

THE EPIDEMIOLOGY OF BACTERIAL INFECTION OF THE GENITALIA IN RAMS

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ABSTRACT

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The interrelationship between the various bacteria isolated from the genital tract of rams and their host animals was studied. The pathogenicity of the different isolates varied. Several of these bacteria could be cultured in a medium consisting of a suspension of pen floor debris solidified with agar, while many organisms survived in the suspension for 10 days. Epidemiological investigations showed that rams kept under intensive systems were subjected to large-scale invasion of their genitalia by bacteria which led to infection of the accessory glands and orchitis and epididymitis. Apart from the preputial cavity, some rams kept on open range were entirely free of bacterial infection of their genitalia, and those that did have bacteria in the deeper parts of their genitalia had a very significantly lower incidence of pathological lesions of their genitalia. Finding bacteria and neutrophils in semen is consistent with the epidemiological findings.

INTRODUCTION

The relevant literature reveals many reports on the isolation of different bacteria from the genital tract of rams. Buddle & Boyes (1953) described the isolation of *Brucella ovis* from the genitalia of rams in New Zealand. The same organism has subsequently been recovered from infected rams in several sheep-breeding countries. Jamieson & Soltys (1947) reported the occurrence of 10 cases of orchitis and epididymitis caused by *Pasteurella pseudotuberculosis* in one flock of sheep in Britain. Ekdahl, Money & Martin (1968), who isolated a number of different organisms from the testes and epididymides of rams in New Zealand, list the following bacteria in their publication: *Brucella ovis*, a Gram-negative pleomorph; *Actinobacillus*-like organisms; *Corynebacterium pyogenes*; *Corynebacterium pseudotuberculosis*; *Streptococcus* spp.; *Staphylococcus* spp.; *Pasteurella haemolytica*; *Pasteurella multocida*; *P. pseudotuberculosis*; *Bacteroides* spp., *Brucella abortus*; *Brucella abortus* (S 19).

Baynes & Simmons (1962) isolated *Actinobacillus seminis* from the epididymides of 3 affected rams in Australia. *Histophilus ovis* was isolated from the testes of a ram by Claxton & Everett (1966).

Various organisms have been associated with epididymitis in rams from Idaho and Eastern Oregon. De Long, Waldhalm & Hall (1970) reported the following isolates: *Actinobacillus actinomycetemcomitans*; *Brucella ovis*; *C. pyogenes*. *C. pseudotuberculosis*; *Pseudomonas multophila*.

Jansen (1980 a) reported on the isolation of the following organisms from the genital tracts of rams: *P. haemolytica*, *Staphylococcus* spp., *Corynebacterium ovis*, *C. pyogenes*, *C. paurometabolicum*, *Micrococcus luteus*, *M. varians*, *Streptobacillus* spp., *Flavobacterium* spp., *Lactobacillus* spp., *Streptococcus bovis*, *Staphylococcus epidermidis*.

Although the published reports mention the isolation of the different bacteria and, in some instances, the pathological lesions associated with their presence, an epidemiologic account of all the aspects of infection by a particular organism exists only for *B. ovis* (Meyer, 1980). Bruere, West, MacLachlan, Edwards & Chapman (1977), commenting on epididymitis and orchitis caused by Gram-negative pleomorphic organisms, stressed the lack of knowledge of the method of transmission of these organisms and of successful control of the disease. They also stated that it affected substantial numbers of sale rams. A study of the interrelationship between the different bacteria found in the genitalia of rams and their host animals under various systems of management was therefore undertaken.

MATERIALS AND METHODS

Experimental animals

Since the object was to study the position as it prevails under farming conditions, the co-operation of stud breeders was sought. Breeders willing to co-operate were found in different geographic areas. Their systems of management, the type of grazing and the climatic conditions varied considerably. All the factors and their variations were studied in detail on the farms included in the project. Records were kept of the incidence of clinically detectable disease of the genitalia in the different studs, and non-affected culled rams were obtained for detailed bacteriological investigation of their genitalia.

When necessary, rams were transferred to the laboratory, where they were kept in cages with metal grid floors for the period of study.

Pathogenicity of bacterial isolates

The ability of some of the isolated bacteria to cause epididymitis when introduced into the vas deferens of a ram was investigated by the method described by Jansen (1980 b). A volume of 0.1 ml of a dilute bacterial suspension of known viable count was injected (Table 1). The rams were killed 10 days after the operation and the epididymis examined bacteriologically and histologically.

Bacteriological examination of the genital organs

For routine cultivation of bacteria in this study, tryptose agar medium* containing 10 % horse blood was used. The cultures were incubated for 48 h in a 10 % CO₂ atmosphere at 37 °C.

As soon after killing as possible, the entire urogenital system of the ram was removed and transferred to the laboratory for detailed cultural examination. Sheath washings were prepared by searing the preputial orifice with a hot spatula, introducing 2 ml of Hank's balanced salt solution (HBSS) into the cavity by means of a sterile pipette, withdrawing the solution and preparing cultures from it.

Cultures were prepared from the vesiculae seminales, ampullae, epididymides and testes by searing the surface of these organs with a hot spatula, incising the seared surface with a sterile scalpel and then introducing a sterile bacteriological loop into the tissue.

No special effort was made in this study to discriminate between *Pasteurella* and *Actinobacillus*, because the distinction between these closely related genera is fraught with taxonomic complexities (Kilian, Fredrickson & Biberstein, 1981) and would not make any difference to the final conclusions.

Whenever required, semen was obtained by electroejaculation with sterile precautions. Cultures were prepared from the semen and also smears stained with Giemsa's stain to check on the presence of neutrophils.

Determination of the presence of inhibitory substances in seminal fluid

Wells were punched in tryptose-blood-agar plates with sterile precautions. The wells were filled with sterile seminal fluid obtained by filtering semen through millipore filters (pore size 0,2 μm). After the contents of the wells had diffused into the medium diluted liquid cultures of the different bacteria isolated from the preputial cavity were thinly spread over the surface of the medium in separate Petri dishes. As soon as the surface of the medium had dried, the wells were refilled with sterile seminal fluid to increase its concentration in the medium around the wells. Incubation of the cultures was continued for 24 h, after which they were examined for zones of inhibition around the wells.

The same test was applied to test the inhibitory effect of rabbit antiserum against the corresponding organism.

Detection of antibodies in semen and serum

The gel-diffusion test was applied by covering the bottom of a small Petri dish with a 3 mm layer of 0,5 % agarose in a 0,1 M phosphate buffer at pH 7,0. A central well, surrounded by a ring of wells 5 mm from it, was punched in the gel. The peripheral wells were about 5 mm apart. The central well was used for the antigen and the peripheral wells for the semen and serum. The reagents were preserved with 0,02 % sodium azide. The test was left at room temperature for 72 h before the results were read.

An antigen was prepared from the different bacteria by washing off the confluent growth from the surface of 10 Petri dishes with 10 ml of HBSS. This resulted in a milky white suspension which was subjected to ultrasonic vibration at 6 micron amplitude for 60 s 5 times consecutively.

The same antigen was used for the preparation of an autologous vaccine by mixing equal volumes of antigen suspension and Freund's complete adjuvant.

This type of vaccine was also used to prepare antisera against individual bacteria by injecting 0,5 ml of vaccine subcutaneously into rabbits on 3 occasions at three-week intervals and bleeding them 2 weeks after the last injection.

Determination of the survival and growth of bacteria on pen floor debris

Debris containing manure and feed residues was collected from the floor of a sheep pen and mixed with distilled water. The mixture was filtered through a fine wire mesh to remove the coarse particles. The filtrate, which had a thick, soup-like consistency, was sterilized for 20 min in 500 ml quantities at 115 °C. It was then incubated overnight at 37 °C to allow the bacterial spores surviving the first sterilization to germinate. To some of the filtrate 1,5 % agar was added, the sterilization process was repeated and the finished product poured into Petri dishes to solidify. The rest of the filtrate was dispensed into 20 ml screw-capped McCartney bottles and then subjected to the same sterilization process.

Cultures of a variety of organisms isolated from the genitalia of rams were spread on the surface of the medium in the Petri dishes to detect colony formation, the indication of growth after incubation for 48 h at

37 °C. The same cultures were also introduced into the liquid suspension that was kept at room temperature. Subcultures onto blood-tryptose agar were prepared at regular intervals to determine the survival of the organisms in the suspension.

RESULTS

Bacteria inhabiting the preputial cavity of rams

The following bacteria were recovered from the sheaths of rams derived from different sheep-producing areas: *C. pseudotuberculosis*; *Streptococcus faecalis*; *S. bovis*; *Bacillus* spp.; *Escherichia coli*; *P. haemolytica*; *Lactobacillus* sp.; *Bacillus subtilis*; *Micrococcus roseus*; *Corynebacterium xerosis*; *Pseudomonas aeruginosa*; *P. multocida*; *C. pyogenes*; *C. paurometabolicum*; *Streptococcus acidominimus*; *Staphylococcus aureus*; *A. seminis*; *Flavobacterium* sp.; *Acinetobacter* sp.; *S. zooepidemicus*; *Streptococcus dysgalactiae*; *Enterobacter cloacae*; *Citrobacter intermedius*; *S. epidermidis*. There seemed to be no consistent pattern in the sense that rams from the same district harboured the same bacterial species. No ram had only a single species in its sheath.

Occurrence of orchitis and epididymitis under various farming conditions

Although few farmers keep an exact tally of the number of rams affected by orchitis or epididymitis, stud farmers are fully aware of the condition when it does occur in their rams. The following information could, therefore, be obtained by carefully investigating the conditions on stud farms and questioning the owners about their systems of management and the presence of the disease in their rams.

On Farm A the rams were kept on low-lying lands along the banks of a river. The sward consisted of a mixture of grass and lucerne and there was a fair amount of organic material on the surface underneath the vegetation. The soil was clayey and remained wet for long periods after irrigation and during the rainy season. Epididymitis regularly occurred in the rams kept on these lands. *P. haemolytica* was isolated from the epididymidis, ampullae and vesiculae seminales of affected rams.

On Farm B the rams were kept on lands with well-drained sandy soil. The lands were planted with Kikuyu grass, but since the cover was not dense, the surface readily dried after irrigation or rain. The breeder had no problems with epididymitis or orchitis among his rams, although *C. pseudotuberculosis* was isolated from the ampullae, vesiculae seminales and capita epididymidis of clinically normal rams.

The sheep on Farm C were kept solely on open range in the Karoo district of Richmond. The owner declared that he had never had any cases of epididymitis or orchitis. *C. xerosis*, *M. varians*, *S. epidermidis*, *Acinetobacter* sp. and *Corynebacterium bovis* were isolated from the ampullae of clinically normal rams. *Acinetobacter* sp. was recovered from the vesiculae seminales of these rams. *Acinetobacter* sp. and *C. bovis* were present in the caput epididymidis.

The owner of Farm D kept his rams in small pens under a thatched roof and occasionally they got a turn to graze for a day on grasscovered lands. The straw covering the floor of the pen was renewed once a month. Several cases of epididymitis occurred under these conditions and the presence of neutrophils in the semen of many of the rams was a source of concern to the veterinarian in attendance. *C. ovis* was isolated from the vesiculae seminales of clinically normal rams and *C. ovis*, *C.*

paurometabolicum, *M. luteus*, *P. haemolytica* and *Arthrobacter* sp. from their caudae epididymidis. *C. ovis*, *C. paurometabolicum*, *M. luteus* and *P. haemolytica* were isolated from their capita epididymidis.

On Farm E the rams were kept in small dusty camps with feeding racks in the centre. One or two trees provided shade in each camp. There was a marked accumulation of debris around the feeding racks and under the trees. Many cases of epididymitis occurred in these rams. From the ampullae of normal rams *P. haemolytica*, *M. luteus*, *C. pseudotuberculosis* and *Staphylococcus* sp. were isolated; from the vesiculae seminales *P. haemolytica*, *M. luteus* and *C. pseudotuberculosis*; from the caudae epididymidis *P. haemolytica*, *C. pseudotuberculosis* and *Staphylococcus* sp.; from the capita epididymidis *P. haemolytica*, *C. pseudotuberculosis* and *M. luteus*.

The system of management on Farm F in the Douglas district included the feeding of 12-month-old Dorper rams in groups of about 200 for about 60 days in small camps. Up to 30% of these rams were affected by acute orchitis caused by *H. ovis*.

On Farm G the rams were kept in pigsties that had fallen into disuse. Around the sties was a small camp in which stood the feeding rack. During the day the rams spent most of the time lying down in the sties, which provided the only available shade. The floor of the sties was covered with a thick layer of debris. Cases of acute orchitis were regularly experienced. From the caput epididymidis of a ram derived from this stud were isolated the following organisms: *P. haemolytica*; *C. xerosis*; *C. paurometabolicum* and *Arthrobacter flavescens*.

It seems relevant to mention that *A. flavescens* is generally recognized as an organism normally occurring in soil and with no pathogenic potential.

A comparison producing significant results was made on 2 stud farms about 20 km apart in the Graaff-Reinet district. Both studs consisted of top-quality Merinos that had won several merit awards. The owner of Stud H consistently applied a management policy in which the rams were never "pampered by being protected and fed in stalls". He believed that, for a sheep to be genetically suited to a particular environment such as the Karoo, it should be born, reared and selected for adaptability and productive capacity in that particular environment. He applied scientific methods of pasture management and preservation and kept all his sheep on open range permanently. Of the 200 rams reared to the age of 15 months during 1982, one animal only was culled on account of clinical epididymitis.

Stud I was kept on a low-lying farm with abundant water. The rams were born and reared in small camps on regularly irrigated lucerne lands and given supplementary pellets in self-feeders. The vegetation on the lands was particularly lush and the rams spent much time resting around the self-feeders. Out of a group of 140 rams, 71 had to be culled on account of epididymitis during 1982 when they reached the age of 15 months. A detailed bacteriological examination of the genitalia of a sample of 5 clinically normal rams from this stud revealed the following bacteria in the organs indicated:

Ampullae: *P. haemolytica*-like organism; *H. ovis*.

Vesiculae seminales *P. haemolytica*-like organisms; *C. pseudotuberculosis*.

Caudae epididymidis *C. pseudotuberculosis*.

Capita epididymidis *C. pyogenes*; *M. luteus*.

Pathogenicity of bacterial isolates

The results of injecting known numbers of 3 bacterial species isolated from the genital tract of rams into the ductus epididymidis of susceptible rams are given in Table 1.

TABLE 1 The effect of introducing bacteria into the ductus epididymidis of rams

Bacterial species	Number injected	Result after 10 days
<i>C. pseudotuberculosis</i>	580	Culturally positive. Several histological lesions.
<i>H. ovis</i>	14	Culturally positive. Several histological lesions.
<i>C. paurometabolicum</i>	19 × 10 ³	Culturally negative. Histologically normal.

From Table 1 it can be seen that a large variation exists in the pathogenicity of various organisms as tested in this particular manner.

Comparison of the bacterial flora of the genitalia of rams kept intensively and on open range

The results of a detailed bacteriological examination of the component parts of the genital tracts of 2 groups of 12 rams are set out in Tables 2 & 3. The rams in Table 2 were randomly selected from different studs kept intensively in small camps around sheds or on lands. The rams in Table 3 were likewise chosen randomly from animals kept entirely on open range. All the rams were free of clinically detectable lesions.

From these tables it is clear that, while in the group on open range there were 5 rams entirely free of bacteria in the deeper parts of the genital tract, in the group kept intensively all the animals showed infection of the deeper parts of their genitalia. In the open range group only 3 showed infection of the caput epididymidis, while in the other group the caput epididymidis was infected in 8 rams.

Although in this experiment only the different types of bacteria and not their numbers, were determined, it is clear that rams kept intensively are subject to more bacterial migration into the deeper parts of their genitalia than rams kept extensively.

An unusual phenomenon is that in 9 out of the 24 rams examined the caput epididymidis was infected, while the cauda epididymidis was free of bacteria.

Ecology of bacterial invasion of the genital tract

The study of the ecology of bacterial invasion of the genital tract, evidenced by the presence of bacteria and neutrophils in the semen, presented a rather irregular picture.

Some rams had no bacteria in their semen. Others showed bacteria in their semen on culture, but no neutrophils. The semen of one ram, for instance, was infected with *M. luteus*, *C. bovis* and *Streptococcus faecium* without any neutrophils being present in smears.

In some rams neutrophils appeared in the semen after a bacterial infection had been present for a while. On its first examination one ram showed the presence of *C. pyogenes* and a *P. haemolytica*-like organism without neutrophils in its semen. At a subsequent examination, one month later, neutrophils were present together with some organisms. In most instances the bacteria persisted in the semen for at least 3 months in the presence of numerous neutrophils, e.g. a ram infected with an *A. seminis*-type of organism.

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TABLE 2 Bacteria found in subdivisions of the genitalia of rams kept intensively

Ram	Ampulla	Vesiculae seminales	Cauda epididymidis	Caput epididymidis
1.	—	<i>H. ovis</i> <i>C. enzymicum</i> <i>C. pseudotuberculosis</i>	—	<i>C. pseudotuberculosis</i> <i>C. xerosis</i>
2.	<i>C. pseudotuberculosis</i> <i>C. xerosis</i> <i>C. pseudodiphtherium</i>	—	—	<i>C. enzymicum</i> <i>C. xerosis</i> <i>C. pseudotuberculosis</i> <i>P. haemolytica</i>
3.	—	—	<i>C. paurometabolicum</i>	—
4.	—	<i>C. paurometabolicum</i> <i>M. luteus</i> <i>M. varians</i>	<i>M. luteus</i>	<i>M. luteus</i>
5.	<i>P. haemolytica</i>	<i>P. haemolytica</i>	<i>P. haemolytica</i>	—
6.	<i>Staph. sp</i>	<i>Staph. sp</i>	<i>Staph. sp</i>	—
7.	<i>C. bovis</i> <i>C. renale</i>	<i>Micrococcus sp.</i> <i>C. bovis</i>	—	<i>C. bovis</i> <i>C. renale</i> <i>Micrococcus sp.</i>
8.	—	<i>C. bovis</i>	—	<i>C. bovis</i> <i>M. luteus</i>
9.	<i>M. luteus</i>	<i>C. bovis</i> <i>M. luteus</i>	—	—
10.	—	—	<i>P. haemolytica</i> <i>C. xerosis</i> <i>A. flavescens</i> <i>C. paurometabolicum</i>	<i>P. haemolytica</i> <i>C. xerosis</i> <i>A. flavescens</i> <i>C. paurometabolicum</i>
11.	—	<i>M. luteus</i>	—	<i>C. bovis</i>
12.	<i>C. bovis</i>	<i>C. bovis</i>	—	<i>C. bovis</i> <i>M. luteus</i>

TABLE 3 Bacteria found in the subdivisions of the genitalia of rams kept on open range

Ram	Ampulla	Vesiculae seminales	Cauda epididymidis	Caput epididymidis
1.	<i>C. xerosis</i>	<i>Acinetobacter sp.</i>	—	—
2.	—	—	—	—
3.	<i>C. pseudotuberculosis</i>	<i>C. pseudotuberculosis</i>	—	<i>C. pseudotuberculosis</i>
4.	—	—	—	—
5.	—	—	—	—
6.	—	—	—	<i>C. pseudotuberculosis</i>
7.	<i>Acinetobacter sp.</i>	—	—	<i>Acinetobacter sp.</i>
8.	<i>Bacillus sp.</i> <i>Acinetobacter sp.</i>	—	—	—
9.	—	—	—	—
10.	—	—	—	—
11.	<i>Acinetobacter sp.</i>	<i>S. epidermidis</i>	—	—
12.	<i>Streptobacter sp.</i>	<i>M. roseus</i>	<i>Corynebacterium pseudodiphtherium</i>	—

In several rams infected with *A. seminis*-like and *P. haemolytica*-like organisms in their semen, no antibodies against the organism responsible for the infection could be detected in the semen by means of the gel-diffusion test. One ram showed in its semen an infection of *S. faecalis* as well as antibodies against this organism detectable by means of immunodiffusion. The infection persisted in spite of the presence of antibodies and neutrophils. Pathological lesions, found on histological examination of the vesiculae seminales and ampullae of this ram, showed the accumulation of plasma cells in the lamina propria and disruption of the epithelium with exposure of the plasma cells to the lumen (Fig. 1 & 2). This

was obviously the source of the antibodies in the semen. Bacteria and neutrophils could be found in the semen of rams of all ages.

Bacillus licheniformis and a *Lactobacillus sp.*, whose growth is inhibited by seminal plasma, could not be found in semen.

Anatomy of the prepuce

Rams differ with regard to the anatomy of their prepuce. The ram in Fig. 3 had a short prepuce firmly attached to the body with its opening pointing forwards and downwards. The ram in Fig. 4 had a long, pendulous

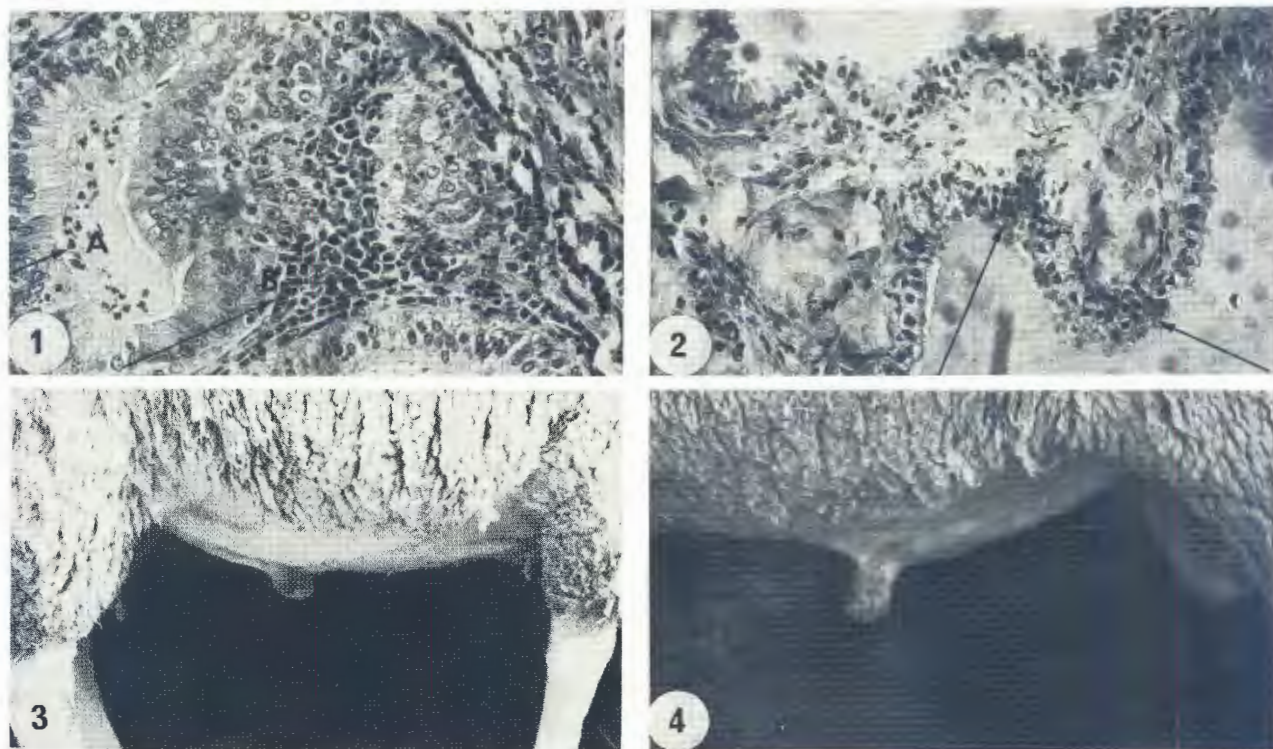


FIG. 1 A: neutrophils in lumen of ampulla. B: accumulation of plasmacells in lamina propria of mucosa of ampulla: HE \times 500

FIG. 2 Plasma cells adjacent to lumen of ampulla in sites indicated by arrows: HE \times 500

FIG. 3 Ram with short, firm prepuce

FIG. 4 Ram with long, pendulous prepuce

prepuce with its opening pointing downwards. After these 2 rams had been kept in a pen with a hard floor for 6 weeks, the one with the long sheath had developed an ulcer at the anterior aspect of the mucocutaneous junction.

Observations on the habits of rams

The preputial opening of a ram lying down touches the ground.

Rams kept in pens are usually fed concentrated feeds and thus do not take long to satisfy their appetite. They are usually in a rather heavy body condition, with the result that their legs have to bear an above average strain. Furthermore, they are usually physically less fit because of lack of exercise. All these circumstances cause them to lie down for long periods in the same confined area on a floor covered with organic matter.

Rams on open range spend at least 10 out of 24 h grazing. They are physically fit and seldom overfat. When they have to lie down, they select a suitable spot according to the prevailing weather conditions. Seldom do rams on open range lie down in the same spot for 2 consecutive resting periods, with the result that any manure shed in such places is allowed time to become pulverized and have its bacterial content reduced by desiccation and solar radiation.

Growth of bacteria on solidified floor debris suspension

The following organisms were found to grow on medium consisting of a sterile suspension of pen-floor debris plus agar: *C. pseudotuberculosis*; *C. pyogenes*; *C. xerosis*; *P. haemolytica*; *M. luteus*; *S. epidermidis*; *Acinetobacter* sp.

Survival of bacteria in floor debris suspensions

The periods of survival of the bacteria introduced into pen floor debris suspensions are given in Table 4.

TABLE 4 Survival of bacteria in suspensions of debris from pen floor

Bacteria	Survival time (days)			
	1	4	6	10
<i>C. ovis</i>	+	+	+	+
<i>H. ovis</i>	+	+	-	-
<i>P. haemolytica</i>	+	+	+	+
<i>C. xerosis</i>	+	+	+	+
<i>Lactobacillus</i> sp.	+	+	+	+
<i>A. actinomycetem-comitans</i>	+	+	+	+
<i>S. bovis</i>	+	+	+	+
<i>S. epidermidis</i>	+	+	+	+
<i>M. luteus</i>	+	+	+	+
<i>C. pyogenes</i>	+	+	+	+
<i>C. paurometabolicum</i>	+	+	+	+
<i>Acinetobacter</i> sp.	+	+	+	+
<i>P. multocida</i>	+	+	-	-

+ = alive

- = dead

From Table 4 it can be seen that most of the bacteria isolated from the genital tract of rams could survive for 10 days at room temperature in moist debris derived from pen floors. The only 2 exceptions are *H. ovis* and *P. multocida*, which survived for only 4 days.

DISCUSSION

The entry of bacteria into the preputial orifice that is in contact with the outside environment and particularly with the surface on which a ram lies is obvious. This phenomenon was confirmed by the country-wide survey of the bacteria present in the sheath cavities of rams. Jansen's conclusions (1980 a) that the bacteria in the sheath cavity can migrate into the deeper parts of the

genital tract along the lumen in the reverse direction to the flow of the semen implies that the infection of the genital tract bears a direct relation to the degree of bacterial contamination of the environment. The fact that bacteria from the environment can be found in the deeper parts of the genital tract is borne out by the isolation of *A. flavescens* from the caput epididymidis of a ram. This organism is generally recognized as an inhabitant of soil with no pathogenic potential.

The degree of bacterial contamination of the environment in which rams are kept is largely determined by the system of management applied. When rams are kept in pens on an intensive system there is always an accumulation of manure and feed residues on the floor. Experiments presented in this paper show that several of the species of bacteria isolated from the genital tract can actively grow on a medium consisting of a solidified suspension of debris collected from a pen floor. Many other species harboured in the genital tract can survive for at least 10 days on the material found on the floor of a sheep pen. This is, of course, only possible while the material contains sufficient moisture to support bacterial multiplication or survival. But the organic material on pen floors is kept moist by urine, overflow from drinking-troughs and even rain.

Sheep, persistently subjected to invasion of the sheath cavity by bacteria, are inclined to show a preputial discharge. This discharge attracts flies which transmit the infected material to the preputial opening of other rams or to the organic material on the floor and thus serve as a further source of contamination.

Rams, confined to pens and kept on a high nutritional plane, are more inclined to mount each other than rams on open range. In doing so they contaminate the fleece of their companions with preputial exudate and semen and thus provide infected material for flies to transmit to the preputial opening of other rams and to debris on the floor. Flies are usually abundant around sheep pens. In mounting other rams a ram also contaminates his penile mucosa with organic material adhering to the fleece of the rams being mounted.

A ram with a long pendulous prepuce is at a disadvantage in so far as his preputial opening is in more intimate contact with the contaminated material on the floor while it is lying down. This was manifested by some of the experimental rams with long sheaths which developed ulcers around the preputial orifice. The possibility that the external ulceration of the prepuce could have been posthitis caused by a high protein diet (Southcott, 1965) can be excluded on the grounds that the experimental animals received a ration consisting of 0.6 kg concentrate containing 12.6% protein plus bagasse *ad lib.* as roughage. Furthermore, none of the rams with a short sheath showed ulceration.

Rams on open range are in an entirely different situation. They are never in contact with accumulated debris which can harbour a rich bacterial population; their droppings are much more thinly spread and therefore subject to rapid drying and solar radiation; flies, which are attracted to moist organic material, are much less of a problem in transmitting infection to the preputial opening of rams. Because of these factors and the grazing habits of sheep on open range, rams kept on this system are exposed to a much reduced bacterial challenge in comparison with their counterparts on an intensive system. Hence the results recorded in Tables 2 & 3.

The conditions prevailing on lands could also be conducive to the invasion by bacteria of the preputial cavities of rams grazing on them. Usually the soil below the plants is kept moist and any organic material on it thus

favours the survival and, in some instances, the multiplication of bacteria. Sunlight does not penetrate to the soil below the plants and therefore does not exercise its sterilizing influence. Rams grazing on lands satisfy their appetite within a relatively short time and thus have ample time for lying down in the land after selection of a shady spot. In practice, the number of shady spots is limited, with the result that organic material is concentrated at the sites where the rams rest.

The overall effect is that rams, grazing in especially small camps on lands, are also exposed to bacterial invasion of the preputial cavity, although not to the same extent as those kept in pens. It stands to reason that well-drained lands with drier vegetation and subdivided into larger camps provide better prophylactic conditions.

Reflection on the results obtained in this investigation leads one to the conclusion that, although a large percentage of rams have bacteria in their genital tracts, the development of pathological lesions is related to the intensity of the infection and also the type of bacterium present. It was shown experimentally, for instance, that as few as 14 *H. ovis* organisms were required to cause a severe epididymitis, while 190×10^3 *C. paurometabolum* caused no pathological lesion. Admittedly, the experimental procedure created a highly artificial situation, but it did serve to show that there is a difference in the pathogenicity of the various organisms encountered in the genital tract.

Certain clearance mechanisms exist in the genital tract which would have the effect of reducing the bacterial infection. The growth of some organisms, e.g. *B. licheniformis* and *Lactobacillus* sp., is inhibited by ovine seminal plasma. The destruction of bacteria by seminal fluid is not unique to sheep. The antibacterial nature of human and canine prostatic fluid against a number of Gram-negative and Gram-positive bacteria has been described (Stamey, Fair, Timothy & Chung, 1969). Reddy & Bhargava (1979) showed that bovine seminal plasma has a microbicidal action on *E. coli*. But in sheep the scope of antibacterial activity of the seminal plasma is too limited to have a significant effect on bacterial invasion into the genital tract. The expulsion of semen during ejaculation would clear the genital tract of a considerable number of bacteria. Several types of bacteria, when present in the lumen of the genital tract, elicit an efflux of neutrophils through the lining epithelium into the lumen. These neutrophils phagocytize and kill many bacteria, as was shown for *H. ovis* by Jansen, Hayes & Knoetze (1983). Urination would also clear the section of the genital tract distal to the bladder of some of the bacteria present on the mucosal surface.

Antibodies, which are effective in killing some bacterial species, cannot be depended upon to reduce the bacterial numbers in the genital tract on account of the blood-genital tract barrier. Although Johnson & Setchell (1968) could identify IgG₁, IgG₂ and IgM in the rete testis fluid of rams by concentrating it up to seventyfold and applying a sensitive cellulose acetate electrophoresis method, Jansen, Hayes & Knoetze (1983) could not detect any antibody against *H. ovis* in the seminal fluid of rams hyperimmunized against this organism. They used immunodiffusion on unconcentrated seminal fluid. It seems reasonable to assume that, for antibodies to be effective against an organism, they should at least be present in a concentration detectable by the less sensitive method. In any case, it has been shown that antibodies to a particular bacterium appear in the lumen of the genital tract only after the development of fairly advanced pathogenic lesions of the mucosa. Furthermore, some bacteria grow well in the presence of antibody.

In spite of the clearance mechanisms that do exist, sufficient bacterial migration and multiplication for the production of pathologic lesions can take place. The site at which the multiplication takes place determines the nature of the lesion.

Bacteria arriving in the rete testis in the mediastinum of the testis have free access to all the seminiferous tubules through the tubuli recti. This explains why an orchitis caused by an organism such as *H. ovis* affects the entire testis.

Since the diameter of the ductus epididymidis is small, the products of bacterial multiplication in the rich contents of the ductus remain in close contact with the sensitive cells of the mucosa. Furthermore, because of the tortuous nature of the ductus, the likelihood of removal of these bacterial products by free drainage is limited. When the mucosa is sufficiently damaged by the bacterial metabolic products, blockage of the tubule results, with accumulation of semen proximal to the blockage site. Evagination of the mucosa follows with spermatozoa escaping into the interstitial tissue. This process causes a granulomatous lesion, with hardening and enlargement of the epididymis. The changes taking place in the epididymis have been described by Jansen (1980 c).

Because of the size of the lumen of the ampullae bacterial infection of the ampullae and vesiculae seminales does not lead to an obstruction to the flow of the semen. The epithelium of affected accessory glands does show hyperplasia, degeneration and infiltration of neutrophils. Neutrophils and plasma cells collect in the lamina propria of the epithelium and usually these glands show enlargement when affected. Neutrophils accumulate in the lumen and the saccular dilatations formed by the epithelium (Jansen, 1980 c).

It is not known exactly to what extent the lesions of the mucosa of the accessory glands change the composition of the seminal fluid and consequently affect the fertility of the ram. Veselsky (1981), referring to various animals, states that the importance of the accessory sex gland secretions for fertilization may assure long-term survival of the sperm in the male reproductive system, successful copulation of male and female, survival of the sperm in the female reproductive system and their successful transport to the oocytes. Unphysiological function of one of the genital tract glands can impair fertilization. He mentions the following immunological components of seminal plasma: scaferrin, haemolytic factor, haemagglutinating factor, protease inhibitors, an immunosuppressive substance and ribonuclease. He specifically mentions the presence of a haemagglutinating factor and an acrosin inhibitor in the seminal vesicle fluid of rams. Pathologic lesions of the accessory glands with consequent deranged function should, therefore, have a detrimental effect on the fertility of rams. It seems, however, that a bacterial infection of these glands is the major source of neutrophils in the semen, which is regarded as a sign of reduced fertility.

The detection of neutrophils and bacteria in semen on pre-sale examination of rams presents a problem to veterinarians. As is shown in this study, this can be true for rams of any age and one is likely to find bacteria and, less frequently, bacteria and neutrophils in the semen of some members of any group of rams. Bacteria and neutrophils are more commonly found, however, in the semen of rams kept intensively in pens or on those types of lands which favour the survival of bacteria. Bacteria and especially neutrophils are less commonly found in rams kept on open range. But the presence of bacteria and neutrophils in semen does not necessarily mean that the ram concerned is sterile, although intensive systems

of management are associated with a higher incidence of bacteria and neutrophils in the semen and a higher incidence of pathologic lesions of the genital tract than extensive systems of management. These pathologic lesions may be clinically detectable or very frequently affect only the accessory glands.

These conclusions also suggest an alleviation of the problem of bacterial infection of the genital tract of rams by keeping the animals on open range as far as possible. The value of this measure is borne out by a comparison of the findings on Stud H with those on Stud I.

With respect to an organism such as *H. ovis* which has invasive properties and can cause pathologic lesions in relatively small numbers, keeping rams on open range may not entirely eliminate the infection. Under such circumstances a vaccine which has been developed against *H. ovis* infections (Jansen, Hayes & Knoetze, unpublished data, 1983) may offer a solution.

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