Protection of Goats, with a Sheeppox Vaccine, Against a Virulent Field Capripoxvirus with High Affinity to Goats

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Abstract:

An attenuated vaccine incorporating the Romanian sheeppoxvirus strain is used to immunize sheep and goats in Saudi Arabia. Recently, a severe outbreak of a capripoxvirus was seen only in goats in a farm containing a mixed flock of sheep and goats. Experimental infection of sheep and goats with that virus strain confirmed its high affinity to goats.

The present study aimed at examining the efficacy of the currently used sheeppox vaccine, in protecting goats, from the virulent field capripoxvirus which caused the recent outbreak. To do so, we followed the vaccination regimen of sheep and goats, against capripox, employed in Saudi Arabia. The results confirmed that vaccination conferred solid maternal immunity against the field virus to goat kids, born to vaccinated dams, up to the age of two months. At the age of 4 months they lost their immunity.

Forty five percent of the adult goats, which were vaccinated at the age of four months, succumbed to infection when challenged with the field virulent capripoxvirus, after twelve months post vaccination.

Result of the present study, indicated that the sheeppox vaccine, currently used in Saudi Arabia, could be continued for both sheep and goats. However, it is proposed that goat kids be vaccinated at the age of three months and boostered nine months later to ensure adequate protection following the decline of maternally-derived immunity.

Key Words :
host- specific ; goatpox virus ; sheeppox vaccine ; protection;

Introduction:

Goatapox Virus (GPV) is a member of the genus capripoxvirus of the family poxviridae (Mathews 1982), which also includes the Sheep-Pox virus (SPV) and the Lumphy Skin Disease Virus (LSDV) of cattle.

The host specificity of GPV and SPV was reported to vary according to the host breed and the virus strain involved (Davies 1976; 1981; Kitching and Taylor 1985; Abu Elzein, *et al.* 2004).

According to the FAO Yearbook of Production, (FAO 1986), mutton lies on the top of the list of meat consumption in Saudi Arabia, followed by goats meat. Goats are also kept as fancy animals and could be highly expensive.

In spite of the great efforts towards increasing local production of sheep and goats in Saudi Arabia, huge numbers are still annually imported to meet the day-by-day consumption demands and to cover the requirements of Al-Hajj (pilgrimage) season.

Capripoxvirus infection in Saudi Arabia (SA) is creating great nuisance to sheep and goats keepers. Although the economical losses in both species are not studied, field observations of veterinarians indicate that losses in the younger stock are enormous.

Very little published information is so far available regarding capripoxvirus infection in Saudi Arabia (Abu Elzein *et al.* 2004).

The present study was prompted by the occurrence of a highly virulent capripoxvirus which struck specifically on goats of all ages, in the presence of unaffected sheep in the same flock.

Since sheep and goats are vaccinated with a sheeppox vaccine, in this country, we thought to examine the protection that the vaccine might confer on goats against this virulent virus which has great affinity to goats.

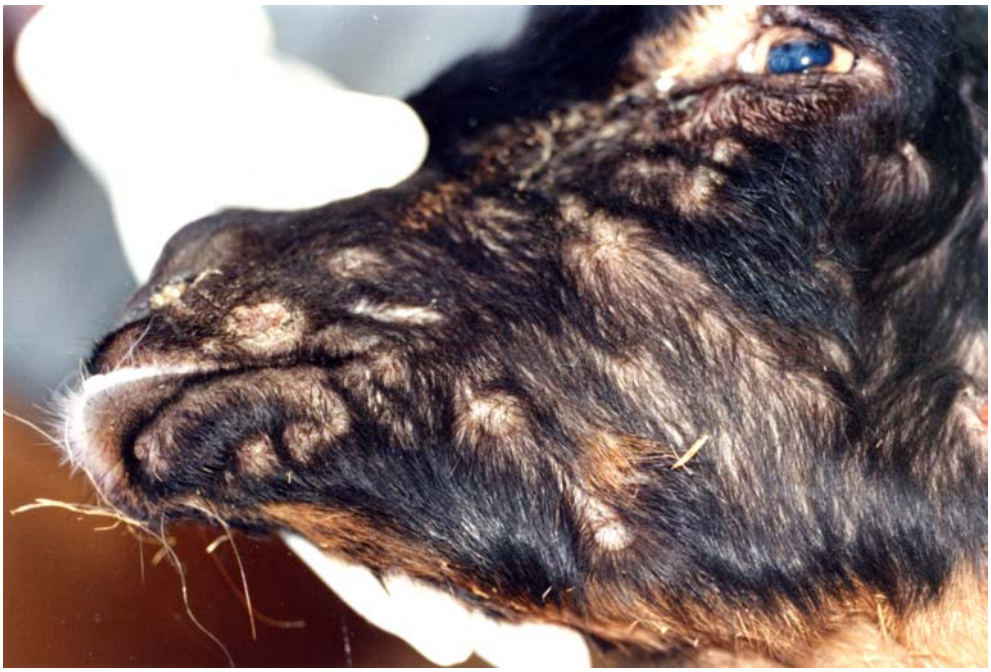
Materials and Methods

The vaccine and the challenge virus

The capripoxvaccine in SA, is formulated from a Romanian strain of sheeppoxvirus which was attenuated by 33 passages in cell culture. This

strain is widely employed in many countriers (Ramyar 1965; Sabban 1957). The dose given to a sheep or a goat is 0.5 ml, subcutaneously. It is given routinely to sheep and goats at the age of 4 months, then repeated annually (Anon 2000).

The challenge virus was a virulent field capripoxvirus which was isolated from outbreaks in goats at Al-Ahsa (Fig. 1). This virus, which was found to show great affinity to goats (Abu Elzein *et al.* 2004), was designated GPZ/SAU/1/99. The virus was used as the challenge virus and also in the cross-serum neutralization tests in this study. For the challenge experiments it was used as a 50% (W/V) suspension of scab material prepared in phosphate buffered saline PBS (pH 7.4).



Legend to Figure (1) : A naturally-infected adult goat showing generalized pox lesions caused by the GPZ/SAU/1/99 capripoxvirus strain (Abu Elzein *et al.* 2004)

Vaccination of the Dam Goats:

A sedentary herd of adult female goats was vaccinated routinely according to the policy of sheeppox vaccination regimen, as laid out by the Ministry of Agriculture, in Saudi Arabia (Anon 2002). A month later the goats were mated.

Forty kids born to the vaccinated dams were used in further experiments as described below. Great care was taken to ensure that these kids suckled ample colostrum from their dams.

The Challenge Experiments:

Twenty of the 40 kids, described above, were divided into two groups of 10 kids each (groups A and B). Kids of group A were challenged at the age of 2 month as described by Abu Elzein *et al.* (2004). Those of group B were challenged at the age of 4 months.

The remaining 20 kids (group C) were vaccinated routinely, using the sheep pox vaccine, at the age of 4 months. Sera were collected before vaccination, and at three monthly intervals thereafter.

A 12 months post vaccination, (group C) goats were bled for serum and then challenged. Two seronegative non-vaccinated goats were similarly challenged as controls. They were placed in a separate room. All the challenged animals were provided with food and water ad lib and kept under observation for clinical signs.

The Serum Neutralizing Test for Antibody Detection:

All the tested sera were heated at 56^{o}C for 30 min before testing. The micro serum neutralization test (SNT) as described by Precausta *et al.* (1979) was followed using microtitre plates, vero cell culture and F-12 medium for dilution of the sera.

The examined sera were collected from the non-vaccinated 2 and 4 months old goat kids (Groups A and B) and from group C goats. The SNT titres were calculated according to Reed and Muench (1938).

Production of Hyperimmuune sera against each of the GPZ/SAU/1/99 Virus and the vaccine strain of sheeppox in Rabbits:

Two groups of adult New Zealand rabbits, each comprising four heads, were used in the production of hyperimmune sera. One group was inoculated with a suspension of 50% GPZ/SAU/1/99 Virus of titre $\log_{10}$ 5.6 homogenized with complete Freund's Adjuvant (V/V). Each rabbit received a primary dose of 0.5 ml intramuscularly followed by a similar booster on day 15 post primary inoculation and after two weeks a third shot was given. In the second and third shots incomplete Freund's Adjuvant was used instead of the complete Freund's used in the first shot. A week following the last vaccination the rabbits were exsuanguinated, and sera were collected, inactivated at 56^{o}C for 30 min and stored at -20^{o}C until used. The other group of four rabbits were similarly inoculated with the vaccine sheep-pox virus.

Titration of the Rabbit Hyperimmune sera:

Each of the rabbit hyperimmune sera was titrated against its homologous virus in microtitre serum neutralization test (SNT) as described by Precausta *et al.* (1979). The end point titres were calculated as described by Reed & Muench (1938).

Antigenic comparison of the GPZ/SAU/1/99 Virus with the Vaccine Strain:

Cross micro SNT based on that of Precausta *et al.* (1979) was performed on the rabbit anti-sheeppox vaccine strain hyperimmune serum against both its homologous virus and the GPZ/SAU/1/99 Virus strain. Alternatively, both virus strains were reacted against the rabbit hyperimmune serum to the GPZ/SAU/1/99 Virus. The SNT titres were calculated according to Reed and Muench (1938).

The criteria employed to differentiate between the two virus strains were made according to Brooksby (1968); and Abu Elzein and Newman (1981). In this respect, the quantity called "r" value is defined as the ratio of heterologous to homologus serum titres. For the two compared viruses (r_1 and r_2) are obtained and the antigenic relationship is expressed as a percentage (R%-values) and is calculated according to Archetti & Horsfall (1950) and Ubertini *et al.*(1960) using the following formula:

$$R = \sqrt{r_1 x r_2} \quad \text{x } 100$$

Results:

Table (1) shows the results of the challenge experiments. The challenged 2-month old kids did not show any clinical signs of pox and their temperatures were within the normal range throughout the span of the experiment.

All the challenged four-months old kids, gave clinical signs typical of capripoxvirus infection. Pyrexia began on day six post inoculation, continued for five days and dropped. The maximum reached body temperature ranged from 41.30^{o}C to 41.95^{o}C. One kid was severely affected and died (mortality rate was 10%).

Challenge of the goats one year post vaccination (group C), indicated that 55% were protected and the remainder succumbed to the disease. Secondary lesions were seen but without generalization. No deaths were encountered. The ailing goats gave pyrexia that ranged from 40.07^{o}C to 41.34^{o}C with a mean of 40.65^{o}C.

The control non-vaccinated goats gave typical lesions of goatpox with generalized lesions and body temperature ranging between 41.05^{o} C and 41.6^{o}C.

The serum antibody titres of the two months old kidsat challenge ranged between 2.4 and 3.0 $\log_{10}$; while in the 4 month old the at challenge ranged between less than 0.3 and 0.6 $\log_{10}$.

Serum antibody titres of the vaccinated goats remained almost steady with a range between 2.7 and 3.0 $\log_{10}$ up to the point just prior to challenge. The two non-vaccinated sero-negative control goats gave titres of less than 0.3 $\log_{10}$.

Table (1)

Results of challenging the 2 and 4-months old non-vaccinated goat kids and the vaccinated adult goats with the virulent GPZ/SAU/1/99 virus

Age of kids and goats	No. challenged	Protection	Clinical Signs	Rectal Temperature °C	
				Mean	Range
Two months old (non-vaccinated)	10	10*/10 (100%)	-	38.64	38.35-39.22
Four months old (Non-vaccinated)	10	0/10 (0%)	++++	41.41	41.30-41.95
Adult goats challenged a year post initial vaccination	20	11/20 (9 Non-Protected)	- +	38.6 40.65	38.1-39.2 40.07-41.34

* = Number of animals protected over the total number challenged.

\- = No clinical signs.

\+ = Mild signs (No generalization)

++++ = Severe generalized clinical signs.

Antigenic relationships between the GPZ/SAU/1/99 strain and the vaccine virus:

Table (2) depicts results of r and R% values. The r value for each virus was 100, while R value was 100% for each virus strain.

Table (2)

The "r" and "R" values: for the GPZ/SAU/1/99 and the vaccine strains:

r value

Antisera	Viruses	
	Vaccome voris	**GPZ/SAU/1/99**
1. Vaccine virus	1.00	1.00
2. GPZ/SAU/1/99	1.00	1.00

R% value

Antisera	Viruses	
	Vaccome voris	**GPZ/SAU/1/99**
1. Vaccine virus	100	100
2. GPZ/SAU/1/99		100

Discussion:

The aim of this study was to understand whether the sheeppox vaccine routinely used in SA, can protect goats from the field virulent capripoxvirus GPZ/SAU/1/99,which has high host affinity to goats.

In order to arrive at a reliable conclusion with respect to the above question, it was necessary to set up experiments to examine the following:

Firstly, whether the goat kids born from vaccinated dams have protective maternal immunity; and when does that immunity wane?

Secondly, whether vaccination of seronegative goats, at the age of 4 months, will protect them until the next vaccination after 12 months as is done routinely with sheep in Saudi Arabia?

Thirdly, to examine the antigenic relationship between the sheep pox vaccine virus, used in SA, and the virulent field Goatpox Virus, GPZ/SAU/1/99, in cross-serum neutralization tests.

Results of challenging the two month - old goat kids indicated that they were protected from the virulent field capripoxvirus. These results confirmed that dams vaccinated with the currently used sheep pox vaccine could pass protective colostoral antibodies to their young (Kitching 1986).

The challenged four month old goats were non-protected and presented typical clinical signs of capripoxvirus infection, namely rise in reaching 41.95^{o}C, and presentation of the different stages of clinical capripoxVirus infection (Nyange and Machange 1983; Kitching and Taylor 1985). These results indicated complete waning of the maternal immunity at the age of four months.

Fifty five percent of the adult goats that were challenged at 12 months post primary vaccination, were protected against challenge. The remaining 45% goats were non-protected, though their lesions were milder than those of the four month old challenged goat kids and from the challenged non-vaccinated controls of the same age.

It is well documented that the level of humoral antibodies in sheep and goats to capripoxVirus is not always correlated to protection (Kitching 1986; Kitching *et al.* 1987; Carn 1993). Therefore, the immune status of a previously infected or vaccinated animal cannot be related to the titre of serum neutralizing antibodies. It was also confirmed that the current serological tests are not reliable in distinguishing between protected and non-protected animals (Carn, 1993). This indicated that immunity is predominantly cell mediated.

Although the vaccinated goats of group C had antibodies that ranged between 2.7 and 3.0 $\log_{10}$ at the time of challenge, still 45% of them showed mild disease signs. In spite of the fact that these signs were not of systemic nature, still such animals may transmit the disease to in-contact susceptible ones (Kitching *et al.* 1987).

The antigenic comparison between the GPZ/SAU/1/99 field strain and the vaccine virus indicated that both viruses had similar r values and that their R value was 100%. According to the criteria of Brooksby (1968) and Pereira (1977), these two viruses are antigenically identical.

From the fore-going it could be concluded that, although the virulent field vapripoxvirus, GPZ/SAU/1/99, showed high preference in virulence to goats under both natural and experimental conditions (Abu Elzein *et al.* 2004), still it showed identity with the vaccine strain in the cross SNT tests; and the vaccine strain could protect gaots against it.

The routine schedule of sheep pox vaccination which is implemented in sheep and goats in SA, starts at the age of four months and is repeated annually. Based on results of the present study, it was observed that the maternal immunity waned at the age of 4 months. Accordingly, we recommend that young goat kids are to be primarily vaccinated at the age of 3 months and then to be boostered after 9 months instead of 12 months. Such a regimen is expected to protect goats before the build up of a large population of seronegative animals at both the ages of four months and sixteen months. Further annual vaccinations are expected to establish a population of high herd immunity in the country.

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مقدرة لقاح جدري الغنم في صدر فيروس جدري معز حقلي شديد الضراوة نحو المعز

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الملخص :

ظهرت في منطقة الأحساء حالات وبائية ضارية لمرض جدري المعز خلال العام ١٩٩٩م وقد لوحظ بأن المرض كان شديد الضراوة نحو المعز فقط، ولم تصب الغنم في القطيع نفسه، مع العلم بان القطيع كله لم يكن محصنا ضد مرض جدري الغنم.

أوعزت هذه الملاحظة بإجراء دراسة لمعرفة مدى مقدره لقاح جدري الغنم المستخدم في المملكة في صد هذه العترة الضارية في المعز. أكدت النتائج بان أمهات المعز المحصنة استطاعت تمرير أجسام مناعية واقية لصغارها ضد الفيروس الضاري، وأن المناعة الأمية تفقد تماما عند بلوغ المواليد الشهر الرابع.

كماأكدت الدراسة بأن اللقاح يقي الحيوانات المحصنة حتى الشهر التاسع بعد التحصين . كما يقيها من الصورة المرضية ذات الانتشار الكلي في جسم الحيوان حتى الشهر الثاني عشر بعد التحصين. بينت نتائج دراسة المقارنة بين الفيروس الضاري وفيروس اللقاح بأنهما متشابهان تماما.

يوصى الباحثون بالاستمرار في استخدام لقاح جدري الغنم الحالي في المعز بعد اقتراح بتعديل في جدول التحصين. وقد تم مناقشة النتائج وأدرجت التوصيات.