

Induced udder orf infection in sheep and goats

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ABSTRACT

Typical orf lesions were successfully produced (experimentally) on the udder dermis of lactating sheep and goats. The animals were subjected to daily clinical examination and the developing lesions were monitored and recorded. The lesions involved were erythema, papules, pustules and firm irregular scabs on the skin of the udders. One goat developed unilateral moderate udder fibrosis and two of the sheep developed bilateral similar lesions. Examination of milk samples from infected animals by California Mastitis Test (CMT) and Mastitis Yellow Card Test was found positive for mastitis. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus mirabilis* and *Arcanobacterium pyogenes* were recovered from the milk of affected animals. All the infected animals were sero-converted to orf infection and produced neutralizing antibodies. The causative orf virus was re-isolated from the scabs of their lesions. The bionomics of the disease was discussed.

Key words: orf virus, sheep, goats, experimental infection

Introduction

Orf or scabby mouth is a skin condition of sheep and goat caused by the orf virus, a member of the family *Poxviridae*, genus *Parapoxvirus* (ROBINSON and BALASSU, 1981). The disease has a worldwide distribution and occurs wherever sheep and goats are raised. Primary sites of infection are lips, mouth and to a lesser extent, other sites such as nostrils, ears, udders and the coronary bands. Udder and teat orifice orf infection, in sheep and

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goats, are always associated with an increase in the number of deaths among orf infected kids and lambs.

Mastitis of sheep and goats is often described to occur in individual animals or sometimes appears in the form of an outbreak (SCOTT and MURPHY, 1997). Losses in dairy goats' production, as a result of mastitis, in Saudi Arabia have increased over the last ten years.

The present study reports clinico-pathological examination of experimentally induced udder orf infection in lactating goats and sheep.

Materials and methods

Twenty lactating animals were used in the study; 10 Najdy sheep and 10 Ardy goats. All the animals were two years old and had not been exposed to orf disease before. The animals were randomly divided into four groups, A, B, C and D; each consisted of five animals. Groups A and B were used for experimental infection with the virus. Group C and D were kept as controls. Each group was kept in a separate unit. Food and drinking water were available *ad libitum*.

Virus inoculum and experimental infection. A 20% suspension of goat udder scab material from a natural orf outbreak in sheep and goats, was made in phosphate buffer saline PBS (pH 7.4), containing penicillin/streptomycin (1,000 unit/mL) and mycostatin (50 IU/mL (HOUSAWI et al., 1993). All animals were scarified in a single line fashion on different sites around the udder. Each animal received 30 scars. The scab inoculum was applied to the scarified sites in animals of group A and B and phosphate buffer saline was applied on scarified sites of the control animals of groups C and D. The experimental procedures were carried out in accordance with the directions of the Animal Ethics Committee, College of Veterinary Medicine, King Faisal University.

Rectal temperature was recorded for every animal from day zero and until the end of the experiment.

Milk sampling. Milk samples were collected from the sheep and goats, two weeks post-infection (p.i.). Three collections were made at an interval of two days. The teat of the udder was cleaned with 70% alcohol. The first few milk streams were discarded and milk from the mid-stream was collected in sterile containers and divided into two portions. The first portion was used directly for the California mastitis test (CMT) and yellow card test. The other portion was sent for immediate bacteriological examination (SENA et al., 2000; KHALIQ et al., 2001).

Virus isolation. Twenty percent of the collected scab materials from infected udders were prepared as described previously and used to inoculate primary monolayers of lamb testicle cells, in which two passages were made, followed by further two passages in Vero cell monolayers (HOUSAWI et al., 1998; HOUSAWI and ABU ELZEIN, 2000).

Identification. Reference rabbit anti-orf hyperimmune serum (HOUSAWI et al., 1991) was used in both the agar gel immunodiffusion (AGID) (HOUSAWI and ABU ELZEIN, 2000) and the serum neutralisation tests (SNT) (TRUEBLOOD et al., 1963; HOUSAWI et al., 1993). Rabbit non-immune serum was used as a negative control for the above two tests.

Results

Gross lesions. All the infected animals developed and showed classical orf lesions at the dermis of the udder (site of infection). The lesions were seen as erythema, papule-pustules and firm irregular scabs. The mean incubation period was 4 days. The erythema, papule and pustule stage took 9-10 days and the scabs developed and remained intact for 13-17 days. (Figs 1-3).

The infected animals showed a mean rectal temperature of 40 °C. No deaths were encountered. One of the infected goats developed unilateral moderate udder fibrosis and two of the infected sheep developed slight to moderate bilateral udder fibrosis and an udder fistula was noted in one sheep. No treatment was given to the animals.

Staphylococcus aureus, *Staphylococcus epidermidis* and *Proteus mirabilis* were recovered from the milk of the infected sheep. *Arcanobacterium pyogenes*, *Staphylococcus aureus*, *Staphylococcus* spp. were isolated from the milk samples of the goats. When the samples examined by the California mastitis test (CMT) and yellow card test, they were found to develop persistent gel-like in the CMT and produced pH ranging between 8.5-10.5 in the yellow card test. All the inoculated goats had evidence of clinical and sub-clinical mastitis (Table 1). The mean course of the disease ranged between 25 to 33 days with a mean of 29. 2 days.

Table 1. Recovered bacteria from the milk samples of experimental animals

Animal group	Species	Isolated bacteria	Field experimentation test	
			CMT	Yellow card pH (alkali)
Group A	Najdi sheep	<i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i> <i>Proteus mirabilis</i>	5/5	5/5
Group B	Ardy goats	<i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i> <i>Arcanobacterium pyogenes</i>	5/5	5/5
Group C	Najdi sheep (controls)	No growth	- 0/5	- 0/5
Group D	Ardy goats (controls)	No growth	- 0/5	- 0/5



Fig. 1. Papulo-pustular stage 8 days post-infection of experimentally infected Najdi sheep



Fig. 2. Early sign of eruptive scabs on the udder of lactating Ardy goats 11 days post-infection



Fig. 3. Recovery of the experimentally infected sheep, however, note sign mark of udder fistula

Antibody response. Examination of serum for the presence of antibodies showed that all the infected sheep and goats sero-converted to orf virus. All the five infected sheep showed a similar pattern of serological response (Fig. 4). A gradual increase in the neutralising antibody titre was noted to start from day 7 post-infection.

The infected goats produced a similar neutralization antibody profile, three goats produced 1: 512 titre and the other two goats produced 1:256. It is interesting to note that one goat (goat No. 4), which produced the highest SNT titre, also produced the highest proliferative eruptive udder lesions, thus correlating between the severity of the lesions developed and the higher humeral antibody response of the affected animal. The control sheep and goats neither showed clinical signs nor seroconverted.

The mean rectal temperature for both groups of the infected sheep and goats was $40^{\circ}\text{C} \pm 0.5$, while that for the controls was $38^{\circ}\text{C} \pm 1$.

Virus re-isolation. The inoculated primary lamb testicle cells (PLT) started to develop a cytopathogenic effect (CPE) from day three post-infection (PI). The CPE appeared as cell rounding. The completion of the CPE and destruction of the whole monolayer cells occurred by day nine PI.

Virus identification. Udder scab material from the infected goats gave a precipitation line, against the hyperimmune orf serum, which completely merged with a line produced by the concentrated virus from the cell culture isolated virus, to make a line of complete identity.

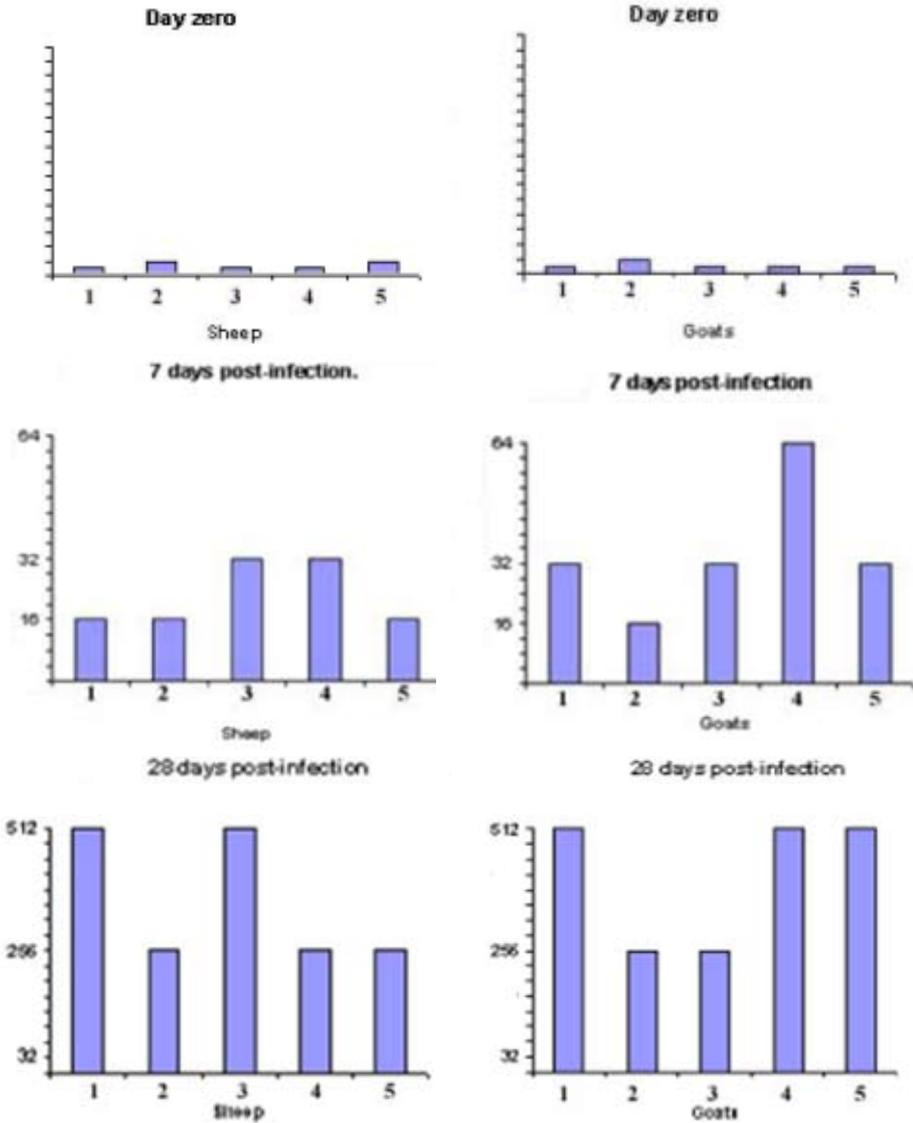


Fig. 4. Antibody titres in infected sheep and goats determined by serum neutralisation test

In the neutralisation test, the anti orf hyper-immune serum completely neutralised the viral infectivity, while the non-immune control serum failed to do so, confirming that the isolated agent from the dermis of the affected sheep and goats was an orf virus.

Discussion

Development of clinical udder orf lesions was accomplished after experimental infection. The incubation period and the clinical signs observed in the udder dermis of the sheep and goats in the present study were found to have no difference from those reported elsewhere, involving either the skin of the mouth or flank region or thigh (BUDDLE and PULFORD, 1984; HOUSAWI et al., 1993; ZAMRI et al., 1993; NETTLETON et al., 1996).

It worth mentioning that no previous experimental study has simultaneously investigated the effect of udder orf infection in sheep and goats.

The gross lesions seen on the udder of the sheep (group A) in the present study were similar in severity to those observed in the goats (group B), in every way nonetheless, one goat developed moderate unilateral udder fibrosis whereas two sheep produced slight to moderate bilateral udder fibrosis in group B.

SCOTT and MURPHY (1997) investigated the occurrence of Staphylococcal dermatitis outbreak in lactating ewes; the disease involved about sixty-eight percent of the ewes. They were of the opinion that ewes bedded in deep bare straw causing superficial skin trauma, is the main key factor which leads to the occurrence of the disease. However, we believe that the scarification of the udder, made in the present work, could have created similar suitable conditions for isolated bacteria (Table 1) to invade the udder of the sheep and goats. Moreover, the orf virus is well known to possess genetic capability that subverts key components of the host protective immune response (HAIG et al., 1996). This might have helped the bacteria to gain access to the teat canal, influencing the incidence of clinical and sub-clinical mastitis.

The general antibody profile of infected sheep and goats was similar. It was interesting to note that the response of some animals, within the group, was quicker than others, in particular goat No. 4 and sheep No. 3, which produced a higher antibody response in the first 3 weeks post-infection, but the peak of neutralizing antibodies for both species was detected by day 28 PI. Control sheep and goats did not seroconvert.

The gradual increase in the neutralizing antibodies detected in the serum of orf infected sheep and goats in the present study, was not surprising and it is in accordance with the findings of DUBEY and SAWHNEY, (1979); HOUSAWI et al., (1993) who demonstrated increase in antibody titre post orf infection.

In conclusion, the present work confirms the following points: firstly, it supports the previous suggestion that udder orf lesions have a direct contribution to increasing the rate of clinical and sub-clinical mastitis among infected ewes and goats (ROBINSON and

BALASSU, 1981; LEWIS, 1996). Secondly, orf disease poses a serious problem to lambs and kids, preventing them from suckling and thus causing starvation. Thirdly, mastitic orf lesions can become complicated, leading to amputation of the udder in severe cases or death of the affected animals.

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SAŽETAK

Pokusna infekcija virusom orf za vrijeme laktacije očitovala se je tipičnim promjenama na koži vimena u ovaca i koza. Životinje su bile dnevno klinički promatrane, a sve uočene promjene zabilježene. Promjene su se očitovale pojavom eritema, papula, pustula i čvrstih nepravilnih krasta na koži vimena. U jedne koze razvila se jednostrana umjerena fibroza vimena, a u dvije su se takve promjene razvile obostrano. Pretraga uzoraka mlijeka kalifornijskim mastitis testom i mjerenjem pH pokazala je da se u zaraženih životinja razvio mastitis. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus mirabilis* i *Arcanobacterium pyogenes* izdvojeni su iz vimena zaraženih životinja. U svih zaraženih životinja dokazana su neutralizacijska protutijela za virus orf. Virus je bio ponovo izdvojen iz krasta nastalih nakon pokusne infekcije.

Ključne riječi: virus orf, mastitis, koza, ovca
