Ulcerative balanitis and vulvitis of Dorper sheep in South Africa: a study on its aetiology and clinical features

A Kidanemariam[a], J Gouws[b], M van Vuuren[b*] and B Gummow[c]

ABSTRACT

Ovine ulcerative balanitis and vulvitis in sheep of the Dorper breed has been observed in South Africa since 1979. Its aetiology has not been conclusively resolved, and there is some discrepancy in descriptions of its clinical features. In order to identify the pathogenic microorganism/s that contribute to the occurrence of the disease, the microflora in the genital tracts of both clinically healthy and affected sheep were isolated and compared. Bacteriological examination of materials from affected and unaffected sheep resulted in the isolation of *Arcanobacterium pyogenes* from 44.2 % and 17.2 % of them respectively. This difference is statistically significant ($P < 0.01$). Seventy-four per cent of the isolates originated from severe clinical cases. Mycoplasmas were isolated from 49.3 % of 116 clinically normal sheep and 78.2 % of 104 affected sheep. There were significant differences in their rates of isolation in clinical groups ($P < 0.05$). Of all the mycoplasma isolates, *Mycoplasma mycoides mycoides* large colony variant (*Mmm*LC) was isolated from 61.5 % of clinically diseased sheep while 6.0 % of the isolates were from apparently healthy animals ($P < 0.05$). The study threw light on the prevalence of mycoplasmas in the genital tract of apparently healthy sheep and, at the same time the identity of the mycoplasma pathogen associated with ulcerative balanitis and vulvitis was revealed. The findings of this investigation therefore confirmed the involvement of mycoplasma, particularly that of *Mmm*LC large colony, in the disease in Dorper sheep in South Africa, and it was concluded that this microorganism is an important pathogen of balanitis and vulvitis in them. The study furthermore demonstrated a probable synergism between *A. pyogenes* and *Mmm*LC. Finding these 2 organisms together occurred 53.4 times more frequently in the affected sheep than in the unaffected, which emphasises the probable multifactorial nature of the disease. The association between age and the presence of clinical signs was statistically significant. It was found that young sheep were more likely to have lesions than adult sheep. Clinical observations showed that the typical ulceration appears to be confined to the glans penis and lips of the vulva; no ulceration was observed on the shaft of the penis and prepuce or vaginal vestibule. In uncomplicated cases inflammation of the prepuce and vaginal vestibule is not a regular feature of the disease. Therefore the names ulcerative balanitis and vulvitis most accurately describe the nature of the disease in South Africa.

Key words: *Arcanobacterium pyogenes,* balanitis, Dorper sheep, *Mycoplasma mycoides mycoides* large colony, South Africa, vulvitis.

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INTRODUCTION

Ulcerative balanoposthitis and vulvovaginitis of sheep is a venereal disease characterised by erosion and ulceration on the glans penis and vulval labiae. Although it has been recognised in South Africa since 1979[36,37], publications dealing with it have only appeared during the last decade. Sheep of the Dorper breed appear to be the most frequently affected [4,15,36]. The distribution of the disease in South Africa, in general, corresponds with that of Dorper sheep and therefore tends to be restricted to the dry areas of the Northern Cape, Western Cape, Eastern Cape, KwaZulu-Natal and Free State provinces[15].

Ulcerative conditions of the genitalia of sheep have been described in Australia, Canada, Britain and India[2,7,11,12,19,21,37], and in goats in India, New Zealand, Australia and Nigeria[6,14,16,31,33]. Although no infectious agent or agents have been consistently isolated[24,37,38], there is some evidence that conventional bacteria[3,36], *Ureaplasma* spp.[2,11,27,28] and *Mycoplasma* spp.[7,11,21,25,26,36] may be involved and have been recovered in some cases from clinical specimens.

Several authors have intimated that the disease only occurs in ewes as no mention was made in their publications of the simultaneous involvement of associated rams[2,11]. On the other hand, others have described the occurrence of the disease in both ewes and rams running together[4,8,12,36,38].

Since the first report on the occurrence of the disease in South Africa, issues relating to the epidemiology, aetiology and control have not been adequately addressed. However, several investigators were of the opinion that ulcerative balanoposthitis and vulvovaginitis could be associated with a *Mycoplasma* sp. infection[4,15,36].

The principal objectives of this study were to determine whether ulcerative balanoposthitis and vulvovaginitis in South Africa is a multifactorial disease in which the major causative organism is a mollicute, and whether the disease manifests mainly in sheep of both sexes under the age of 36 months.

MATERIALS AND METHODS

Study area

The study was conducted between September 2001 and March 2002 in 4 districts, namely Namaqualand, Hay, Barkly West and Hopetown, in the Northern Cape Province, South Africa and Beaufort West in the Western Cape Province (Fig. 1). A total of 15 sheep farms (Table 1) in these 5 districts was visited to study the clinical features of the disease and to collect specimens for microbiological investigation.

Study animals

The study focused exclusively on Dorper sheep, as this breed is the one primarily affected by the disease in South Africa. It is a breed with a white body and a black head (Fig. 2). Fat deposits on the rump, tail and brisket are absent. Under good management conditions mature rams can weigh 82–91 kg and ewes

[a]National Animal Health Research Centre, PO Box 04, Sebeta, Ethiopia.

[b]Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Private Bag X04, Onderstepoort, 0110 South Africa.

[c]Department of Production Animal Studies, Faculty of Veterinary Science, Private Bag X04, Onderstepoort, 0110 South Africa.

*Author for correspondence.
E-mail: moritz.vanvuuren@up.ac.za

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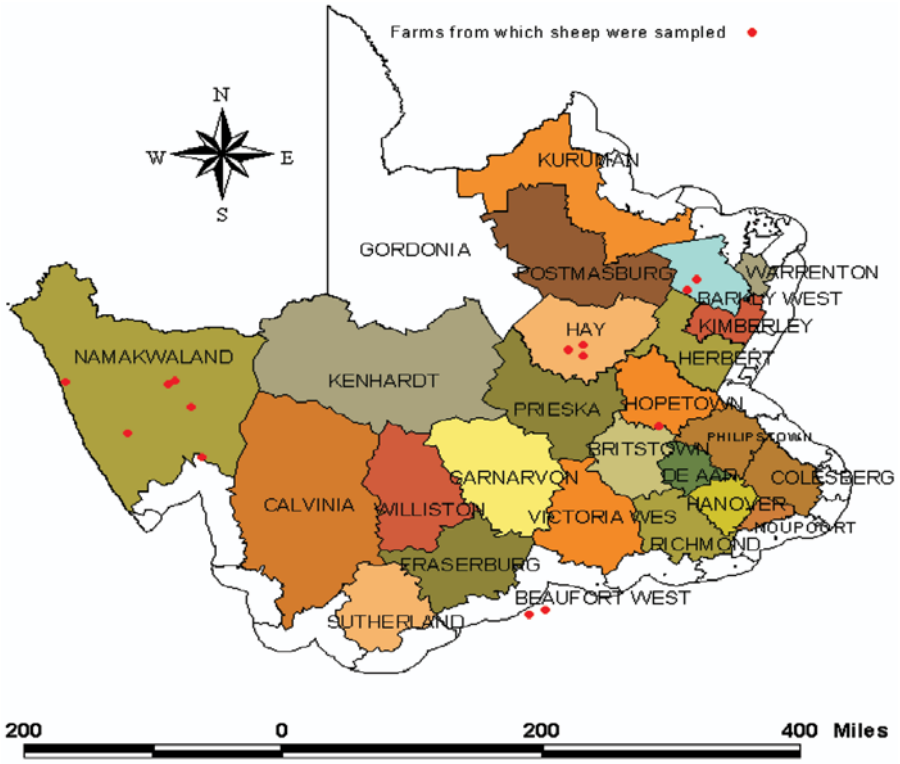

Fig. 1: **Map of the Northern Cape Province indicating the study sites.**

54–63 kg. Lambs can reach a live weight of 34 kg at about 6 months of age[10]. Where possible the number of animals sampled on each farm was at least 5 affected and 5 healthy for each sex group. The age of each animal sampled was estimated according to the number of temporary and/or permanent incisor teeth. Thus sheep with no permanent incisors were judged to be less than 12 months of age, those with 2, 4 or 6 permanent incisors to be 12–14, 18–20 or 26–30 months of age respectively, and the sheep with 8 were considered to be adults. In addition a record was made on the presence or absence of the lesions associated with the disease and of the location and severity of the lesions in those sheep showing them.

Study design

The study was designed as a case-control study in which diseased animals (cases) and non-diseased animals (controls) were selected and compared in terms of the presence of the risk factor[34]. The data were recorded on a standard data sheet before they were entered into a computer database.

The variables analysed included temporal and spatial data, population variables such as age, sex and disease status and microbiological data. A dichotomous variable with either yes (Y) or no (N) as an entry was used to denote the disease status of each animal sampled and to describe if certain microorganisms were isolated from the genital swabs examined.

Table 1: **Coordinates of the selected study sites in each district.**

District	Farm	Coordinates*
Namaqualand	Grootvlei	30°12′41″S, 17°47′34″E
	Matjiesfontein	30°31′41″S, 18°37′58″E
	De Beers	29°35′00″S, 17°05′58″E
	Smorgenskadu	29°30′S, 18°15′E
	Grootkou	29°34′19″S, 18°18′48″E
	Dooddrink	30°35′S, 18°45′E
	Koppieskraal	29°55′S, 18°25′E
Hay	Tolo	22°43′S, 29°08′E
	Dam	22°54′S, 29°05′E
	Gladiam	22°54′S, 29°12′E
Barkly West	Melkvlei	28°24′S, 24°21′E
	Hondevlei	28°17′S, 24°09′E
Strydenburg	Kortkop	30°07′45.2″S, 23°43′32. 4″E
Beaufort West	Bellevue	22°24′35.6″S, 32°28′10″E
	Springfontein	32°30′17. 8″S, 22°17′4.01″E

*S = south latitude, E = east longitude.

Specimen collection

Genital swab specimens were collected from both clinically normal and diseased sheep using commercial sterile cotton swabs. A total of 220 vulval and penile swabs consisting of 116 and 104 swabs from healthy and clinically affected sheep, respectively, were examined. The methods used for taking swabs, and procedures for the detection and identification of microorganisms, have been described in detail by Kidanemariam (2003)[22].

Specimens were obtained from the vulvae by spreading the vulval lips, inserting a swab and rubbing it back and forth gently before removing it. Swab samples from rams were obtained by gentle rubbing of the glans penis and preputial mucosa after manual extrusion of the penis from the prepuce.

Swabs were placed into cryovials containing transport medium appropriate for the different selected microorganisms. The transport media included Hayflick's broth for mycoplasma isolation, brain heart infusion broth for isolation of bacteria, and phosphate buffered saline for viral isolation. Transport medium was not used for swabs to be used for *Chlamydophila* antigen detection. The cryovials were placed in liquid nitrogen and transported to the laboratory where they were stored at –85 °C until processed.

Isolation and identification of bacteria

Swabs were streaked onto plates containing Columbia blood agar (Difco) enriched with 5–6 % horse blood which were then incubated in an atmosphere of 5 % CO_2 at 37 °C for 24 hours, with further re-incubation for 36–72 hours if no growth was observed. Single colonies of the different colony types recognised on each plate were selected and re-inoculated onto plates containing blood agar and MacConkey (Difco) agar. This was repeated until pure growth was obtained. Smears of the bacterial cultures were stained by Gram's method for light microscopy and the cultures were subjected to the catalase and oxidase tests and oxidation-fermentation (O-F) reactions. Cultures that conformed to certain criteria were subjected to additional biochemical tests.

Biochemical characterisation of isolates was done using conventional methods that included a panel of sugars, and 3

Fig. 2: **A flock of Dorper sheep photographed near Beaufort West.**

commercial analytical systems, API 10S (bioMérieux), Microbact 12A and 12B (Medvet Science Pty. Ltd.) and API Coryne (bioMérieux).

Isolation and identification of mycoplasmas

The standard growth media used for the propagation of field isolates and reference strains of *Mycoplasma* were Hayflick's agar and broth, Chalquest agar and broth, ureaplasma agar and broth, and rabbit meat infusion broth. The latter was also used to grow *Mycoplasma* reference strains that were used as antigens to inoculate adult white New Zealand rabbits for the production by standard means of hyperimmune sera for the indirect immunofluorescent antibody test.

Thawed swab specimens were both streaked on plates containing mycoplasma and ureaplasma media and used to inoculate the broth media. These were incubated in a moist chamber at 37 °C in an atmosphere containing 5 % CO_2. Evidence of growth in broth medium was monitored by turbidity of the medium. When satisfactory growth had been obtained, aliquots of broth culture were inoculated onto mycoplasma agar plates. All plates were examined daily or on alternate days, using a dissection microscope at ×20–40 magnification. The colonies were subsequently identified by the indirect immunofluorescent antibody test using the antisera produced in the rabbits.

The reference *Mycoplasma* spp. used as antigens for the production of positive serological controls were 8 reference *Mycoplasma* spp. supplied by the National Collection of Type Cultures, Central Public Health laboratory, London, UK. These comprised *Acholeplasma laidlawii* (10116 PG8), *Mycoplasma bovigenitalium* (10122 PG11), *Mycoplasma arginini* (10129 G230), *Mycoplasma* species group 7 (10133 N29), *Mycoplasma mycoides capri* (10137 PG3), *Mycoplasma capricolum* (10154 California kid), *Mycoplasma mycoides mycoides* LC type (11706 Y-goat) and *Mycoplasma agalactiae* (101223 PG2), and were grown in rabbit meat infusion broth.

For the indirect staining procedure employed in this study, agar blocks containing colonies *in situ* were used. Rabbit antiserum was used as the primary antibody, followed by addition of fluorescein-conjugated goat anti-rabbit IgG (The Binding Site, Birmingham, UK) as secondary antibody. Phosphate buffered saline (PBS) at pH 7.2 was used as a diluent for the conjugate, antisera and Evans' blue, and PBS with 0.5 % Tween twenty was used for washing.

Fluorescence microscopy was achieved with the use of a binocular microscope equipped with an epi-fluorescent attachment and Osram HBO 100 W/2 high-pressure mercury vapour lamp (Nikon corporation, Japan). A green excitatory filter (G 2A) and yellow barrier filter were used. The microscope was fitted with a photomicrographic apparatus (Model UFX-DX).

Isolation of viruses

Primary and secondary lamb foetal kidney (LFK) cell cultures were used in an attempt to isolate a virus or viruses. The LFK cells were grown in Eagles' Minimal Essential Medium (MEM) with Earle's salts supplemented with 5 % heat-inactivated foetal bovine serum (FBS). Gentamicin was added to the medium at a final concentration of 50 μg per mℓ.

Chlamydophila antigen detection

The Clearview® Chlamydia MF (Clearview®, Unipath Limited, Bedford, UK) test kit was used in an attempt to detect *Chlamydophila* antigen from genital swabs collected for this purpose from the sheep.

Although, the test was developed to detect *Chlamydia trachomatis* antigen from human genital swabs, it has been shown that it also detects *Chlamydophila psittaci* subspecies *ovis* antigen. It provides a simple direct detection assay which is highly sensitive, specific and rapid.

Data analysis

All relevant data generated in the study were recorded in a data-capturing format and entered into a computer database for subsequent analysis. The statistical package used to store and analyse the data was EpiInfo 2000 version 1.0 (Centers for Disease Prevention and Control, Department of Health and Human Services, USA). The variables to be assessed were included in an EpiInfo questionnaire and were analysed using the ANALYSIS and STATCALC facilities of the EpiInfo software. Descriptive statistics were employed to designate the different types of microorganisms encountered in both the healthy and clinically infected sheep. The association of each isolate (risk factor) with the disease process was assessed using an odds ratio. The differences were analysed using the chi-square test, and the levels of significance were taken as $P < 0.05$ and confidence intervals (CI) were set at 95 %.

RESULTS

Clinical observations

Early signs of vulvitis were characterised by swollen and reddened vulvae visible at a distance in short-docked Dorper ewes (Figs 3, 4). In some ewes the swelling was accompanied by the development of discrete mucosal ulcers at the mucocutaneous junction of the vulval labia.

On closer examination, shallow, blister-like wounds covered with scabs could be found. No lesions were observed in the vestibule of the vulva/vagina.

On manual withdrawal of the penis of affected rams, the characteristic lesions could readily be seen involving the soft glans of the penis (Figs 5, 6). This comprised the presence of 1 of more discrete ulcers on the glans penis, each of which had a sharply defined edge. Hyperaemia

of the glans penis was invariably present. The rest of the penile tissue and preputial mucosa appeared unaffected.

Isolation and identification of bacteria

Bacteria isolated from healthy and affected sheep

The microbiological flora of 104 clinically affected genitals of both ewes and rams from sheep in 15 flocks was compared with that of 116 clinically unaffected genitals. A total of 20 species of bacteria was identified from the 220 specimens. Only 6 (2.7 %) of the 220 samples showed no growth of bacterial organisms. The isolates were usually mixed populations and only 8 specimens yielded pure cultures of *Arcanobacterium pyogenes.* A summary of the bacteria isolated from the genital swabs of the sheep is shown in Table 2.

Arcanobacterium pyogenes was isolated from sheep affected with balanitis or vulvitis significantly more often than the other species of bacteria ($P < 0.001$). Of the isolated strains of *A. pyogenes,* 73.9 % (34/46) were from sheep showing severe balanitis or vulvitis whilst 21.6 % (12/46) were isolated from sheep that showed mild lesions. This difference is statistically significant ($P < 0.01$) and the odds ratio analysis showed an association between *A. pyogenes* isolation and the degree of severity of the lesion (OR = 3.36; 95 % CI = 1.75, OR < 6.47; chi-square = 15.97; $P = 0.000$).

The bacterial species isolated most often from apparently healthy sheep were *Enterococus faecalis* (22.4 %), *Pasteurella multocida* (17.2 %), *Rhodococcus equi* (14.7 %), *Staphylococcus epidermidis* (16.4 %) and *Streptococcus* species (25.9 %). None of these differences in microbial colonisation are statistically significant ($P > 0.05$).

Isolation and identification of mollicutes

A total of 222 mollicutes were isolated from genital swabs of 104 clinically affected and 116 unaffected sheep. Of the 222 isolates, *Mycoplasma* represented 153 (69.0 %), *Ureaplasma* 54 (24.3 %) and *Acholeplasma* 15 (6.7 %). The isolation rates of mycoplasmas from healthy and infected sheep varied significantly ($P < 0.05$). The likelihood that mycoplasmas would be isolated from affected sheep was 3.86 times greater than it was from apparently healthy sheep. *Mycoplasma mycoides mycoides* large colony variant (*Mmm*LC) was the most frequently isolated mycoplasma (71 of 153; 46.4 %). Thirteen specimens (18.3 %) yielded pure cultures of

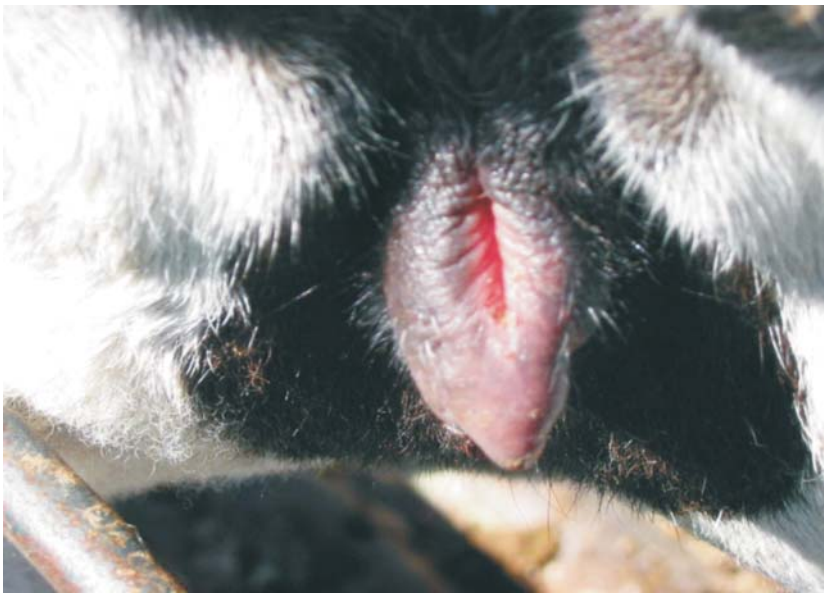

Fig. 3: **Swollen, oedematous and reddened vulva**.

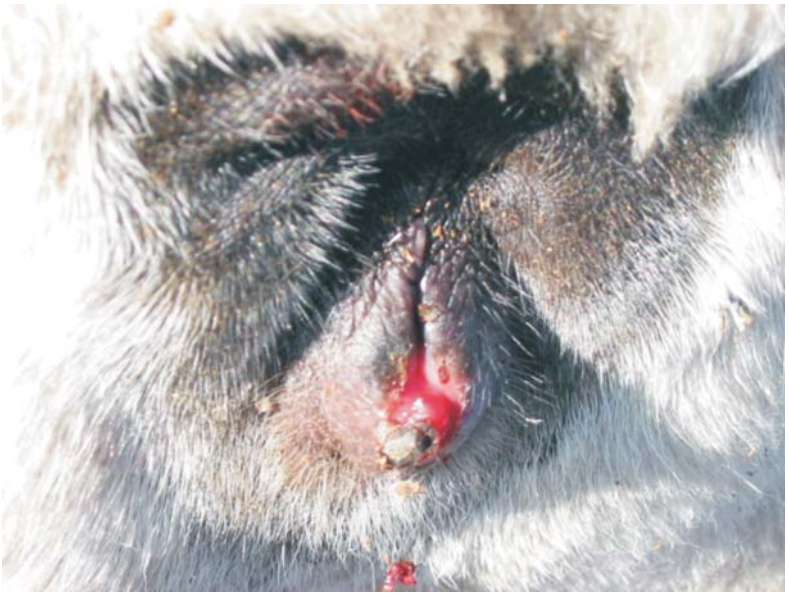

Fig. 4: **Scabs and blister-like ulcers on the labia and haemorrhagic lesions at the ventral commissure.**

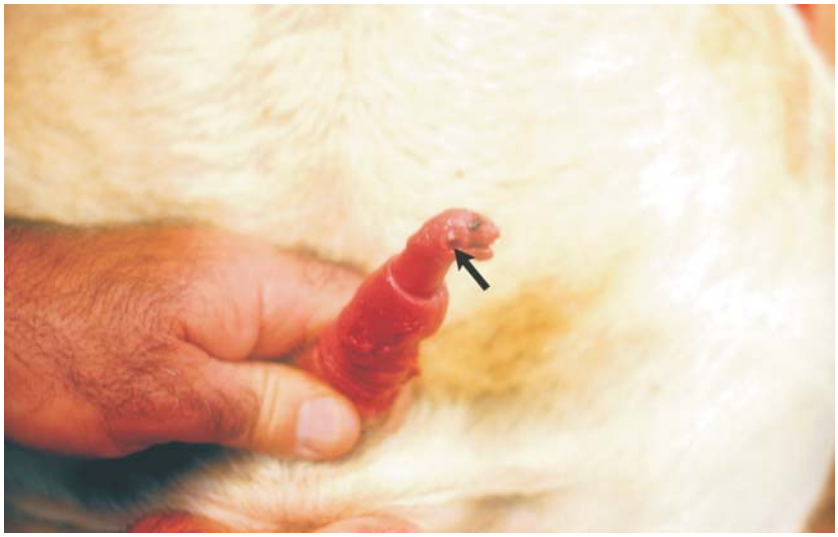

Fig. 5: **Small circumscribed ulcer in the soft tissues of the *glans penis.***

Fig. 6: **Ulcer that damaged a large part of the *glans penis.***

*Mmm*LC while the remaining 58 (81.7 %) were found in mixed cultures with 1 or more species of mycoplasma. Of the 54 ureaplasma strains isolated, 24 (45 %) were from affected and 30 (54 %) were from healthy sheep.

When the isolates from individual clinically affected sheep were compared, 64 (61.5 %) sheep had *Mmm*LC. The difference in the isolation of this species between healthy and affected groups is statistically significant ($P < 0.01$; chi-square = 7.6; $P = 0.000$). Sheep with clinical lesions were 24.46 times more likely to have been infected with *Mmm*LC than healthy sheep (95 % CI = 9.76–64.01).

Twenty of the affected sheep (19.2 %) sheep were infected with *Mycoplasma* species group 7, 20 with *M. bovigenitalium*, 7 (6.7 %) sheep with *M. capricolum*, and 4 (3.8 %) sheep with *M. arginini* (Table 3).

Of the 64 isolates of *Mmm*LC from the 104 clinically affected sheep, 46 (71.9 %) were from sheep manifesting severe clinical signs with varying degrees of ulceration, and 18 (28.1 %) were from mildly affected sheep (Table 3).

When the association between the isolate and degree of disease severity was evaluated, a significant association ($P < 0.05$) was evident between the degree of the disease severity and the rate of isolation of *Mmm*LC (odds ratio = 2.89; 95 % CI = 1.54 < OR < 5.43; chi-square = 12.9; $P = 0.000$). In other words, sheep from which *Mmm*LC was isolated were 2.89 times more likely to have manifested severe lesions than mild lesions.

The association between age and the presence of clinical signs was statistically significant ($P < 0.05$). It was found that young sheep (0–6 teeth) were more likely to have lesions than adult sheep.

As *A. pyogenes* and *Mmm*LC were the 2 most common isolates in sheep with clinical signs of the disease, their possible synergistic role in the disease process was assessed. They were simultaneously isolated from 33 (31.7 %) sheep out of 104 clinically affected sheep and from 1 (0.9 %) sheep out of 116 healthy sheep (Table 4). The odds ratio analysis showed that when *A. pyogenes* and *Mmm*LC were present together, they were 53.5 times more likely to occur in clinically affected sheep than in unaffected sheep (Table 5).

Table 2: **Summary of the bacterial species isolated from 104 clinically affected and 116 unaffected sheep.**

Bacterial isolates	Total isolates			
	Affected sheep ($n = 104$)		Unaffected sheep ($n = 116$)	
	n	**%**	***n***	**%**
Acinetobacter lwoffi	5	4.8	14	12.1
Actinobacillus actinomycetemcomitans	7	6.7	10	8.6
Actinobacillus seminis	2	1.9	4	3.4
Alcaligenes odorans	15	14.4	18	15.5
Arcanobacterium pyogenes	46	44.2	20	17.2
Corynebacterium pseudotuberculosis	12	11.5	14	12.1
Corynebacterium renale	3	2.9	2	1.7
Corynebacterium species	15	14.4	15	12.9
Enterococcus faecalis	9	8.6	26	22.4
Erysipelothrix rhusiopathiae	15	14.4	7	6.0
Escherichia coli	3	2.9	12	10.3
Flavobacterium multivorum	8	7.7	13	11.2
Lactobacillus species	6	5.8	11	9.5
Moraxella species	3	2.9	14	12.1
Pasteurella multocida	16	15.4	20	17.2
Rhodococcus equi	24	23.1	17	14.7
Staphylococcus aureus	11	10.6	7	6.0
Staphylococcus epidermidis	15	14.4	19	16.4
Streptococcus agalactiae	6	5.8	9	7.8
Streptococcus species	22	21.2	30	25.9
Total	243	100.0	282	100.0

Examination for *Chlamydophila* and viruses

No *Chlamydophila* antigens were detected in 20 selected swabs each from affected and unaffected sheep, using the Clearview® chlamydia antigen detection ELISA kit.

No viruses were isolated in the cell cultures from the 160 specimens examined from affected sheep.

DISCUSSION

Although, Trichard *et al.*[36] and Trichard and Van Tonder[37] described posthitis and vaginitis as part of the disease syndrome, the involvement of the prepuce, vaginal and vulval vestibule was essentially absent in the affected sheep examined in this study (Figs 3–6). This observation is consistent with the description of the disease given by Dent[9], Webb and Chick[38] and Deas[8]. In almost all the cases examined the tissues affected were the soft tissue of the glans penis of rams and the muco-cutaneous junction of the vulval lips of ewes. According to Deas[8] the fibro-elastic tissues of the shaft of the penis are less likely to be affected by such lesions than the soft tissues of the *glans penis.* The name ulcerative balanitis and vulvitis as suggested by Deas[8], Linklater and Smith[24], Dunn[12] and Greig[13] is therefore a more appropriate description of the disease as seen in South Africa.

In the present case-control study, mycoplasmas were isolated from 59.1 % (130/220) of the sheep, with *Mmm*LC being the predominant species (61.5 %). There was a difference in the isolation of mycoplasma organisms between healthy and affected sheep. Mycoplasma organ-

Table 3: **Summary of the isolated *Mycoplasma* species from the genital swabs of 116 clinically unaffected and 104 affected sheep.**

Mycoplasma species	Affected		Unaffected		Total	
	n	%	*n*	%	*n*	%
M. agalactiae	1	0.96	1	0.86	2	0.9
M. arginini	4	3.8	1	0.86	5	2.3
M. bovigenitalium	20	19.2	13	11.2	33	15.0
M. capri	1	0.96	4	3.4	5	2.3
M. capricolum	7	6.7	1	0.86	8	3.6
*M. mm*LC	64	61.5	7	6.0	71	32.3
M. species group 7	20	19.2	5	4.3	25	11.4
Unidentified *Mycoplasma* spp.	1	0.96	3	2.6	4	1.8

Table 4: **The combined isolation of *Arcanobacterium pyogenes* and *Mmm*LC from 104 clinically affected and 116 unaffected sheep.**

Isolates	Affected		Unaffected		Total	
	n	%	*n*	%	*n*	%
A. pyogenes alone	13	12.5	19	16.4	32	14.5
*Mmm*LC alone	31	29.8	6	5.2	37	16.8
A. pyogenes + *Mmm*LC	33	31.7	1	0.9	34	15.4

Table 5: **Odds ratio analysis to determine the association between the mutual isolation of *Arcanobacterium pyogenes* and *Mmm*LC and clinical disease status.**

Disease status	Isolates		Odds ratio	95 % CI	Chi-square	*P*-value
	Yes (*n*)	No (*n*)				
Affected	33	71	53.45	7.57 < OR < 1073	39.9	0.000
Unaffected	1	115				

isms were isolated 3.86 times more often from the affected sheep than from the unaffected sheep. This result points to an association of these mollicutes with ulcerative genital disease of sheep in South Africa. It confirms the suppositions expressed in earlier publications that mycoplasma organisms are important contributing pathogenic agents in ulcerative balanoposthitis and vulvovaginitis in sheep[4,15,36].

The results of this study provide strong evidence for the association of *Mmm*LC with a clinical genital tract infection in Dorper sheep in South Africa. This postulation is supported by the fact that there was a higher rate of isolation of *Mmm*LC from clinically affected sheep compared with the apparently normal flock mates (Table 3). Furthermore, the absence of viruses, *Chlamydophila* species and inconsistent isolation of different species of bacteria from clinically infected sheep indicates that these organisms are probably not responsible for the clinical signs observed. However, it seems probable that bacteria may contribute by initiating or aggravating the condition when present in association with mycoplasmas. This is substantiated by the combined isolation of *A. pyogenes* and *Mmm*LC from 31.7 % of sheep with clinical signs of the disease (Tables 4, 5).

The study has shown, for the first time, an association of *Mmm*LC and *A. pyogenes* with genital tract infection of sheep in South Africa. Mycoplasmas are well-defined pathogens acting singly or in consort with other agents. Although it cannot be assumed with certainty, it is possible that on the basis of the results obtained in this investigation, the concurrent isolation of *A. pyogenes* and *Mmm*LC supports the concept that the bacterial pathogen is the primary factor that 'opens the door' for mycoplasmal invasion with subsequent pathological consequences. *Arcanobacterium pyogenes* is considered as the most pathogenic bacterium residing on mucosal surfaces as it can cause tissue damage by means of its virulence factor, the exotoxin pyolysin[5]. The organism has for example been isolated from 3 of 10 pus specimens taken from goats suffering from vulvitis in Nigeria[17]. Furthermore, *Mmm*LC has an endotoxic activity which is able to induce damage to epithelial cells of the genital tract[30]. It is therefore probable that these endotoxins can be held responsible for aggravating the cellular damage of the genital system of sheep colonised by this *Mycoplasma* species.

It seems likely that *A. pyogenes* and *Mmm*LC act synergistically to induce the characteristic lesions of the disease. A synergistic interaction of *A. pyogenes* with other organisms in a variety of other clinical conditions has been described[27,29,32].

In this study, *Mycoplasma capricolum* was isolated from 7 sheep with lesions of ulcerative balanitis and vulvitis (Table 3). This organism is usually associated with polyarthritis in sheep and goats[19], but has also been isolated from the genital tract of sheep in England in an outbreak of vulvovaginitis and balanoposthitis[20]. However, from the low isolation rate detected in the investigation reported here, it appears unlikely that *M. capricolum* played a primary pathogenic role.

Although *Mycoplasma* species group 7 was isolated from 23 % of affected sheep with an odds ratio of 5.19 (Table 3), it is difficult to establish whether or not it has any significant role in the clinical signs of ulcerative balanitis and vulvitis. Moreover, there are no reports in the literature which incriminate this organism as a causal agent of any disease.

Nine of the 15 strains of *A. laidlawii* isolated were from animals showing balanitis or vulvitis (data not shown). Although, *A. laidlawii* is usually regarded

as a non-pathogenic organism, there are reports associating it with reproductive disease conditions[1,21,35]. In the light of increasing evidence of the association of this organism with disease conditions there is a need for further assessment of its pathogenic potential.

In this survey the prevalence of ureaplasmas in the apparently healthy sheep was higher than in affected sheep. These results are in agreement with earlier reports that many apparently healthy sheep carry ureaplasmas in their urogenital tract[11,18,27]. Therefore, it is possible that the ureaplasmas were either commensals within the vagina or prepuce of the sheep as suggested by Jones and Rae[18], or alternatively, that no pathogenic serotypes were amongst them (H J Ball, Veterinary Research Laboratories, Belfast, Ireland, pers. comm., 2002)[24,25].

Ten field strains of MmmLC and 9 field strains of *A. pyogenes* isolated in this investigation were tested for their *in vitro* sensitivity to several antimicrobial drugs[22,23]. The results indicated that the MmmLC strains were susceptible to all antimicrobials used which included enrofloxacin, florfenicol, oxytetracycline and spiramycin and that *A. pyogenes* strains were susceptible to all 3 antimicrobial drugs used which were enrofloxacin, oxytetracycline and tilmicosin[22,23].

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