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CONTROL OF LUMPY SKIN DISEASE OUTBREAK IN EGYPT WITH ROMANIAN SHEEP POX VACCINE

(With One Table)

By

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مقاومة وباء مرض الجلد العقدى فى مصر بلقاح جدرى الأغنام العترة الرومانية

عزيز إسحق ، محمد صابر ، سعاد سليمان ، على موسى ، سيد سلامه ، عادل فايد ، منيره نصار ، جيمس هاوس

حقن لقاح جدرى الأغنام (العترة الرومانية) بمقدار جرعة واحدة وعشرة أضعافها فى الماشية التى خضعت لاختبار تحدى المناعة بحقنها بالعترة الضارية لفيروس الجلد العقدى. الأبقار المحصنة لم تعطى الآثار المرضية مقارنة بالحيونات الضوابط غير المحقونة. فى الأبقار المحقونة بعشرة أضعاف جرعة جدرى الأغنام بقيت الآثار المرضية بعد التحدى لفترة (١٥) يوم تقريبا مقارنة بالأبقار التى حصنت بجرعة واحدة حيث بقى بها الآثار المرضية لمدة (٢١) يوم. أربعة أبقار من خمسة (٨٠٪) من كلا المجموعتين المحصنتين (بجرعة واحدة أو عشرة جرعات لكل منها) أكتسبت وقاية من الآثار المرضية العامة (الشاملة) بينما أربعة أبقار من الخمسة الضوابط الغير محصنة (٨٠٪) أظهرت ردود فعل عام مرضى للجلد العقدى بعد إجراء التحدى ومات واحد من العجول الضوابط. أعطت العترة الرومانية للقاح جدرى الأغنام حماية تبادلية كافية للأبقار ضد التحدى بفيروس مرض الجلد العقدى. ويتميز لقاح جدرى الأغنام هذا بأنه لا توجد خطورة منه على الأغنام المخالطة على عكس إستخدام جدرى الأغنام الضارى لتحصين الأبقار ضد مرض الجلد العقدى.

SUMMARY

A sheep pox (SP) vaccine (Romanian strain) was inoculated (one and ten doses) into cattle that were subsequently challenge inoculated with a virulent lumpy skin disease (LSD) virus. Vaccinated cattle had markedly reduced lesions compared to non-inoculated controls. In cattle receiving ten sheep doses of vaccine, the duration of LSD lesions was about 15 days compared to approximately 21 days in the cattle given one sheep dose of vaccine. Four out of five cattle inoculated with one dose and four out of five inoculated with ten doses of vaccine were protected from generalized LSD, while, all four nonvaccinated controls experienced generalized LSD following challenge inoculation, one control died. The Romanian strain of sheep pox vaccine induced sufficient cross protective immunity to protect cattle against severe LSD virus challenge. This SP vaccine has the advantage of not being a risk to contact sheep in contrast to situation, where virulent SP is used to immunize cattle against LSD.

Key words: *Lumpy skin disease- Control-Vaccination*

INTRODUCTION

Lumpy skin disease (LSD) was first described in Egypt in 1988 (Anon, 1988). In 1989, severe and widespread outbreaks caused extensive economic losses. The disease was diagnosed by clinical signs, histopathology, and virus isolation and identification (House et al. 1990).

The application of sheep pox (SP) virus for immunization of cattle against LSD was first reported in Kenya by Capstick and Coackley (1962) who used virulent SP virus. This work reports the protection afford by SP vaccine (Romanian strain) against challenge inoculation with LSD virus (LSDV).

MATERIALS and METHODS

Animals:

Fourteen bulls 18 to 24 months old were housed in insect-proof isolated units and observed for one week before starting the experiment. Ten male Barki lambs four to five months old were obtained from local flocks known to be susceptible to sheep pox.

Sheep pox vaccine:

Desiccated live SP vaccine (Romanian strain of virus), prepared in lambs as described by Sabban (1960), was obtained from the Pox Research Department, Veterinary Serum & Vaccine Research Institute, Abbasia, Cairo. The vaccine had a titer of 10^5 sheep infectious doses 50% (SID_{50}) per 0.1 ml dose as determined by titration performed by inoculating tenfold dilutions of the vaccine intradermally (ID) into susceptible lambs (Sabban 1960).

Lumpy skin diseas viruses:

The Ismailia strain of LSD virus was used for challenge inoculation; the virus was passaged three times in primary fetal bovine lung (FBL) cells. The Kenya B 2390 strain of LSDY was used in the cell culture virus neutralization test; the virus had been passage approximately 18 times in lamb testicle, lamb kidney and FBL cell cultures.

Virus neutralization tests:

(a) Neutralization index (NI) test in lambs: The NI test was performed using constant serum and variable virus dilutions using the guidelines of Boulter (1957). Briefly, 10 lambs were divided into five groups of two lambs each. Sera taken from the five experimental calves in group 1 before vaccination, at the day of challenge inoculation, and 40 days post challenge (dpc), inoculation were used to form three pools respectively. Serial tenfold dilutions of Sp vaccine virus were made and an equal amount of serum was added to each tenfold dilution of SPvaccine virus (Sabban 1960). After incubation at 37°C for one hour, 0.1 ml of each serum-virus mixture was inoculated ID, using three sites for each virus dilution. Two lambs were used for each serum pool, the control virus and the hyperimmune serum control were prepared according to Sharma and Dhanda (1971).

(b) Virus neutralization (VN) test in cell culture: The microneutralization test was performed on individual samples using two fold serum dilutions 110 $TCID_{50}$ of LSDV in FBL cells.

Experimental design:

The five bulls in Group 1 (numbers 1 to 5) were vaccinated ID in the tailfold with one dose of SP vaccine. The five bulls in Group 2 (numbers 6 to 10) were similarly vaccinated with 10 doses SP vaccine. Four bulls in Group 3 were kept as unvaccinated controls (numbers 11 to 14).

On the 40 th day post vaccination (dpv), the cattle in Groups 1, 2 and 3 were challenged by ID inoculation with LSDV following the procedure described for challenge of sheep vaccinated with sheep pox vaccine (Davies and Mbugwa 1985). Briefly, the challenged virus was diluted tenfolds from

10^{-2} to 10^{-7}. The hair was clipped at the site of inoculation and each dilution of challenge virus was inoculated ID into three sites in the neck of each animal. Dilutions 10^{-2}, 10^{-3}, 10^{-4} were inoculated on the left side of the neck, and dilutions 10^{-5}, 10^{-6}, 10^{-7} on the right side of the neck. Animals were observed daily for five weeks for clinical signs, including rectal temperature, swelling at the site of inoculation, or generalized skin lesions. Titers of the LSD lesions were calculated using the Spearman-Karber method of estimating 50% endpoints (Cotrral 1978). Sera were collected and NI and VN tests were performed as indicated in Table 1.

RESULTS

Clinical observations following vaccination of cattle.

A rise in average temperature of about 0.5°C for cattle in Group 1 was recorded eight to ten dpv. For group 2, the average temperature rise did not exceed 0.8°C between nine to thirteen dpv. No pox- type lesions were noted at the site of vaccination, although a slight thickening of the skin was observed.

Clinical observations following challenge inoculation:

Thermal reaction: The average rectal temperature of cattle in Group 1 rose about 0.6°C from five dpc, increased to 1°C by 11 dpc, and returned to normal by 16 dpc. The cattle inoculated with 10 doses of vaccine (Group 2) had an average of 0.5°C rise seven dpc. Through nine dpc with all bulls, temperatures returing to normal by 11 dpc.

In the nonimmunized control group, an average of 0.6°C rise was observed on three dpc, increasing to 1°C, six through 14 dpc, and returning to normal by 18 dpc.

Clinical signs of LSD: A firm, circumscribed nodule 5 to 7 cm in diameter, accompanied by hyperemia, cellular infiltration, and warmth, appeared at the site of inoculation of the lowest dilution of challenge virus in two bulls in Group 1 on the three dpc and in one bull in Group 2 on four dpc. By Five dpc all the bulls in Groups 1 and 2 had lesions or inflammation at the inoculation site. The lesions at the inoculation sites had subsided to the point of not being clearly detected, however, at lower dilutions (10^{-2} and 10^{-3}), only a scar-like lesions was present by 21 dpc for Group 1 and 15 dpc for Group 2.

One bull in each of Groups 1 and 2 immunized with SP vaccine developed generalized LSD, but the lesions were not as severe or extensive

as those noted in the control Group. The generalized lesions in the two affected bulls in vaccinated groups had resolved by twenty dpc.

In the control bulls, fever preceeded the development of nodules, which first appeared at the sites of inoculation six dpc. Generally, by 10 dpc extensive hyperemia surrounded the nodule, and by 20 to 30 doc, the nodule became necrotic. Eventually, thick scab formed and became hard, dry and sloughed from the tissue, often leaving a deep scar or hemorrhage followed by development of a further scab. The early nodules were circular and less than 1 cm in diameter and later achieved a maximum size of 10 cm in diameter. By 14 dpc, extensive generalized spreading was evident. was evident . The nodules were most numerous on the neck, eye lids, nostrils, around the mouth, the trunk, under the tail, ventral aspect of the body, perineum and scrotum. Nasal discharge and lacrimation were observed. The limbs were swollen with edema and cellulitis, and the animal moved with difficulty. The dewlap was edematus with gross enlargement of the prefemoral and prescapular lymph nodes. One control animal had very severe, generalized lesions and on the fifteen dpc had labored breathing, mucopurulent nasal discharge, profuse lacrimation and depression. This control animal died on twenty dpc. Gross pathological and histopathological observations were supportive for generalized LSD. The lesions in the remaining three control bulls resolved between 40 to 50 dpc.

The means of the titers of the LSD lesions following challenge inoculation recorded five to 21 dpc were $10^{4.9}$. $10^{3.3}$ and $10^{3.8}$ respectively, for groups 1,2 and 3. Using the least significant difference method, the average titer of lesions of group 2 was significantly less ($p \leq 0.05$) than groups 1 and 3.

Serological Results:

The NI for the prevaccination pool was negative (NI < 1.7 [Cottral,1978]), equal to 2.3 for the 14 dpv serum pool, and ≥ 4.3 for the one month post challenge pool. The titer of the virus control was 10^4, and the NI of the positive control serum was ≥ 4.3.

The preimmunized serum samples and the samples up to 40 dpv had no detectable VN antibody at a final serum dilution 1:4 in the microneutralization test (Table 1). Antibodies titers were detected seven dpc in the vaccinated cattle and 14 dpc for control one. The average antibody titers in both vaccinated groups of cattle were higher than that of the control one.

DISCUSSION

In this experiment, Romanian strain of SP vaccine provided protection against generalized LSD in eight of ten vaccinated cattle which were challenged with an Egyptian LSD field isolate. The febrile reaction to challenge inoculation in unvaccinated control calves (0.5 to 1°C) extended from the three to 18 dpc. Following challenge inoculation, the bulls which received one dose of vaccine were mildly febrile from five through 16 dpc, and the febrile reaction of animal receiving ten doses of vaccine were short and delayed (seven through 11 dpc), demonstrating the protective effect of the SP vaccine.

The local reaction in unvaccinated controls at the sites of challenge inoculation appeared after a febrile response and on six dpc, followed by a generalized disease typical of an active LSD infection in nonimmune cattle.

On the other hand, the local skin thickening in the vaccinated bulls following challenge inoculation appeared early (three to four dpc) and before any temperature rise. The earlier response prior to a febrile reaction may clearly reflect cell mediated immune response (CMI) to viral antigens.

The greatest response to challenge inoculation, as measured by local lesions, was observed in the control animals which had the highest titer of lesions. The viral lesions resolved with time, slowly in the control group (40 to 50 days), more rapidly in Group 1 (21 days) and most rapidly (15 days) in Group 2. There was also significant difference ($p \leq 0.05$) in the overall mean titer of lesions of Group 2 compared to Groups 1 and 3. These differences, along with 80% protection from generalized LSD in the 10 vaccinates compared to 100% generalized in the controls including the death of one control indicated that, the attenuated Romanian SP vaccine affords a significant immunity against a severe LSD challenge. The advantage of this vaccine compared to the a virulent SP virus is that, even if SP vaccine virus spread to sheep it is an attenuated strain. Virulent SP virus is used for vaccination of cattle against LSD (Capstick and Coackley, 1961; Davies, 1976); However, the virulent SP virus used as a vaccine has the potential to infect sheep and goats in contact with vaccinated cattle.

No prevaccination antibodies were detected in either the NI test done in lambs nor the NV test performed in cell culture. However, at 40 dpv the NI was 2.3 in the lamb test but negative in cell culture test. This may be due. to CMI in corporation in the invivo test. The potential importance of CMI is emphasized by the significant protection against the severe LSD challenge in the absence of virus neutralizing antibody in SP vaccine- inoculated cattle.

This study showed that the Romanian strain SP vaccine induces immunity in cattle against LSD. It further substantiates its close relationship to capripox viruses, demonstrated in cross-protection studies (Capstick, 1961; Capstick and Coackley, 1962) serologically (Davies and Otema, 1981) and in studies of their viral DNA (Black et al. 1986).

Studies are in progress on the duration of immunity to LSD infection in SP-vaccinated cattle so that recommendations can be made as to the proper time to revaccinate cattle. Field studies on the use of the Romanian strain of SP vaccine in the face of LSD epidemic are also in progress.

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VIRUS NEUTRALIZING ANTIBODY TITERS OF CALVES VACCINATED WITH ONE AND TEN DOSES OF SP VACCINE.

	Average titers[a]		
Time of sampling	Group 1 one sheep dose of vaccine	Group 2 ten sheep doses of vaccine	Group 3 control (nonimmunized)
prevaccination	< 4[b]	< 4	NT[c]
7 dpv[d]	< 4	< 4	NT
14 dpv	< 4	< 4	NT
21 dpv	< 4	< 4	NT
40 dpv =0dpc[e]	< 4	< 4	< 4
7 dpc	11[f]	11	< 4
14 dpc	177	63	66
21 dpc	349	292	80
28 dpc	304	181	23
35 dpv	474	252	191

a -Average titer of sera tested with 110 TCID50 of LSD strain B 2490.

b - No antibody detected at a final serum dilution 1:4.

c - Not tested.

d - Days post vaccination.

e - Days post challenge.

f - Titers were calculated using the Spearman-Karber method of estimating 50% endpoints and are expressed as the reciprocal of that titer.