudan and looked into its relationship with vaccinia and goat pox virus. Sheep pox is widespread in the Sudan, mostly occurrs during winter, affects all age groups and causes serious losses, especially in feed lots, (Muzichin and Ali ,1979). The disease may concomitantly occur with lumpy skin disease (Ali and Obeid , 1977). However, sheep pox was recently observed to occur all the year round, (Sheihk – Ali *et al*., 2004). The objective of this study was to investigate the immune responses elicited by the Sheep Pox experimental thermostable vaccine, produced locally in the Sudan, by plaque purification of the Kenyan sheep and goat pox vaccine strain 0240 and clone selection for heat resistance .

Materials and Methods

Vaccine and challenge strations:

The Vaccine (strain 0240) was obtained from the vaccine bank, which was developed recently at the viral vaccines production unit of the Central Veterinary Research Laboratories Adminstration (VRLA), Khartoum, Sudan.

Soba Sheep pox virus field strain was isolated in 1980 at the VRI, Khartoum, Sudan by Prof. Babiker H. Ali and kept lyophilized at –20^{o}C.

Lamb Testis Cells (LT):

Lamb testis cells were prepared as described by Lennett and Schmidt (1979), and used for virus propagation.

Sheep pox hyperimmune serum:

Three rabbits were hyper-immunized using 4 injections of SPV 0240 as described by Lennett and Schmidt (1979). Briefly, the animals were first injected subcutaneously with SPV (10 $^{2.5}$ $TCID_{50}$) and an equal volume of Freunds' complete adjuvant thereafter, a second dose that contained SPV and Freunds incomplete adjuvant was given intramusularly 10 days .This was then followed ten days later with two doses of SPV intradermally (10 days apart) .The animals were bled 10 days after the last dose and the collected serum was kept at -20^{o}C till used.

Vaccination and Challenge Test:

This test was performed as outlined by the OIE of Standards (2004). Briefly, ten sheep with no previous vaccination history were purchased from a local market. Pre-immunization sera were collected, then 5 of them were vaccinated using recommended field dose ($10^{2.5}$ $TCID_{50}$) and other 5 sheep were inoculated with diluent (PBS) and kept as controls. Animals were clinically observed for 21 day post vaccination (PV) during which temperature was recorded daily and sera were collected by the end of the period. All 10 sheep were kept for another 24 days and then challenged using 10^{4} $TCID_{50}$ of Soba field strain. All sheep were daily observed for another 21 days for pyrexia (rectal temperature), clinical signs of disease and lesions or mortality.

Serum Neutralization Test:

The test was carried out as outlined by OIE (2004). The thermostable vaccine was titrated against a constant dilution of hyperimmune serum and also against pre-immunization and post-immunization sera. All sera were diluted (1:5) in GMEM, while10 fold dilutions of the virus were made using a serum–free GMEM. Neutralization indices were calculated according to Karber (1931). Results were analyzed using Student- Test. P value of ≤ 0.05 was considered significant.

Immuno - capture Enzyme Linked Immunosorbent Assay (Ic. ELISA):

The aim was to measure the magnitude of humoural immune response of IgG as whole molecule. This test was perfomed according to Rao *et al*., (1997) with slight modifications as described by Ahmed *et al*. (2007a).

Field Trial

Flocks of 250 Hamari sheep of different age groups and sexes were vaccinated in 2009 in Al Obeid, (Kordfan state) with $10^{2.5}$ $TCID_{50}$ of thermostable SPV. These animals were mixed with other un-immunized animals at grazing areas and watering points and all were subjected to a natural challenge with a wild type SPV.

Results

Challenge Test:

Comparison means of daily rectal temperature of challenged both control and vaccinated sheep are shown in Fig 1. Table 1 shows post - challenge reactions of sheep. Unimmunized control sheep, showed generalized pox lesions, with the exception of sheep No 139 which showed only a few nodules (one to three watery nodules under the tail), Animal No 108 in the immunized group showed, most likely a delayed hypersensitivity reaction. The calculated protection percentage was 80% (Table 1).

Serum Neutralization Test:

Comparison means of pre-vaccination and post-vaccination indices using Student – t test, showed a probability of 0.021, which is significant.

Ic. ELISA:

The mean Optical Density values (OD) of pre-vaccinated, post-vaccinated and post-challenged sheep are shown in Table 2. Student- t Test result of vaccinated and un vaccinated was highly significant as shown below:

1. Control and post-vaccination samples: $P < 0.05$.
2. Post-vaccination and post-challenged samples: $P < 0.5$.
3. Control and post-challenged samples : $P < 0.05$.

Field Trial:

All immunized 250 sheep did not experience any morbidities or mortalities, while unimmunized flocks (2045 sheep of different age groups) showed a morbidity of 18.9% and a mortality rates 1%, respectively.

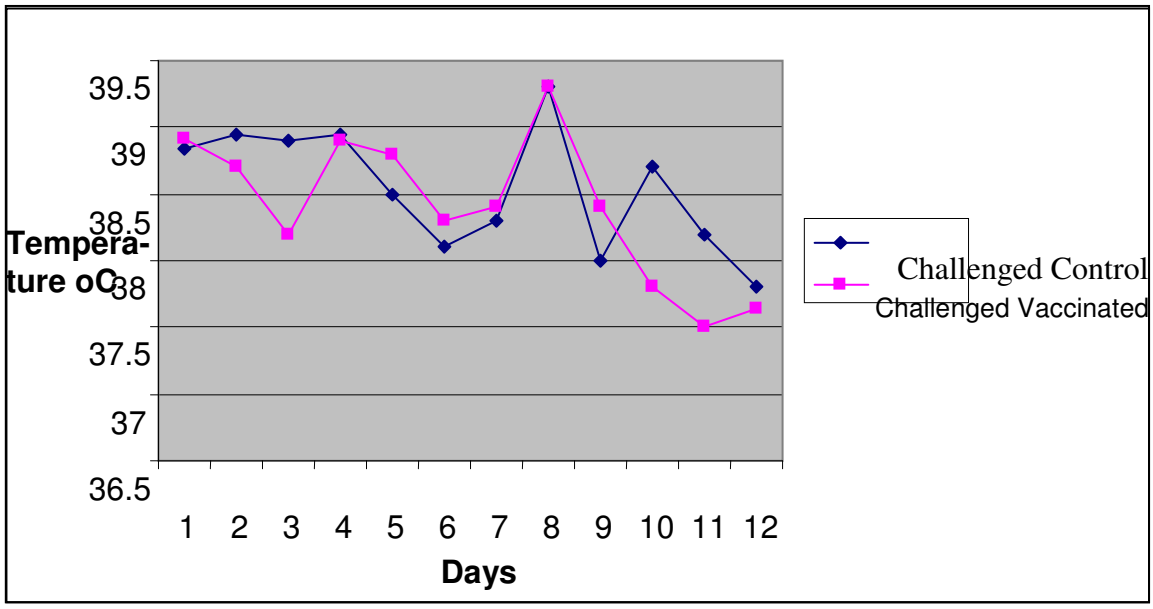

Fig.1:Mean daily temperature of challenged vaccinated and challenged control sheep.

Table 1: Post-challenge reactions observed in unvaccinated control sheep and sheep vaccinated with pox thermostable vaccine.

Sheep No.	Pox Lesions
Status	**Days Post Challenge**
Unvaccinated control	***5***
136	*3+*
142	*3+*
117	*3+*
110	*3+*
139	+
vaccinated	
111	-
109	-
108	-
101	-
143	-

- No lesions; + 1-2 Pox lesions under the tail; 2+ Multiple nodules under the tail and on ears.;3+ generalized pox lesions.

Table 2: Mean Antibody response of sheep vaccinated with sheep pox thermostable vaccine ($10^{2.5}$ $TCID_{50}$) and challenged with Soba field (10^4 $TCID_{50}$) using Ic-ELISA.

Post-challenge OD	Pre-vaccination OD	Post-vaccination Control OD	Post-challenge Vaccinated OD
0.248±0.00	0.347 ±0.00	0.542 ±0.04	0.386 ±0.02

O.D.= Optical density

Discussion

The present study was designed and carried out for the assessment of the immune response of sheep to an experimental sheep pox thermostble vaccine. The mean neutralization indices of pre-vaccination sera (0.41) and post- vaccination sera (1.24) reveal quite clearly the occurrence of a significant sero - conversion (P= 0.021), provided that neutralization index of the rabbit hyperimmune serum is 1.0 (heterologous to TV). Neutralization activity of hyperimmune serum, however, was incomplete (data not shown) in conformity with Muzichin and Ali (1979) who reported the same phenomenon.

This result also compares very well with mean optical densities obtained by Ic-ELISA (Table 2), that showed significant difference (P= 0.04) between pre-vacination and post-vaccination sera. which implies potency of thermostable vaccine. A highly significant difference (P= 0.001) was noticed when OD of pre-vaccinated compared with post -vaccinated sheep sera using the same technique of Ahmed *et al*. (2007 a). In this experiment,comparison of OD of post**-** vaccination and post- challenged samples clearly indicate an absence of a booster effect (P = 0.4 , table 2). The explanation of this, is that all challenge virus was neutralized; this is contrary to the results of

Chaudary *et al*. (2009) who reported that sheep immunized with SPV showed a booster response on challenge with virulent SPV.

Although, challenge test was performed using a high titre of a hot virus (10^4 $TCID_{50}$) and at day 45 post vaccination, protection percentage was still 80 % (Table 1). Reactions shown by sheep 108 were much likely delayed hypersensitivity reactions , but no other clinical signs were observed . This result was similar to that recorded by Ahmed *et al* . (2007 b) in the Sudan, when sheep were vaccinated with Kenyan Sheep and Goat Pox Vaccine strain 0240 and challenged with the same hot virus used in our experiment. The results were also similar to those recorded by Mukhopadhyay *et al*. (1999) where sheep vaccinated with $10^{3.5}$ $TCID_{50}$ per 0.2 ml of Kenyan Sheep and Goat Pox Vaccine strain 0240 and challenged with 2×10^4 of a virulent sheep pox virus (Ranipet strain), showed no signs of clinical disease. Similarly, vaccinated, animals with Hyderabad strain gave 83.3% protection and NI of 1.54 (Das and Mallick , 1986).

Fig 1 displays the mean daily temperature of challenged unimmunized (control) and immunized sheep. Initially the former's body temperature may be due to virus replication, which was higher than that of the latter (most probably a period of virus neutralization). Later on, a state of equilibrium was reached, where there is neither virus replication nor neutralization, and body temperature of both groups was nearly similar and resembled body temperature of a clinically normal animal.

Usually, in unvaccinated herds a morbidity rate greater than 30% and a mortality rate higher than 1 % are reported during winter. This study indicated that annual vaccination may lead to either disease control or eradication. Some animals challenged under laboratory conditions are expected to show clinical signs and lesions much readily than those challenged naturally, this is because the formers receive high equal doses of a virulent SPV virus and via the same route, which is contradictory to the latter. Natural challenge is thus the mirror which reflects the real value of any vaccine.

The vast majority of sheep and goats in the Sudan are kept under nomadic conditions, in remote areas where electricity power supply and cold chain facilities are lacking. Therefore a thermostable vaccine will help alleviate the need for such prerequisites. The results of the present immunization trial clearly manifest the value of using such a vaccine to control and possibly eradicate the disease.

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