Onderstepoort J. Vet. Res., 60:29–37 (1993)

The identification of *Mycoplasma mycoides mycoides* LC as the aetiological agent of balanoposthitis and vulvovaginitis in sheep in South Africa

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ABSTRACT

TRICHARD, C. J. V., JORDAAN, P., PROZESKY, L., JACOBSZ, ELSIE P. & HENTON, MARIJKE, M. 1993. The identification of *Mycoplasma mycoides mycoides* LC as the aetiological agent of balanoposthitis and vulvovaginitis in sheep in South Africa. *Onderstepoort Journal of Veterinary Research,* 60:29–37 (1993)

Clinical ulcerative balanoposthitis and vulvovaginitis was experimentally reproduced in 14 sheep infected with a *Mycoplasma mycoides mycoides* LC field strain, isolated from the Straussheim Dorper stud. The study encompassed a series of field observations, a therapeutic trial and experimental investigations. A wide range of bacteria and various mycoplasma spp., but no viruses, were isolated from a large number of infected animals.

INTRODUCTION

Since 1979 a disease referred to as ulcerative balanoposthitis/vulvovaginitis (ub/vv) has been encountered on numerous farms in the Cape Midlands, eastern Cape, southern Orange Free State and the north-eastern and north-western semi-arid parts of the Cape Province in South Africa (E. M. van Tonder, Regional Laboratory, Middelburg, Cape, unpublished data 1979; C. J. V. Trichard, unpublished data 1979). These observations were later confirmed by R. W. Muir, State Veterinarian, Kimberley, unpublished data (1981); P. Jordaan, State Veterinarian, Upington, unpublished data (1985).

Ulcerative balanoposthitis/vulvovaginitis is a venereal disease mainly found in Dorper sheep and characterized by ulcerations on the mucous membranes of the penis, prepuce, vulva and vagina. Clinically affected rams and ewes refuse to mate. The morbidity in primary outbreaks is high, especially in rams. Conception rates are significantly reduced with ensuing economic implications. The disease is readily transmissible to experimental rams and ewes by mating them to naturally infected sheep or by the direct application of infective material obtained from diseased animals to the mucous membranes of the penis, prepuce, vagina or vulva of experimental animals. These observations left little doubt that an infectious agent is responsible for the disease.

Somewhat similar conditions have been reported in other countries. In Britain, a venereal disease affecting both rams and ewes has been described (Ball & McCaughey 1982; Jones, Rae, Holmes, Lister, Jones, Grater & Richards 1983). Vulvovaginitis, granular vaginitis, vulvitis and vaginitis have been observed in Australia, Canada, the USA and India (Cottew, Llyod, Parsonson & Hore 1974; Doig & Ruhnke 1977; Livingstone jr & Gauer 1983; Kapoor, Singh & Pathak 1984).

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Received 30 October 1992—Editor

Livingstone *et al.* (1983) isolated *Mycoplasma* sp. 2D in 2 flocks with reproductive problems. However, isolations were also made from flocks with normal reproductive performances. As yet, the aetiological role of this mollicute has not been verified. According to Cottew *et al.* (1974) this mollicute appears to be related to *M. mycoides mycoides. M. capricolum* and Acholeplasma axanthum (Jones *et al.* 1983) have been isolated during sporadic outbreaks of vulvovaginitis and vulvitis. *M. mycoides mycoides, M. mycoides capri, M. bovigenitalium, M. agalactiae, M. alkalescens, M. arginini, Acholeplasma laidlawii, A. axanthum, A. granularum* and *A. modicum* have been recovered from infertile sheep with vaginitis and ewes that aborted (Kapoor *et al.* 1984). Ureaplasma strains have also been isolated from sheep with genital lesions (Doig & Ruhnke 1977; Ball & McCaughey 1982). Due to the high incidence of this disease in South Africa since 1979, this study was undertaken to establish the role of mollicutes as possible aetiological agents of ub/vv in sheep.

MATERIALS AND METHODS

Field survey

During the course of this study 19 infected flocks were investigated. Within these flocks, 930 animals were examined and 484 specimens collected for further evaluation. Specimens entailed vulva, vagina, sheath and penis swabs and semen. Reference specimens, which served as controls, were collected from clinically sound animals in each herd. On each farm approximately 9 clinically affected ewes and 3 rams, and 10 clinically sound ewes and 4 rams were selected for sampling. All specimens collected were examined for the presence of bacteria, viruses and mycoplasma organisms *(vide infra).*

Pilot study

Two Dorper rams and 1 ewe with clinical lesions were submitted to the Onderstepoort Veterinary Institute (OVI). These animals served as a source of infective material during transmission trials.

Four locally bred Dorper rams, proven negative for mycoplasmas on specimen cultivation, were selected as experimental animals. Swabs were taken from the penile and vulvar lesions of the donor animals and applied directly to the penile mucous membrane of 2 experimental rams. Filtered material, prepared by rinsing 3 swabs dabbed on the genital mucous membranes of the donor animals in 2 mℓ phosphate buffered saline (PBS) and passed through a millipore filter (0,45 nm), was applied with the aid of sterile cotton wool swabs to the penile mucous membranes of the remaining 2 experimental rams.

Infectivity trial

Experimental animals and procedure

Ten normally cycling maiden yearling ewes and 4 maiden yearling rams were used in this study (Table 2).

The animals were divided into 2 groups (Group 1 & Group 2). Each group consisted of 5 ewes and 2 rams which had been vasectomized a fortnight previously. The genital tracts of all the animals were screened for the presence of mycoplasmas and pathogenic bacteria 10 d before infection on Day 0 *(vide infra).*

Ten d after the animals had been screened for bacteria and mycoplasmas, a strain of *Mycoplasma mycoides mycoides* LC, which had been isolated from the Straussheim Dorper stud *(vide supra)* and cloned and stored in liquid nitrogen prior to the study, was used to infect the ewes in Group 1 (Day 0). Each ewe was infected by administering 1 mℓ culture medium containing 10^{10} organisms intravaginally. The animals were examined daily and specimens for mycoplasma isolation were collected on Days 3, 7, 14, and 25 post infection (Table 2) and for bacterial isolation on Days 0, 3 and the day of slaughter.

On Day 25 ewes of Group 2 were infected intravaginally with a pure culture of *M. mycoides mycoides* LC that was isolated from 1 of the ewes in Group 1 (Sheep 2). Each animal received 10^8 organisms intravaginally. The animals were examined daily and specimens were collected for mycoplasma isolation on Days 3, 7, and 14 post inoculation (Table 2) and for bacterial isolation on Days 0, 3 and the day of slaughter. (The rams in both groups were not artificially infected.)

Animals in Group 1 were slaughtered on Day 56 and those in Group 2 on Day 30. Specimens were collected from the anterior vagina, uterus, preputium, ampulla, seminal vesicles and tail of the epididymis for bacteriological and mycoplasma isolation. The intact urogenital tracts were examined macroscopically and specimens from the uterus, cervix, vagina, vulva, prepuce and glans penis were collected in 10 % buffered formalin, routinely processed and stained with haematoxylin and eosin (HE).

Therapeutic trial

During the period of investigation an acute outbreak of ub/vv in a Dorper stud (Straussheim stud) was reported. This flock offered an opportunity to initiate a field trial to establish the efficacy of 2 antibiotics, viz. enrofloxacin (Baytril, Bayer A.H.) for local application or systemic treatment, and oxytetracycline long-acting (LA) (Liquamycin LA, Pfizer) for systemic treatment. The products were selected

because of their mycoplasmacidal and mycoplasmastatic properties.

Seventy-four animals (69 ewes and 5 rams) which proved positive to mycoplasma on specimen isolations taken from the genitalia were used in this trial. No attempt was made to identify the spp. concerned, except for 5 randomly selected animals. *M. bovigenitalium, Mycoplasma* spp. Group 7 and *M. mycoides* were isolated from the latter sheep. The 74 experimental animals were divided into 5 groups and treated according to a schedule summarized in Table 1. Group 5 (15 animals) served as a control group and was treated with 4 mℓ glycerine/water solution intravaginally (3 × 24 hourly).

Vaginal and penile swabs were taken on Days 2, 7, 16 and Day 28 after the last treatment and processed within 8 h after sampling. Nine ewes that still proved positive for mycoplasmas 28 d post-treatment, were treated by combining the regime utilized for Groups 3 and 4. Vaginal swabs were collected from the latter animals 14 d and 3 months after the last treatment.

Sampling techniques

Specimens from ewes

Vulva specimens were taken by dabbing a sterile cotton swab on a mucocutaneous erosion or intact mucous membrane. Vaginal specimens were collected by parting the vulvar lips, inserting the cotton bud the full length of the vagina and rotating it serveral times before removal. Two drops of Hayflick's broth (Hayflick 1965) were added to each specimen after collection. Each swab was replaced in the plastic tube which was sealed with the stopper provided. The plastic tubes were immediately packed in a cool-bag containing pre-frozen freezer packs and taken to the laboratory.

Specimens from rams

Preputial specimens were collected by dabbing a sterile cotton bud on a mucocutaneous erosion or intact mucous membrane adding 2 drops of Hayflick's broth after collection and storing as described for the ewes. The same technique was employed for penile samples. Semen was collected by electro-ejaculation after the penis had been extruded and immobilized by placing a piece of gauze bandage well behind the glans penis.

Culture and identification of micro-organisms

Mycoplasmas

Hayflick's agar (Hayflick 1965), Hayflick's broth (Hayflick 1965), Chalquest's agar, Chalquest's broth (Trichard & Jacobsz 1985), ureaplasma agar (TA 13) and ureaplasma broth (TB 13) were used throughout the study.

The ureaplasma media consisted of the following:

UREAPLASMA AGAR TA 13

Purified agar (Oxoid)[1] ..... 9 mℓ
Distilled water ..... 405 mℓ
Autoclave 10 1b–10 min
10 × Medium 199 (Sigma)[2] ..... 45 mℓ
Hartley's Digest Broth (Difco)[3] ..... 200 mℓ
Yeast extract (Difco)[3] (5 g) ..... 100 mℓ
Pig serum (inactivated) ..... 200 mℓ
Urea in distilled water 20 %
(PAL Chemicals)[4] ..... 5 mℓ
Dithiothreitol in distilled water 10 %
(Sigma)[2] (0,5 g) ..... 5 mℓ
DNA (calf thymus) 0,2 % (BDH)[5] ..... 10 mℓ
Phenol red 0,4 % ..... 15 mℓ
$MnSo_4$ 1,5 % ..... 10 mℓ
Penbritin 100 mg/mℓ (Beecham)[6] ..... 10 mℓ
Set ph at 6,0–6,2

Filter through a 450 nm Millipore filter and add to the purified agar when the latter attains a temperature of 60 °C.

UREAPLASMA BROTH TB 13

10 × Medium 199 (Sigma)[2] ..... 45 mℓ
Distilled water ..... 405 mℓ
Hartley's Digest Broth (Difco)[3] ..... 200 mℓ
Yeast extract (Difco)[3] ..... 100 mℓ
Pig serum (inactivated) ..... 200 mℓ
Urea in distilled water 20 %
(PAL Chemicals)[4] ..... 5 mℓ
Dithiothreitol 10 % (Sigma)[2] ..... 5 mℓ
DNA (calf thymus) 0,2 % (BDH)[5] ..... 10 mℓ
Phenol red 0,4 % ..... 15 mℓ
$MnSo_4$ 1,5 % ..... 10 mℓ
Penbrin 100 mg/mℓ (Beecham)[6] ..... 10 mℓ

Set pH at 6,0–6,2

Sterilize by passing through a 450 nm Millipore filter.

Mycoplasma isolates were identified by the direct fluorescent antibody technique (Baas & Jasper 1972) using conjugated gammaglobulin prepared at the OVI. The following monospecific hyperimmune rabbit antisera were used: *Acholeplasma laidlawii* PG8 (sewage A), *Mycoplasma arginini* G230, *Mycoplasma bovigenitalium* PG11, *Mycoplasma bovis* Donetta and *Mycoplasma* species group 7 PG50. These type cultures were obtained from the National Collection of Type Cultures, London. Additional type cultures were obtained from

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[1&4] Protea laboratory Services, Sandton, RSA

[2] Saarchem (Pty) Ltd, Muldersdrift, RSA

[3] Difco Laboratories, Detroit, Michigan, USA

[5] BDH, Chemicals Ltd, Poole, England

[6] Beecham Research Laboratories, Beecham Pharmaceuticals (Pty) Ltd, Sandton, RSA

the Collaborating Centre for Animal Mycoplasmas, Institute of Medical Microbiology, University of Aarhus, Denmark, viz. *Mycoplasma agalactiae* PG2, *Mycoplasma capricolum* California kid, *Mycoplasma mycoides capri* PG3 and *Mycoplasma mycoides mycoides* LC type or Y-goat.

Bacteria

The samples were inoculated within 2 h of receipt onto blood tryptose agar (BTA) prepared with bovine blood, MacConkey agar and serum broth. Two BTA plates were inoculated in each case, one being incubated in an atmosphere of 10 % CO_2 for possible isolation of *Haemophilus* and *Actinobacillus* spp. and the other in a normal atmospheric incubator. All cultures were incubated for a minimum of 3 d. The isolates were identified according to standard criteria, as described in Razin & Freundt (1984) and Stephens, Humphrey, Little & Barnum (1983).

Viruses

The following technique was employed for the cultivation of viruses. Primary foetal lamb kidney (FLK) and foetal spleen (FSPL) cells were established and stored in liquid nitrogen. The cells were cultivated in Eagle's medium containing 5 % irradiated bovine serum, benzyl penicillin (400 iu/mℓ), streptomycin sulphate (200 µg/mℓ), vancomycin (0,5 mg/mℓ) and amphotericin B (2,5 µg/mℓ).

Material from preputial scrapings and vaginal swabs were inoculated onto monolayers of FLK and FSPL cells in roller tubes. The latter were incubated at 37 °C and subcultures were made after 10 d or when the cells showed signs of degeneration. The tubes were inspected regularly for cytopathic changes indicative of virus infection and were discarded after the 3rd passage.

RESULTS

Clinical manifestations

Since the first outbreak of ub/vv in 1979, 19 field outbreaks were investigated. Clinically these outbreaks presented a regular pattern. Lesions developed 4–10 d after the introduction of rams to the ewes for breeding. Reluctance or complete refusal to serve is the first sign of the disease in rams. Some animals are depressed and may stand to one side with their backs arched. If still inclined to mate, free blood discharges from the preputial opening. The skin around the prepuce is often blood-stained and haemorrhaging may occur after urinating. The prepuce is swollen, erosions occur at the mucocutaneous junction and on extrusion the penis is hot, painful to the touch and the mucous membrane may easily tear and bleed. Small scattered erosions are visible. At times the erosions are extensive, often forming ulcers covering most of the glans and the processus urethralis (Fig. 1). Paraphimosis may occasionally develop. This is followed by extensive trauma and soiling of the penis. In primary outbreaks up to 100 % of the rams may be affected.

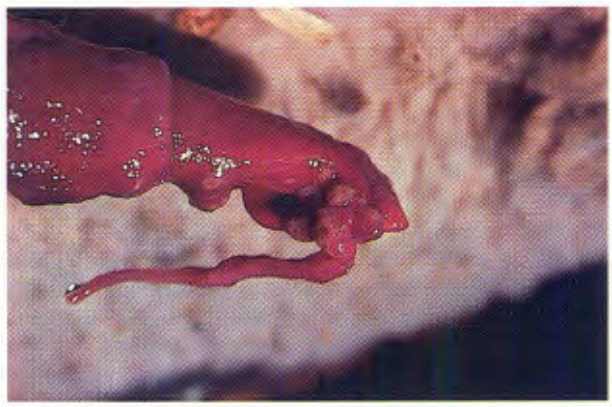

FIG. 1 Erosions often forming ulcers are present on the prepuce and processus urethralis

Some ewes presented blood-stained hindquarters, particularly around the vulva and tail area. In Dorper sheep where the tail is docked short and the vulva is visible, the vulvar lips are swollen, oedematous and red (Fig. 2), while blood-stained fluid may ooze from the external orifice. The vaginal mucous membrane bleeds easily when handled, particularly after vaginoscopic examination. Frequent wriggling of the tail-butt and urination are seen in some cases. Flies are attracted to the inflamed genitalia which further agitates the animals. After

TABLE 1 Treatment of ewes with Enrofloxacin and Oxytetracycline LA

Group	No. of sheep	Chemotherapeutic agent	Dosage rate	
1	16	Enrofloxacin	5 mg/kg bm i.m.	3 × 12 hourly
2	13	Enrofloxacin	100 mg i.vag.	3 × 24 hourly
3	14	Enrofloxacin	200 mg i.vag.	3 × 24 hourly
4	16	Oxytetracycline LA	20 mg/kg bm i.m.	1 × 24 hourly
5	15	Placebo-glycerine/water	4 mℓ i.vag.	3 × 24 hourly

i.m. — intramuscular
bm — body mass
i.vag. — intravaginal

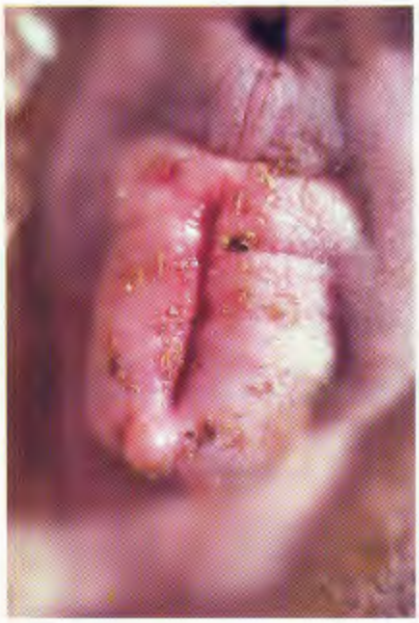

FIG. 2 The vulvar lips are oedematous with crust formation

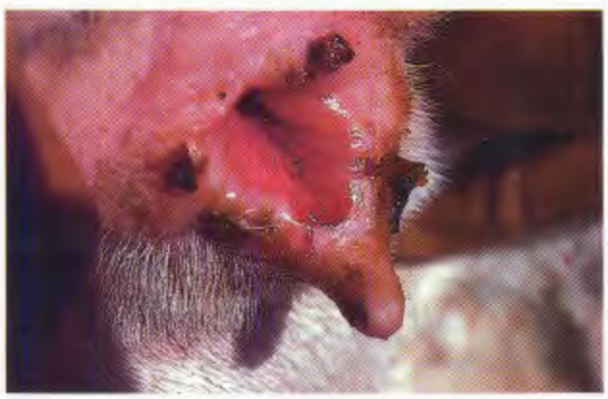

FIG. 3 Scab-covered ulcers are evident on the vulvar lips particularly at the mucocutaneous junction

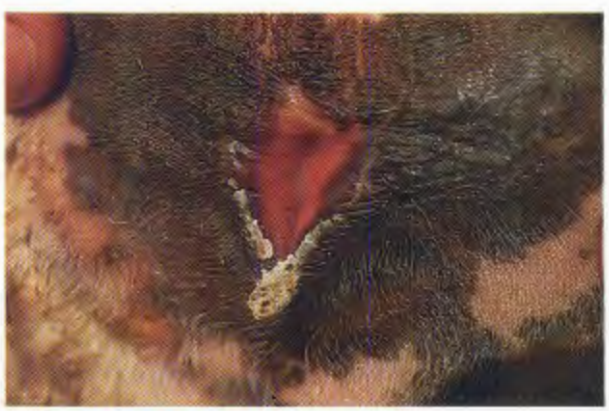

FIG. 4 A mucopurulent discharge developed 3–4 d after infection with *M. mycoides mycoides* LC

the acute signs have abated, scab-covered ulcers are visible on the vulvar lips particularly at the mucocutaneous junction and the ventral commissure (Fig. 3). In primary outbreaks the morbidity may reach 70–80 %.

Field survey

A vast variety of bacteria were isolated from the genitalia of clinically healthy and diseased rams and ewes. These included *Haemophilus somnus; Actinomyces pyogenes; Corynebacterium renale; C. xerosis; C. pseudotuberculosis; Fusobacterium necrophorum; Enterococcus faecalis; Streptococcus dysgalactiae; Staphylococcus aureus; S. epidermidis; Pasteurella haemolytica; Escherichia coli; Enterobacter agglomerans; Klebsiella* sp.; *Micrococcus luteus; Citrobacter diversus; Bacillus* sp.; *Acinetobacter alcaligenes* and *Pseudomonas aeruginosa.*

Mollicutes were isolated from 83 % of the diseased sheep and 36 % of the controls. The spp. involved were *Mycoplasma arginini, M. bovigenitalium; M.* species group 7; *M. mycoides capri; M. mycoides mycoides* LC, ureaplasmas as well as a number of mycoplasmas that could not be identified. No viruses were isolated.

Pilot study

All 4 experimental rams developed clinical lesions of ub 4–5 d after infection. However, lesions in the 2 rams infected with the filtered material were less conspicuous compared to those in the 2 rams infected with the crude unfiltered material.

Infectivity trial

The clinical observations and mycoplasmal and bacterial isolations are given in Table 2. The most prevalent clinically recognisable changes were oedema and hyperaemia of the genital mucous membranes and the presence of small epithelial erosions in conjunction with a distinct mucopurulent discharge 3–4 d after infection (Fig. 4). These changes gradually subsided from Day 25. At slaughter *M. mycoides mycoides* LC was isolated from the vaginal specimens of 7 ewes, viz. Sheep 1, 2, 4 & 5 in Group 1 and 8, 9 & 12 in Group 2. All uterine specimens were negative.

A mucopurulent discharge was present in Sheep 1, 4, 5, 8 and 9 at slaughter. Of the series of specimens taken from the 4 rams, only 1 preputial sample, (Sheep 14) was positive for *M. mycoides mycoides* LC on the day of slaughter.

Bacteriological examination revealed that all sheep were found to harbour non-pathogenic organisms with the exception of 1 ram (Sheep 13) which was

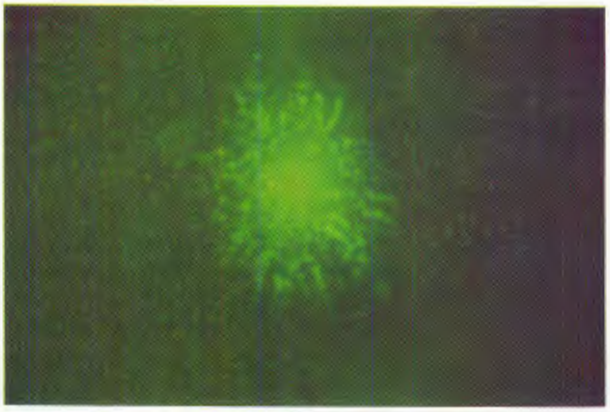

FIG. 5a Direct fluorescent antibody technique demonstrating a mixed mycoplasma colony

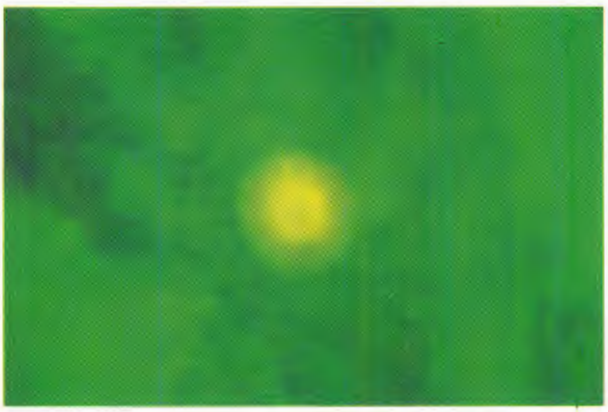

FIG. 5b A pure mycoplasma colony demonstrated by direct immuno-fluorescent antibody technique

also positive for *Haemophilus somnus.* No viruses were isolated.

Macroscopical lesions

At slaughter, single circular ulcerations approximatey 3–5 mm in diameter were present at the vulvar mucocutaneous junction of 1 animal (Sheep 5). The ulcers were covered by a seropurulent crusty exudate. Multiple small erosions/ulcers were present in the vaginal mucosa of the same animal and the entire mucosal surface was covered with a whitish congealed discharge.

Light microscopical lesions

In all the ewes the vaginal mucosa was diffusely infiltrated by mild to moderate numbers of predominantly lymphocytes and isolated plasma cells. The lymphocytic infiltration was particularly noticeable at the muco-epidermal junction and the perivascular spaces in the mucosa often contained the highest concentration of round cells. A mild diffuse lymphocytic exocytosis throughout the vaginal epithelium was a characteristic feature in all the ewes. In a few animals this was accompanied by intra- and extracellular oedema resulting in acantholysis and necrosis of single keratinocytes.

In 2 animals (Sheep 3 and 5) a. diffuse mild to moderate neutrophil infiltration was evident in the vaginal mucosa. Exocytosis of neutrophils resulted in the formation of intra-epithelial micropustules with necrosis of the surrounding keratinocytes and the development of erosions/ulcerations. Basal cells of the vaginal epithelium underlying the erosive areas were hyperplastic and hypertrophic. Similar lesions as described in the vaginas of Sheep 3 and 5 were present in the vulva of Sheep 5. In the rest of the animals no lesions were seen in any of the other tissues examined.

Therapeutic trial

Five of 9 ewes that had been treated by combining the regime utilized for Groups 3 and 4 (Table 1) still harboured mycoplasma organisms a fortnight after the 2nd treatment. A number of different colony types representing various *Mycoplasma* species could usually be distinguished. Due to the occurrence of mixed colonies (Fig. 5a), it was not possible to obtain pure cultures (Fig. 5b). However, in 2 of these animals the various *Mycoplasma* species were now presented as separate entities. These isolations were propagated, cloned and stored in liquid nitrogen for transmission trials.

DISCUSSION

This project was launched in an attempt to identify the aetiological agent responsible for ub/vv in Dorper sheep. It commenced with field outbreaks, infected sheep presented at the OVI (pilot study), followed by field surveys, an infectivity trial and a therapeutic trial.

In the pilot study clinical cases of ub were reproduced by applying crude or filtered material, prepared from the lesions of clinical cases, onto the penile mucosae of 4 experimental animals. No viruses were isolated from clinical cases which suggested that mycoplasmas could be responsible for the disease. Further evidence to support this suggestion was the isolation of mycoplasma organisms from 83 % of the diseased sheep compared to only 36 % of healthy sheep. In more than 90 % of cases the mycoplasma isolates presented as mixed colonies. The spp. included *M. bovigenitalium, M.* species group 7, *M. mycoides capri, M. mycoides mycoides* LC and a number of unidentified spp. The latter often formed the basic structure of the colony, with 1 or 2 of the other spp. being incorporated within the same colony. It was

TABLE 2 Mycoplasmal and bacteriological isolations and clinical observations in sheep infected with *Mycoplasma mycoides mycoides* LC

Isolations and clinical findings	Day 0								Day 3								Day 7							
	M	U	B	MPD	VO	HMM	P	E	M	U	B	MPD	VO	HMM	P	E	M	B	MPD	VO	HMM	P	E	
Sheep No.																								
* 1	–	–	–	–	–	–	–	–	+	–	–	+	+	+	+	–	+	ND	+	+	+	+	+	
G * 2	–	–	–	–	–	–	–	–	+	–	–	+	+	–	–	–	+	ND	+	+	+	+	–	
R * 3	–	–	–	–	–	–	–	–	+	–	–	+	+	–	–	–	+	ND	+	+	+	+	–	
O * 4	–	–	C	–	–	–	–	–	+	–	C	+	+	+	–	–	+	ND	+	+	+	+	–	
U * 5	–	–	–	–	–	–	–	–	+	–	–	+	+	+	–	–	+	ND	+	+	+	+	–	
P ° 6	–	–	–	–	–	–	–	–	+	–	–	+	–	+	–	–	+	ND	+	–	+	+	+	
I ° 7	–	–	–	–	–	–	–	–	+	–	–	+	–	+	–	–	+	ND	+	–	+	+	–	

	Day 0								Day 3								Day 7							
G * 8	–	–	–	–	–	–	–	–	+	–	–	+	+	+	–	–	+	ND	+	+	+	+	+	
R * 9	–	–	–	–	–	–	–	–	+	–	–	+	+	+	–	–	+	ND	+	+	+	+	+	
O * 10	–	–	C	–	–	–	–	–	+	–	C	+	+	+	–	–	+	ND	+	+	+	+	+	
U * 11	–	–	C	–	–	–	–	–	+	–	C	+	+	+	–	–	+	ND	+	+	+	+	+	
P * 12	–	–	Ap	–	–	–	–	–	+	–	Ap	+	+	+	–	–	+	ND	+	+	+	+	+	
2 ° 13	–	–	Ap Ph	–	–	–	–	–	+	–	Ap Ph	+	+	+	–	–	+	ND	+	+	+	+	+	
° 14	–	–	C	–	–	–	–	–	+	–	C	+	+	+	–	–	+	ND	+	+	+	+	+	

	Day 14								Day 17								Day 25							
G * 8	+	ND	+	+	+	+	+		+	–	ND	+	+	+	+	+	+	ND	+	+	+	+	+	
R * 9	+	ND	+	+	+	+	+		+	–	ND	+	+	+	+	+	+	ND	+	+	+	–	–	
O * 10	+	ND	+	+	+	+	+		+	–	ND	+	+	+	+	+	+	ND	+	+	+	+	–	
U * 11	+	ND	+	+	+	+	+		+	–	ND	+	+	+	+	+	+	ND	+	+	+	–	–	
P * 12	+	ND	+	+	+	+	+		+	–	ND	+	+	+	+	+	+	ND	+	+	+	–	–	
2 ° 13	+	ND	+	+	+	+	+		+	–	ND	+	+	+	+	+	+	ND	+	+	+	–	–	
° 14	+	ND	+	+	+	+	+		+	–	ND	+	+	+	+	+	+	ND	+	+	+	–	–	

	Day 56								S
	M	U	B	MPD	VO	HMM	P	E	
G * 8	+	–	C	+	–	–	+		–
R * 9	+	–	C	–	–	–	–		–
O * 10	–	–	C	–	–	–	–		–
U * 11	+	–	C	+	–	–	+		+
P * 12	+	–	C	–	–	–	–		–
2 ° 13	–	–	C	–	–	–	–		–
° 14	–	–	C	–	–	–	–		–

	Day 30							S
G * 8	+	–	C	+	+	+	+	–
R * 9	+	–	C	+	–	–	–	–
O * 10	–	–	C	–	–	–	–	–
U * 11	–	–	C	–	–	–	–	–
P * 12	+	–	C	–	–	–	–	–
2 ° 13	–	–	Hs	–	–	–	–	–
° 14	+	–	C	–	–	–	–	–

Abbreviations

1. M — *Mycoplasma mycoides mycoides* LC
2. U — Ureaplasma
3. B — Bacteria
4. MPD — Mucopurulent discharge
5. VO — Vulvar oedema/Preputial oedema
6. HMM — Hyperemic mucosal membrane
7. P — Petechiae
8. E — Small epithelial erosions
9. C — Contaminants, these include part or all of the following: *Escherichia coli; Enterobacter agglomerans; Staphylococcus epidermidis; Bacillus; Corynebacterium xerosis; Acinetobacter alcaligenes; Citrobacter diversus*
10. Ap — *Actinomyces pyogenes*
11. Ph — *Pasteurella Haemolytica*
12. Hs — *Haemophilus somnus*
13. ND — Not done
14. * — Ewes
15. ° — Rams
16. S — Day of slaughter

TABLE 3 Biochemical properties of *M. bovigenitalium, M.* J14* and *M. mycoides mycoides* LC

Species	Glucose catabolism	Arginine hydrolysis	Digitonin sensitivity	Phosphatase activity	Tetrazolium reduction	Proteolytic activity	Film and spot
M. bovigenitalium	–	–	+	+	+/–	+	+
M. J14	–	–	+	+/–	+/–	+	+
M. mycoides mycoides LC	+	+	+	+/–	–	+	–

*J14 — Unidentified species isolated from genitalia of sheep
\+ — Positive
– — Negative
+/– — Weakly positive

possible to maintain all these mycoplasma spp. simultaneously intravaginally in a single ewe. However, maintenance of these spp. *in vitro* could not be achieved with any of the culturing methods employed during this study.

In *vitro* propagation of pure cultures of previously identified spp. was pursued, but repeated attempts over several years failed. During the process of cloning, colonies of the identifiable spp. failed to maintain themselves and only unidentifiable species remained. Biochemically the latter spp. appeared to be related to *M. bovigenitalium* (Table 3).

The 5 ewes of Group 1 (Table 2), which were infected with the field strain of *M. mycoides mycoides* LC and in which coitus was allowed, developed vv lesions and the corresponding rams developed ub. Since *M. mycoides mycoides* LC could be re-isolated from all the animals in Group 1, and the ewes in Group 2 could be infected with the same strain isolated from an animal in Group 1, it was deduced that *M. mycoides mycoides* LC is the major aetiological agent responsible for ub/vv in Dorper sheep in South Africa. This is the first time that the presence of this mycoplasma spp. has been documented in South Africa. Furthermore, this is the first report that *M. mycoides mycoides* LC has been specifically associated with a venereal disease in sheep. Kapoor *et al.* (1984) did isolate *M. mycoides mycoides* LC in combination with other mollicutes from the genitalia of 24,7 % of ewes which were infertile or aborted, but no proof was tendered that this organism was responsible for the reproductive failures.

Few reports on the natural infection of sheep with *M. mycoides mycoides* LC variant, have been documented (MacOwan 1984). Experimentally the pathogenicity in sheep is similar to that for goats (MacOwan 1984). Subcutaneous inoculation of high-titre cultures is followed by cellulitis, a febrile response and, in the more severely affected animals, a mycoplasmaemia. Intratracheal inoculation causes pleuropneumonia. It is therefore imperative that the pathogenicity of the *M. mycoides* field strain isolated during this study should be assessed further for Dorper sheep.

A lymphocytic exocytosis of the vaginal epithelium associated with a lymphocytic infiltration in the vaginal mucosa was present in all the ewes infected with *M. mycoides mycoides* LC. At the time of slaughter ulcerations of the vulvar and vaginal epithelium were evident in only 1 animal and microscopically in the vagina of an additional animal. However, during the course of the disease small ulcerations of the vaginal epithelium were noted in all the experimental sheep on Day 14.

The clinical, gross and microscopical lesions in field and experimental cases of ub/vv in this study were similar to those of sheep infected with bacteria of the haemophilus/histophilus group (Ball, Kennedy & Ellis 1991). Furthermore, vulvitis in ewes has been shown to be caused by ureaplasmas (Ball *et al.* 1982; McCaughey & Ball 1985). In the present study neither ureaplasmas nor bacteria of the haemophilus/histophilus group were isolated from the experimental animals, except in 1 ram from which *H. somnus* was isolated on Day 17. Bacteria isolated from natural and experimental cases of ub/vv represented a wide variety and the disease could not be associated with any specific spp. isolated.

The therapeutic trial *per se* did not form part of this project, but was initiated solely to explore the therapeutic value of enrofloxacin and oxytetracycline LA in the control of an acute outbreak of ub/vv. What transpired was serendipitous. Five ewes did not respond to treatment. *M. mycoides mycoides* LC, *M. mycoides capri, M. bovigenitalium* and *Mycoplasma* species Group 7 could now be isolated as separate entities from the genital organs of 2 of these animals. This incident made it possible to isolate the *M. mycoides mycoides* LC field strain which was subsequently utilized in the transmission studies of this project.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr A. P. Schutte for his valued advice and continued interest in this project, Dr B. J. H. Barnard and the staff of the Division of Virology, Pathology and Bacteriology for competent technical assistance and Mr J. C. Strauss for making facilities on his farm available for the project.

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