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Observations on natural and experimental infection of sheep and goats with a virulent field *Capripoxvirus* with high affinity to goats

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ABSTRACT

A virulent field *Capripoxvirus* with high affinity to goats was isolated for the first time in Saudi Arabia from a natural disease involving a mixed herd of local breeds of sheep and goats. Observations on both the natural disease and the experimental studies indicated its host preference to goats. The results were discussed in relation to concerns regarding the use of the current Romanian strain of sheeppox vaccine in the country. The epidemiology of *Capripoxvirus* infection in Saudi Arabia was discussed.

Key words: virulent *Capripoxvirus*, affinity to goats, epidemiology, Saudi Arabia

Introduction

Sheep and goats are an economically very important species of livestock in Saudi Arabia. Mutton is the meat of choice in this country. Goat meat is also preferred, but to a lesser extent.

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For instance, the consumption of food animals in Saudi Arabia in 1986 (F.A.O. Yearbook of Production, Rome, 1986) was 3.8 million head of sheep, followed by 2.35, 0.53 and 0.17 million head of goats, cattle and camels, respectively.

Although some sheep and goats are locally-bred in Saudi Arabia, their numbers still fail to satisfy the increasingly high demand of local markets. In order to fill the gap between local production and the immense amount required, sheep and goats are imported annually.

Under certain religious circumstances, e.g. during Al-Hajj (a period of pilgrimage), huge numbers of sheep and goats are imported from Africa, Australia and from elsewhere in the Middle East.

From the foregoing, it could be envisaged that any disease which can specifically affect sheep and goats and which can retard their production in Saudi Arabia is indeed of vital significance, and its control and eradication would constitute a never-ending challenge. *Capripoxvirus* infection of sheep and goats in Saudi Arabia is one such ailment. Unfortunately, the published scientific work on *Capripoxvirus* infection in sheep and goats in Saudi Arabia is very scanty.

In an attempt to gain an insight into the situation of *Capripoxvirus* infection in sheep and goats in Saudi Arabia the present study was undertaken and will hopefully be followed by more work on the subject.

Goatpox virus (GPV) is a member of the genus *Capripoxvirus* of the family *Poxviridae* (MOYER et al., 2000). The genus *Capripoxvirus* also includes the sheeppox virus (SPV) and the lumpy skin disease virus of cattle.

The host range of both GPV and SPV was reported to vary according to the breeds of sheep and goats and the strain of virus. Some authors reported the existence of host-specific viruses to either sheep or goats (AL-BANA, 1978; BENNET et al., 1944; KITCHING et al., 1986). Others have reported a wide host-range for both viruses (BENNET et al., 1944; DAVIES, 1976; KITCHING et al., 1986; KITCHING and TAYLOR, 1985; MOYER et al., 2000).

In the present paper we report on a strain of *Capripoxvirus* which showed high affinity towards goats under both natural and experimental conditions. This situation has not been previously observed in Saudi Arabia.

Materials and methods

The natural disease. During January 1999 a farm containing a mixed sedentary flock of sheep and goats (65 goats and 42 sheep) was struck by a generalized skin disease. The disease was specific to goats only. The ages of both species in the flock ranged from less than one month to over four years. No history of sheep or goatpox infection, or vaccination, was reported in this flock. Generalized pock lesions were evident in goats in

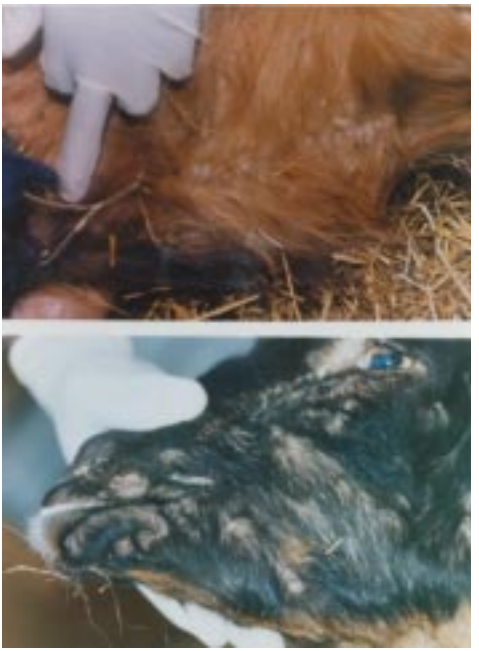

Fig. 1. Naturally-infected goats showing pox lesions

Table 1. The natural disease in the mixed flock of sheep and goats

Animal species	Total N°	Morbidity rate	Mortality rate	Age groups affected
Goats	65	100%	*11%	All
Sheep	42	0%	0%	None

* = deaths were seen in young kids only

all age groups (Fig. 1) (100% morbidity rate), whereas none of the sheep in any age group showed evidence of clinical disease.

The outbreak continued for six weeks during which 11% of goats died (Table 1). Deaths were seen only in the suckling kids (< 3 months). The lesions began as erythema which developed into papules, pustules which in turn dried to form scabs. The rectal temperature of goats in the acute phase reached 41.5 °C although none of the sheep showed thermal reaction.

Sampling. Scabs and vesicular fluid from pock lesions were collected in sterile containers and immediately transported in the cold to the laboratory where they were stored at -86 °C until used. Blood for serum was also collected.

Biopsy samples from the pock lesions for histopathology were collected and processed routinely and sections of 4-6 millimicrons were made and stained with haematoxylin eosin (HE) and periodic acid Schiff (PAS) stains.

Virus isolation. Skin biopsies were ground with sterile sand to create 20% in modified Eagles medium (MEM) without serum. Following low centrifugation at 1500 rpm for 10 minutes the supernatant fluid was collected, antibiotics were added (ABU- ELZEIN et al., 1997). The fluid was then aliquoted into 0.5 ml volumes in small sterile vials and stored at -86 °C until used in further experiments.

A 50% skin biopsy suspension was also prepared as above and stored at -86 °C until used in the experimental infection studies as described below.

The 20% tissue suspension was used to inoculate monolayers of secondary bovine testicle cell culture (SBT) and Vero cells as described by KITCHING and TAYLOR (1985). The monolayers were examined daily for presence of cytopathic effect (CPE). When CPE was detected, two further passages were made and the isolated virus was subsequently adapted to Vero cells in which it was titrated using microtitre plates as described by ABU-ElZEIN et al. (1997). The virus titre was calculated according to REED and MUENCH (1938), in tissue culture infective dose 50 ($TCID_{50}$/0.1 ml).

Virus identification. The serum neutralization test (SNT) as described by PRECAUSTA et al. (1979) was followed using 100 $TCID_{50}$ of the isolated virus reacted against an equal volume of 2-fold dilution series of sheep

hyperimmune serum produced against the vaccine strain of sheeppox virus, which is used in Saudi Arabia.

Transmission experiments. The aim of the present experiments was to establish whether the isolated field *Capripoxvirus* behaves in a similar way to that observed in natural conditions, where it showed high host preference towards goats. Two modes of transmission trials were followed: by inoculation and by contact.

Animals used. Animals were local breeds. The sheep were Najdi and Hejazi breeds. The goats were locally cross-bred (Nubian and Shami) and the dwarf southern breeds. All animals were between 5 and 12 months old, and were sero-negative in the serum neutralization test against the isolated goatpox virus.

The virus. The virus used was the field virulent *Capripoxvirus* which was isolated in the present study and was in the form of scabs collected from goats and made into 50% (w/v) suspension in phosphate buffered saline (PBS) pH 7.4, containing antibiotics (ABU-ELZEIN et al., 1997).

Transmission by inoculation. Twenty animals from each species were used in this experiment. They were inoculated intradermally as described by KITCHING and TAYLOR (1985). Groups were separated and provided with food and water *ad libitum.* Five animals were kept as controls for each group, in isolation from the inoculated ones. The inoculated animals were put under close daily clinical observation.

Transmission by contact. With the appearance of pyrexia and papules in the inoculated goats, five sheep and five goats were introduced to the inoculated goats; also, five sheep and five goats were introduced to the inoculated sheep group. All animals were kept under the daily clinical observation.

Skin biopsies were collected from those animals which showed lesions for virus re-isolation and for histopathology. Blood for serum was sequentially collected from all the animals in the experiment.

Antibody detection. Sera from the acute stage and from convalescent goats in the natural outbreak, and sera from the sheep in the same flock, were collected. Sera were also collected from all the experimental animals prior to inoculation, during the acute phase and at convalescence.

All these sera were examined in the SNT for detection of antibodies to the goatpox virus isolate.

Generally, the method of PRECAUSTA et al. (1979) was followed using microtitre plates and Vero cell culture. The SNT titres were calculated according to REED and MUENCH (1938).

Results

Virus isolation and identification from naturally infected goats. The SBT and Vero cells inoculated with the skin biopsy suspension from the naturally-infected goats, showed cell rounding on day three post-inoculation (PI). This progressed in both cell systems until day 7 PI when more than 90% of the cell sheets were destroyed. In a further two passages, complete destruction of either cell type monolayer was achieved in three days. The virus was subsequently adapted to Vero cell culture. The cell suspensions were then repeatedly frozen and thawed and stored at -86 °C until used in further experiments. This isolate was designated GPZ/SAU/1/99.

Clinical response of the experimentally-infected animals. The 20 goats in group A which were inoculated intradermally showed pyrexia between days 7 and 13 post-inoculation (PI) with a peak mean temperature of 41.9 °C on day 10 P.I. (Fig. 2).

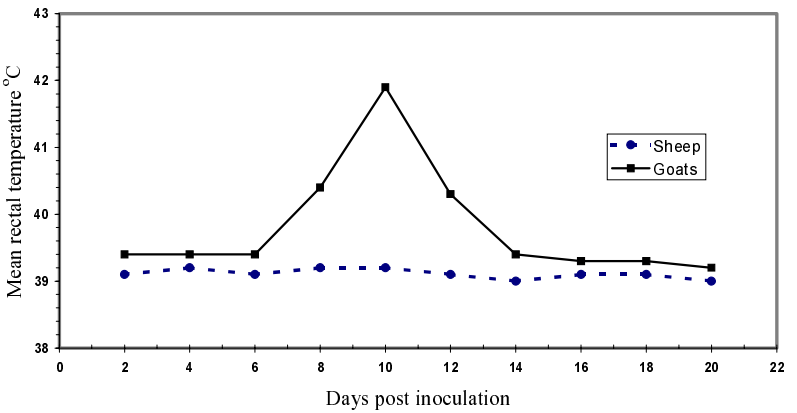

Fig. 2. Rectal temperature in experimentally-inoculated sheep and goats

The pyrexia was accompanied by erythema at the sites of inoculation, which developed into papules by day 12 PI (Fig. 3). The papule stage continued for 4-6 days, which then developed into pustules. The pustule stage continued for 6-8 days at which time secondary lesions developed. The pustules then dried into scabs, a condition which persisted for 13-15 days and after which healing occurred. Eighteen of the inoculated sheep did not show a rise in temperature or clinical signs until the end of the observations, a period of 3 months. The remaining two sheep in this group showed a mild reaction, manifested by pyrexia of 40.9 °C and the appearance of a few papules which dried out after four days with no further development.

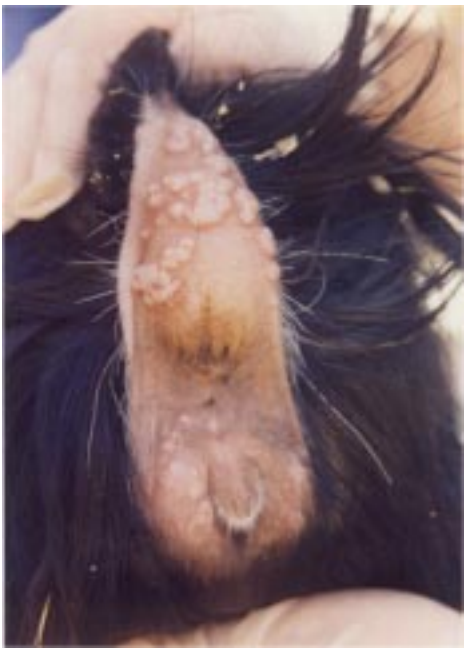

Fig. 3. Pox lesions on the tail of an experimentally-infected goat

Contact transmission experiments. Of the ten in-contact goats which were introduced to group (A) goats, seven showed pyrexia by day 7 post-contact (PC) and three showed pyrexia on day 9 PC. The pyrexia continued for five days and then fell to normal. The maximum temperature reached was 41.6 °C. One day following pyrexia, erythema was evident under the

tail and abdomen of seven goats. The remaining three goats showed similar lesions two days later. The development of the pock lesions was similar to that seen in the inoculated goats.

The in-contact sheep with group (A) goats showed no clinical signs or pyrexia until the end of the experiment, a period of three months. The control sheep and goats remained normal until the end of the experiment Table 2).

Table 2. Experimental inoculation of the sheep and goats with the GPZ/SAU/1/99 field capripoxvirus

Type of exposure	Animal species and N° used	Morbidity	Mortality
Skin scarification	Goats (20) Sheep (20)	20/20 (100%) 2/20 (10%)	3/20 (15%) 0/20 (0%)
Contact with diseased goats	Goats (10) Sheep (10)	10/10 (100%) 0/10 (0%)	0/10 (0%) 0/10 (0%)

Antibody detection. No antibodies against the isolated goatpox virus were detected in the acute phase sera of the naturally infected goats, or in the sera of sheep in the same herd. However, antibodies were detected in the sera of the convalescent naturally infected goats, with titres ranging from between $\log_{10}$ 0.9 and 1.5.

Neutralizing antibodies were detected in the convalescent sera of the goats inoculated intradermally with the virus and showed titres ranging from 1.8 to 2.4 $\log_{10}$. Sero-conversion was also detected in the in-contact goats at lower levels ($\log_{10}$ 1.2 to 1.5) and in the two inoculated sheep which showed mild clinical signs. No neutralizing antibodies were detected in the sera of the remaining 18 inoculated sheep, or in the in-contact sheep with the experimentally infected goats.

Histopathology. Sections prepared from the soft nodules exhibited varying degrees of epidermal hyperplasia characterized by acanthosis, hyperkeratosis and swelling of acanthotic cells. Marked congestion and edema were seen in the dermis and subcutis. Lymphocytes, plasma cells and large macrophages were seen infiltrating the dermis. (Fig. 4).

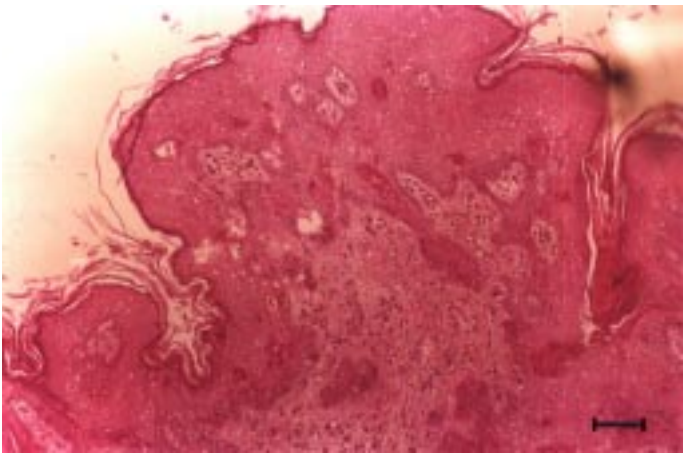

Fig. 4. Skin, goat: Experimental hyperplasia with marked acanthosis and cell swelling. H&E ×100.

The firm nodules mainly showed epidermal hyperplasia, with cellular edema, acanthorrhesis and acantholysis. Underlying dermis and subcutis

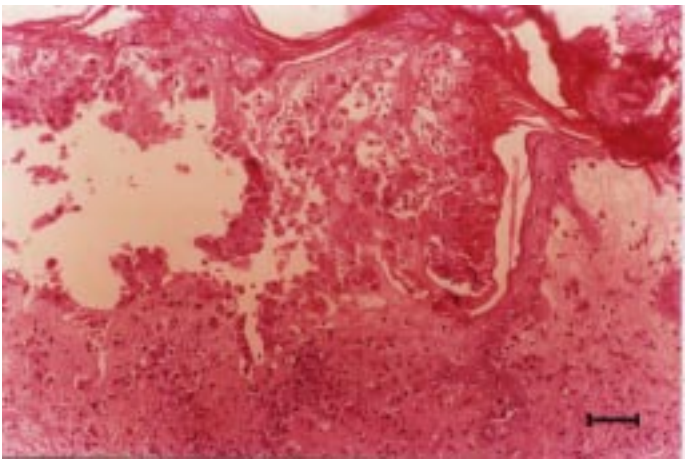

Fig. 5. Skin, goat: Epidermal hyperplasia with acantholysis and acanthorrhesis. H&E ×200.

were intensely infiltrated by inflammatory cells, as previously described, with vasculitis, congestion and occasional haemorrhages; the perivascular cellular infiltration in many places almost effaced the vessel wall. (Fig. 5).

Eosinophilic cytoplasmic inclusions could be seen in large dermal cells with vesiculated nuclei and marginated chromatin (Fig. 6).

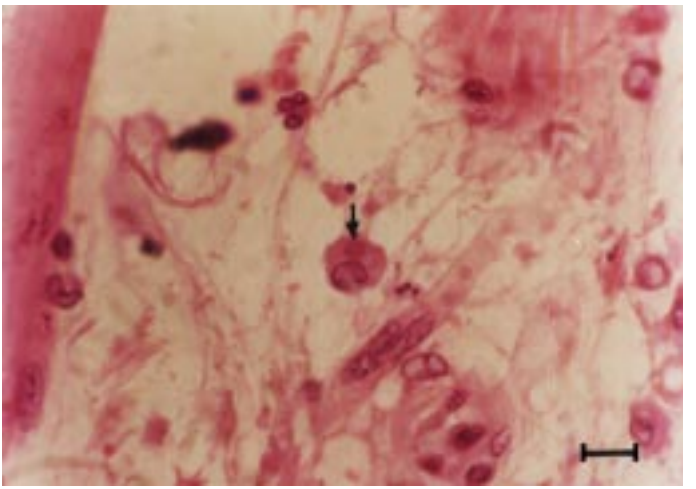

Fig. 6. Skin, goat: Eosinophilic intracytoplasmic inclusions in large dermal cells with marginated nuclei chromatin. H&E ×1000.

Discussion

The clinico-pathological and virological investigations in the present study confirmed that the disease outbreak was due to a *Capripoxvirus* infection. Indeed, this is the first *Capripoxvirus* to be isolated from goats in Saudi Arabia.

This virus strain showed zero percent morbidity to sheep under both the natural and the in-contact experimental conditions with diseased goats. However, 10% of the experimentally-scarified sheep developed a mild form of the disease which ended in the papule stage, without further developments (Table 2).

In the light of the above and from previous clinico-pathological observations (Dr. A. Gameel - personal communication) it appears that outbreaks of *Capripoxvirus* infection involving only goats are common in Saudi Arabia. Nevertheless, clinico-pathological *Capripoxvirus* infections involving only sheep, in the presence or absence of goats, were also seen (Dr. A. Gameel - personal communication).

Despite the fragmentarily gathered observations on the clinical *Capripoxvirus* infections of sheep and goats in Saudi Arabia, no epidemiological study has thus far been conducted in order to understand the peculiarities of the disease. For instance, it is not yet known whether the Saudi *Capripox* field virus strains infect both species, or whether there is some degree of host-preference or host-specificity. On the other hand, although a locally produced live sheeppox vaccine (incorporating a Romanian strain of sheeppox) is used in both sheep and goats in Saudi Arabia, it remains unknown whether it protects goats against virulent goatpox virus infections (Dr. H. Al-Khalaf – Head of the Vaccine Production Unit, Riyadh, Saudi Arabia - Personal communication).

With the influx of imported sheep and goats from various countries into Saudi Arabia it is anticipated that some *Capripoxvirus* strains could be introduced. The epidemiological significance of such strains could be of particular interest. This is because *Capripoxvirus*es vary in their pathogenecity to the different breeds of sheep and goats. For example, certain strains were found to be equally pathogenic for both species (BENNET et al., 1944; DAVIES, 1976; NYANGE and MACHANGE, 1983). A second group was reported to infect both species but with preference to one species rather than the other (AL-BANA, 1978; KITCHING et al., 1986; KITCHING and TAYLOR, 1985). A third group showed host-*s*pecificity to either sheep or goats (ELZEIN et al., 1983; KITCHING and TAYLOR, 1985; ABU-ELZEIN et al., 1997; BENNET et al., 1944). From the above it is felt therefore that a long-term study on *Capripoxvirus* infection in sheep and goats in Saudi Arabia must be conducted. This could entail a complete epidemiological study of the disease outbreaks, virus isolation, pathogenicity study on the various breeds of sheep and goats, and formulation of a potent vaccine to protect against the disease in both animal species.

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SAŽETAK

Virulentni terenski soj *Capripoxvirusa* s visokim afinitetom za koze izdvojen je prvi put u Saudijskoj Arabiji iz prirodno oboljelih mješovitih stada lokalnih pasmina ovaca i koza. Promatranje prirodno i pokusno izazvane bolesti pokazalo je da su koze prirodni domaćin te da ovce nisu oboljevale. Rezultati su razmatrani u odnosu na vakcinaciju protiv ovčjih boginja rumunjskim vakcinalnim sojem. Razmatrana je epidemiologija infekcije *Capripoxvirusom* u Saudijskoj Arabiji.

Ključne riječi: virulentni *Capripoxvirus*, afinitet za koze, epidemiologija, Saudijska Arabija