Onderstepoort J. vet. Res., 54, 107–111 (1987)

THE RELATIONSHIP BETWEEN THE SKIN AND SOME BACTERIAL SPECIES OCCURRING ON IT IN THE MERINO

B. C. JANSEN and MARIANNA HAYES, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

JANSEN, B. C. & HAYES, MARIANNA, 1987. The relationship between the skin and some bacterial species occurring on it in the Merino. *Onderstepoort Journal of Veterinary Research*, 54, 107–111 (1987)

Ten bacterial species were isolated from areas on the skin of Merino sheep affected by a chronic scaly condition leading to retarded wool growth. The growth requirements of the bacteria were studied and it was shown that they could multiply under the conditions prevailing on the skin if moisture was provided. All the bacteria isolated have the ability to stimulate the infiltration of round cells into the dermis, which is the essential feature of the pathological changes in the naturally affected skin. It was concluded that the bacteria have a decided aetiological role.

INTRODUCTION

It is generally accepted that the skin and fleece of the Merino sheep harbour a variety of bacteria because of their exposure to the environment. Merritt & Watts (1978) found the predominant organisms present in the fleece and on the skin to be the following: Gram-positive bacilli, coccobacilli, cocci and Gram-negative pleomorphic rods. Counts of these organisms varied among sheep. Meritt (1981) found that *Pseudomonas, Proteus, Staphylococcus* and *Bacillus* were the most common members of the resident flora in the fleece.

The composition of the bacterial flora with respect to the different genera and the numbers in the individual genera may vary according to external circumstances. Merritt & Watts (1978), for instance, found vast increases in the bacterial counts of fleece and skin samplings within 24 h after wetting under an artificial rain system delivering 25 mm of soaking rain over four 30-min periods each day for 6 to 8 days. The counts remained high while the fleece and skin remained wet. *Pseudomonas* was frequently the predominant organism. Burrell, Merritt, Watts & Walker (1982) showed experimentally that *P. aeruginosa,* when stimulated to multiply by artificial wetting, causes a subacute fleece-rot dermatitis and exudation of sero-purulent material, which in turn provides suitable conditions for the development of fly strike. They found no evidence of invasion of the epidermis and dermis by bacteria and concluded that the growth of the organism is sufficiently luxuriant on the surface of the skin for its soluble products to be responsible for the changes observed.

In the current study, a more chronic relationship between the skin and some bacteria occurring on it and in the fleece was investigated.

MATERIALS AND METHODS

Experimental animals

Five Merino ewes were obtained from a farm in the Stutterheim district of the eastern Cape Province. They showed dark patches on the fleece where the wool tended to be shorter than on the rest of the body. The individual staples had a pointed structure. On closer examination, the skin of the affected patches displayed a reddish-purple colour and was tender to the touch. Pulling the wool fibres apart readily caused the skin surface to lacerate. In some areas, the wool grease had been changed to a yellow sticky material, causing the wool fibres to adhere. Keratinous scales of varying size were present on the skin and among the wool fibres.

The condition is fairly widespread in the Stutterheim area and is a source of concern with the farmers.

Bacteriological investigation

Wool specimens were collected by removing the weathered tip of the wool with sterile scissors and then collecting about 10 mm of wool from the surface of an affected area. About 1 g of wool was taken and suspended in 20 mℓ of a sterile 0,15 N NaC1 solution in a McCartney bottle. Subsequently, the denuded area of the lesion was scraped with a sterile scalpel and the flaky material collected was likewise deposited in the McCartney bottle. The bottle was shaken vigorously by hand for 10 min. Cultures were subsequently prepared from the contents of the bottle. Ten per cent horse blood tryptose agar in Petri dishes was used as medium and the cultures were incubated for 48 h at 37 °C in a 10 % CO_2 atmosphere.

Histological examination of skin biopsies

Biopsy specimens were taken under general anaesthesia from the skin of naturally affected and experimental sheep. They were fixed in 10 % buffered formalin and the cut sections were stained with haematoxylin and eosin and Gram stain.

Skin treatments

Various treatments were applied to the skin of experimental sheep. For this purpose, Merino sheep about 15 months old with visually normal fleeces were selected. Areas of about 400 mm^2 on the side of the ribs were clipped close to the skin and subjected to the following different treatments:

(a) Thorough washing with 10 % SURF[1] detergent and subsequently with commercial ether to remove as much organic material as possible from the surface.

For 14 days the following respective treatments were applied 3 times per day to the cleaned areas: moistening with 2 mℓ of a sterile 0,15 N NaC1 solution; moistening with 2 mℓ of horsemeat extract broth[2]; the application of 2 mℓ of a milky suspension of one of the bacteria isolated.

(b) The clipped area was not cleaned ad a sterile saline solution was applied as above.

Biopsy specimens were taken at the end of each period of application.

Extraction of wool yolk components

To study the ability of the bacteria isolated from the lesions to grow on the excretion of the glands and the material desquamated from the skin, the suint (water soluble part) and the wax (ether soluble part) were separately extracted from speciemens of normal greasy wool.

Received 28 January 1987—Editor

[1] Lever Brothers (Pty) Ltd. 73 Maydon Rd, Durban

[2] Standard product of this laboratory

For the extraction of the water soluble component, 0,25 kg of greasy wool was mixed with 250 mℓ of distilled water and heated to 60 °C. The mixture was kept at this temperature and shaken repeatedly for 30 min. The aqueous portion was poured off and filtered through cheesecloth to remove any coarse particles. It was then dialysed against physiological saline for 24 h at 4 °C to adjust the salt concentration to a level acceptable to bacterial growth. To sterilize the extract, it was then heated to 100 °C for 30 min on 3 consecutive days. Before the last day of heating, sterile agar powder was added to a final concentration of 1,5 % to solidify the end-product when poured into Petri dishes.

The ether extract was prepared by mixing 0,25 kg of wool with 250 mℓ of ether, heating the mixture to 30 °C and shaking it for 30 min. The ether fraction was then poured off and filtered through cheesecloth, and the ether was evaporated by heating the mixture to 60 °C. When most of the ether had evaporated, the wax-like deposit was subjected to a vacuum of −0,006 Pa for 2 h at 60 °C. After this treatment there was no smell of ether left in the residue. The wax-like material was then heated to 100 °C and poured into Petri dishes, where it formed a smooth surface.

Granulocyte chemotaxis

The granulocyte fraction was isolated from ovine blood, according to the method described by Carlson & Kaneko (1976). The granulocytes were suspended in HBSS containing 15 % inactivated foetal calf serum to a concentration of 10×10^6 cells/mℓ. Neutrophils represented 89 % of these cells. The viability of the granulocyte suspension was greater than 94 % as determined by the trypan blue exclusion technique.

The chemotaxis test was done by the agarose method described by Nelson, Quie & Simmons (1975). As attractant, a suspension of the bacteria under investigation, was used in separate tests.

The Petri dishes containing the agarose and the reagents were incubated for 4 h at 37 °C in a 7 % CO_2 moist atmosphere. The cells were then fixed with the agarose in place by the addition of 5 mℓ of absolute methanol and leaving them overnight at room temperature. After fixation, the gel was removed and the cells stained with Giemsa's stain.

The area of migration of the netrophils around the wells was recorded by dark-field microphotography at 80 × magnification.

Variation in media

A medium was prepared by dissolving 1,2 g of agar[3] in 100 mℓ of 0,15 N NaCl solution and allowing it to solidify in Petri dishes after sterilization for 10 min at 110 °C. The object was to determine the ability of the experimental organisms to grow on a poor nutrient medium. According to the Oxoid Manual (1982), the analysis of agar is as follows:

Moisture	7,0 %
Ash	2,0 %
Acid soluble ash	0,1 %
Cl as NaCl	0,1 %
P	0,005 %
CO_4	0,9 %
Total nitrogen	0,1 %
Ca	100 ppm
Mg	40 ppm
Fe	80 ppm

Separate volumes of the aqueous extract from greasy wool were adjusted to pH 7,0; 6,5; 6,0; 5,5 and 5,0, respectively, before the last stage of sterilization and the addition of the agar, to test the influence of pH on the growth of the organisms.

[3] Oxoid

The experimental cultures were incubated at 37 °C and at ambient temperature.

The extent of growth was estimated according to the size of the colonies and the tendency towards confluence and expressed as 1+ to 4+.

Influence on wool growth of the long-term application of a culture suspension to the skin

The wool on 2 adjacent 400 mm^2 areas was clipped to the skin. Two mℓ of a milky-white suspension of *Enterobacter agglomerans* was applied to one of the areas once daily for 6 weeks and then the wool growth on the 2 areas was compared.

Rainfall figures for the Stutterheim area

To obtain an impression of the atmospheric moisture in the area where the affected sheep had been kept, the average rainfall figures for the Stutterheim area were obtained from the South African Weather Bureau.

The data are given in Table 1.

TABLE 1 The average monthly rainfall and number of rainy days per month for the period 1939–1975 (Stutterheim)

Month	Rainfall (mm)	Rainy days
Jan	98,3	14
Feb	101,3	13
March	114,2	14
Apr	57,3	9
May	31,4	6
June	15,4	4
Jul	12,8	3
Aug	31,1	5
Sept	45,0	8
Oct	69,0	11
Nov	80,6	12
Dec	80,3	12
Year total	736,7	111

RESULTS

Bacteria isolated

The following organisms were isolated from the wool and flaky material removed from the sheep:

Enterobacter cloacae
Staphylococcus aureus
Acinetobacter calcoaceticus
Micrococcus luteus

Pseudomonas aeruginosa
Corynebacterium bovis
Arthrobacter sp.
Enterobacter agglomerans
Chromobacterium typhiflavum
Corynebacterium xerosis

Histological appearance of naturally affected skin

There was a general infiltration of cells into the dermis, being more manifest in the area immeditely beneath the epidermis and less so in the deeper areas (Fig. 1).

Deeper in the dermis the accumulation of cells tended to be perivascular. The cells were mainly round cells consisting of macrophages, lymphocytes, plasma cells with small numbers of eosinophils in parts (Fig. 2).

The blood vessels in the dermis were dilated. The dermis showed oedema which was fairly marked in certain areas (Fig. 3).

There was a definite hyperkeratosis (Fig. 1). The epidermis showed an increase in keratohyaline and the Langerhans cells were prominent. No bacteria could be detected in the tissues.

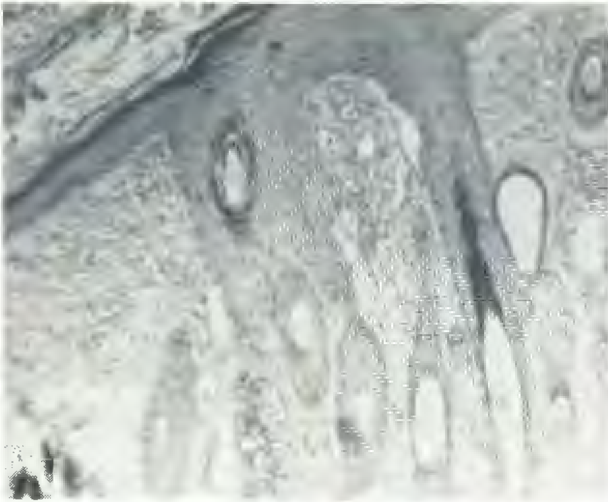

FIG. 1 Infiltration of round cells into the dermis. Hyperkeratosis: HE × 40

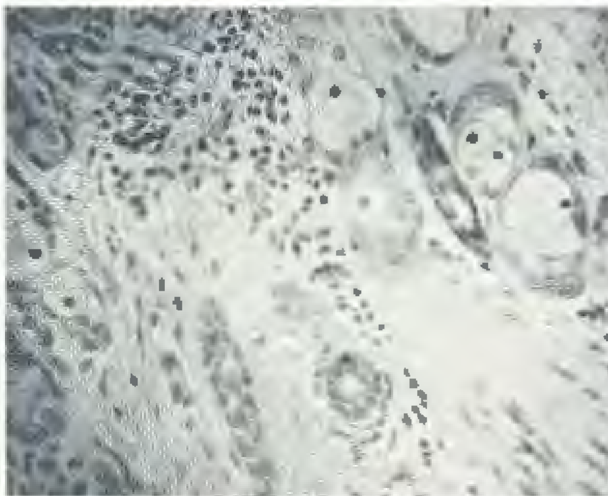

FIG. 2 Infiltration of round cells between the dermal glands and hair follicle roots: HE × 100

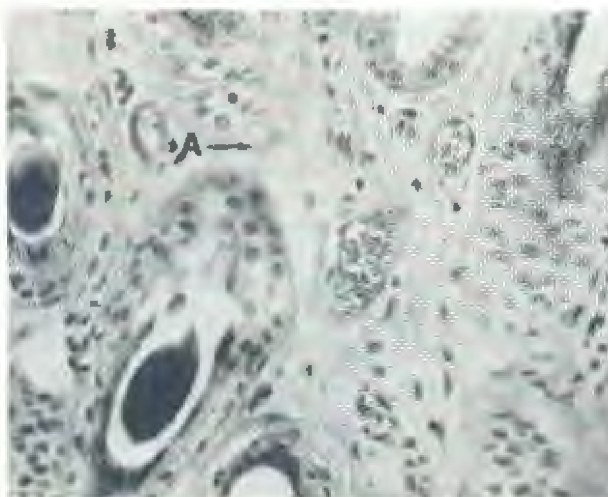

FIG. 3 Dilated blood vessels and area of oedema (A): HE × 100

Skin treatments

The application of a sterile saline solution to the skin cleaned with detergent and ether did not bring about any histological changes in the skin. However, when broth was applied to the skin under the same conditions, a rather marked infiltration of round cells into the dermis was induced. When saline was applied to skin not cleaned with ether and detergent, an infiltration of cells resulted.

The application of suspensions of all the bacterial cultures to the cleaned skin of a sheep caused the infiltration of round cells into the dermis which, in some instances, was massive. As an example may be given the results obtained with *Enterobacter agglomerans*. Fig. 4 shows the extent to which this organism caused an infiltration of cells into the dermis, while Fig. 5 depicts the condition of untreated skin on the same sheep.

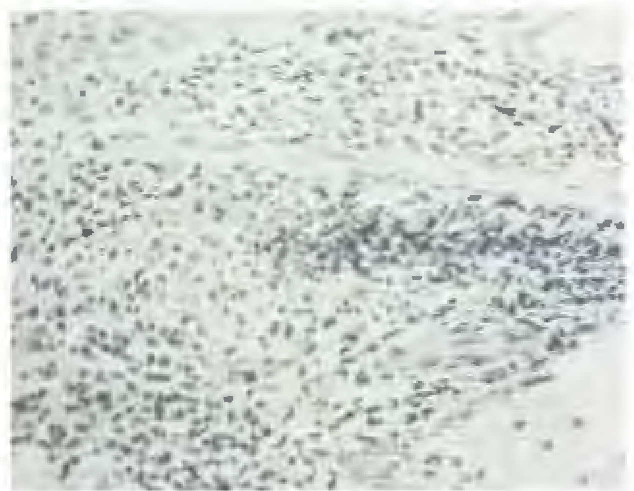

FIG. 4 Infiltration of cells into dermis after application of a suspension of *E. agglomerans:* × 40.

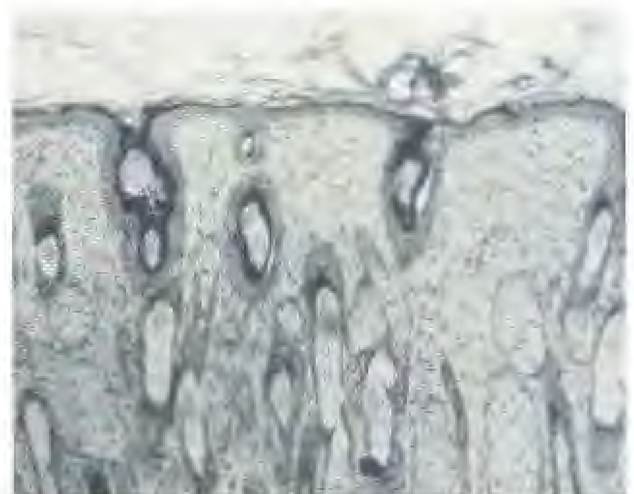

FIG. 5 Histological appearance of untreated skin on the same sheep used to produce Fig. 4: HE × 40

Chemotaxis

All the bacterial isolates elicited a positive chemotactic response towards blood granulocytes. Fig. 6 illustrates the results obtained with *Enterobacter agglomerans*, as an example.

Growth on a poor nutrient medium

The extent to which the different bacterial isolates grew on a medium consisting of agar plus saline is reflected in Table 2.

Growth on extracts from wool yolk components

None of the organisms grew on the waxy component of wool yolk.

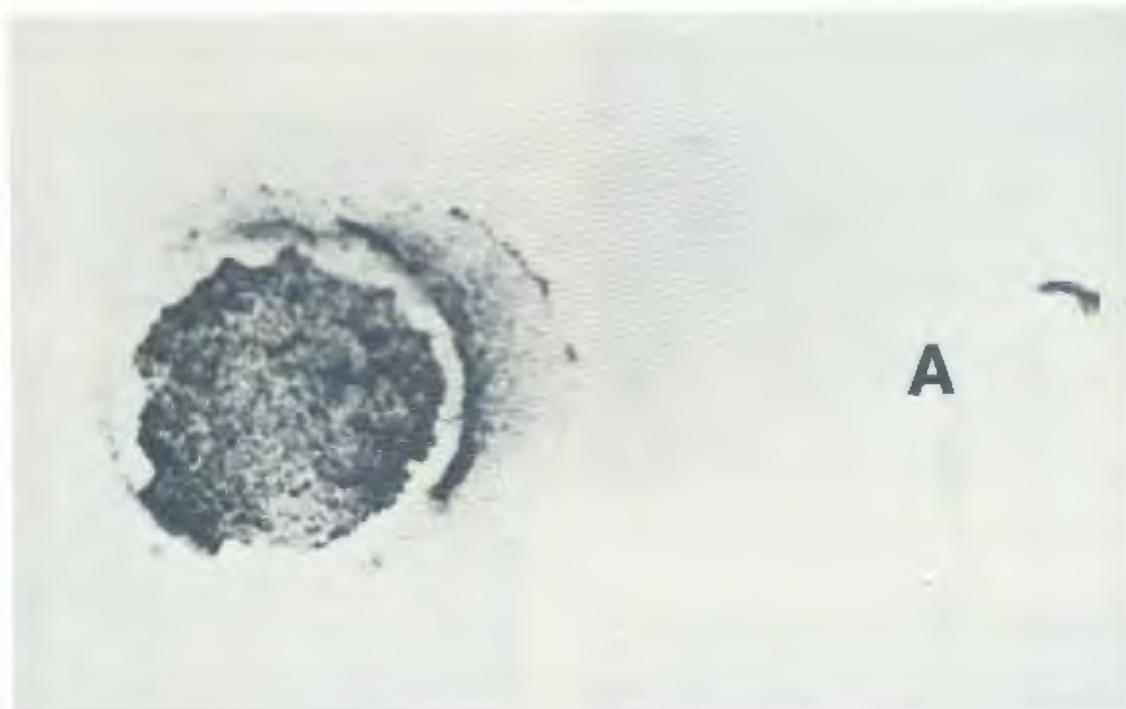

FIG. 6 Blood granulocytes attracted towards the well containing suspension of *E. agglomerans:* (A) × 80

TABLE 2 Growth of bacteria isolated from skin lesions on 1,2 % agar in 0,15 N NaCl

Bacteria	Degree of growth	
	Temp. of incubation	
	37 °C	Room temp.
E. cloacae	2+	1+
S. aureus	2+	1+
A. calcoaceticus	2+	1+
M. luteus	2+	1+
P. aeruginosa	2+	1+
C. bovis	2+	1+
Arthrobacter sp.	2+	1+
E. agglomerans	2+	1+
C. typhiflavum	2+	1+
C. xerosis	2+	1+

The medium prepared from the aqueous extract of wool yolk plus agar supported the growth of all organisms to a much greater extent than the agar alone. The results are recorded in Table 3.

TABLE 3 Growth of bacteria isolated from skin lesions on an aqueous extract of wool yolk solidified with agar

Bacteria	Temp. of incubation	
	37 °C	Room temp.
E. cloacae	3+	2+
S. aureus	3+	2+
A. calcoaceticus	3+	2+
M. luteus	3+	2+
P. aeruginosa	3+	2+
C. bovis	3+	2+
Arthrobacter sp.	3+	2+
E. agglomerans	3+	2+
C. typhiflavum	3+	2+
C. xerosis	3+	2+

The influence of pH on growth

The results of culturing the bacterial isolates on aqueous wool yolk extract at various pH levels are recorded in Table 4.

TABLE 4 The growth response of the bacterial isolates to different pH levels

Bacteria	pH level				
	5	5,5	6	7	8
E. cloacae	—	—	4+	3+	1+
S. aureus	—	1+	4+	4+	4+
A. calcoaceticus	—	—	4+	4+	4+
M. luteus	—	—	4+	4+	4+
P. aeruginosa	—	—	4+	4+	4+
C. bovis	—	1+	4+	4+	4+
Arthrobacter sp.	—	—	4+	4+	4+
E. agglomerans	—	—	4+	4+	4+
C. typhiflavum	—	—	4+	4+	4+
C. xerosis	—	—	4+	4+	4+

The effect of long-term application of a culture to the skin

The application of a suspension of a culture of *Enterobacter agglomerans* to the skin for 6 weeks resulted in a retardation of wool growth. The wool growth over the treated area was irregular and the staples tended to be pointed. The wool fibres were glued together by a yellowish, sticky material. Most of these features can be

FIG. 7 Comparison between wool removed from treated area and that from adjacent untreated area

seen in Fig. 7, where a sample of wool from the treated area is compared with a sample from the adjacent control area.

Discussion

Only 2 of the range of bacterial species isolated from affected areas on the sheep under investigation have been identified as the cause of overt clinical disease. Burrell *et al.* (1982) proved *P. aeruginosa* to be the cause of subacute fleece-rot dermatitis, and Scott, Fraser & Martin (1980) described *S. aureus* as the cause of dermatitis. But the experimental evidence produced in this study leaves no doubt that all the bacteria isolated from the affected areas have the potential to contribute to the chronic condition of the skin observed in these sheep. All these bacteria are ubiquitous, and it appears that they only require suitable circumstances to exert their pathogenic influence on the skin of the sheep.

The application of sterile saline to the skin from which all debris had been removed by thorough cleaning elicited virtually no reaction, while the application of nutrient broth under the same conditions evoked the large-scale infiltration of round cells into the dermis. The most reasonable explanation for this finding is that the debris (including wool yolk) on the surface of the skin, when moistened, promotes the multiplication of the bacteria normally present in the fleece and on the skin. This in turn elicits the skin reaction. The conclusion is confirmed by the reaction resulting from the moistening of the uncleaned skin. By the same token, the nutrient broth applied to cleaned skin promotes the growth of bacteria that produce the skin reaction. Because the dry skin in control patches on the same sheep was not subject to any pathological changes, moisture must be accepted as the trigger in initiating the series of events leading to the chronic skin condition.

According to the results recorded in Table 2, the bacteria isolated from the skin and fleece have limited nutritional requirements, being able to grow on a medium consisting of agar only. Increasing the temperature from the ambient level to 37 °C has a stimulating effect. Table 3 shows that all the bacterial isolates grow well on a medium containing an aqueous extract of wool yolk. Table 4 shows that lowering the pH of the medium to below pH 5,5 inhibits the growth of all the organisms. At pH 7,0, which is more or less the normal level for the material on the surface of the skin, the growth is profuse. One must, therefore, accept that given suitable circumstances, these bacteria can multiply in the debris on the surface of the skin of a Merino sheep.

All these organisms attract blood granulocytes through chemotaxis *in vitro* (Fig. 6). When applied to the skin they also cause the infiltration of a variety of cells into the dermis (Fig. 4). Although in the experimental situation the extent of infiltration of cells into the dermis was much greater than in the natural disease, it is to be understood, because in the experiment a thick suspension of organisms was applied to the skin, while in nature the growth of the bacteria is never so profuse. Nonetheless, pathological changes analogous to those seen in the natural disease were produced. By means of a less intensive treatment over 6 weeks, changes in the fleece similar to those seen in the affected sheep were produced.

The plasma cells in the dermis and the activity of the Langerhans cells in the dermis and epidermis in natural cases indicate that the skin was subjected to an antigenic stimulus—no doubt from the bacterial activity on the skin surface.

One must therefore conclude that the bacteria isolated are the cause of the chronic scaly condition of the skin and of the retarded wool growth in the affected areas on the sheep under investigation.

In comparing the experimental results with the condition as it affects sheep in the Stutterheim district, one can suggest the following sequence of changes: With 111 rainy days per year (Table 1), sufficient moisture is available to wet the fleece of sheep, particularly those with open fleeces as a result of poor staple formation. Sheep with conformational faults, such as a depression between the shoulder blades on top of the withers, will be more prone to wetting of the fleece at such sites. Wetting of the fleece also takes place while sheep are lying down on vegetation moistened by rain or dew. It appears as though sufficient moisture is provided under the conditions prevailing in the Stutterheim area for the bacteria present on the skin to multiply and cause the lesions seen in the affected sheep. The moisture does not appear to be sufficient for the development of fleece-rot in these particular instances.

Control measures suggesting themselves are the following: the selection of sheep with a tight fleece and good staple formation; the culling of sheep with conformational faults conducive to wetting of the fleece; the use of antibacterial substances, particularly those likely to result in an acid reaction of the environment.

Acknowledgements

We are greatly indebted to Dr M. M. Henton for identifying the bacterial isolates. The Section of Pathology, Veterinary Research Institute, Onderstepoort, kindly prepared the histological sections.

References

Burrell, D. H., Merritt, G. C., Watts, J. E. & Walker, K. H., 1982. Experimental production of dermatitis in sheep with *Pseudomonas aeruginosa*. *Australian Veterinary Journal*, 59, 140–144.

Carlson, J. P. & Kaneko, J. J., 1976. Isolation of leucocytes from bovine peripheral blood. *Proceedings of the Society for Experimental Biology and Medicine*, 142, 853–856.

Merritt, G. C., 1981. Precise role of *Pseudomonas aeruginosa* and other organisms. Workshop on Fleece-rot and Mycotic Dermatitis in sheep. University of Sydney. 26–27 May 1981, pp. 18–19.

Merritt, G. C. & Watts, J. E., 1978. The changes in protein concentration and bacteria of fleece and skin during the development of fleece-rot and body strike in sheep. *Australian Veterinary Journal*, 54, 517–520.

Nelson, R. D., Quie, P. G. & Simmons, R. L., 1975. Chemotaxis under agarose: A new and simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leucocytes and monocytes. *Journal of Immunology*, 115, 1650–1656.

Scott, F. M. M., Fraser, J. & Martin, W. B., 1980. Staphylococcal dermatitis of sheep. *Veterinary Record*, 107, 572–574.