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RETARDATION OF WOOL GROWTH IN MERINO SHEEP CAUSED BY BACTERIA

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ABSTRACT

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A condition evidenced by retarded growth of wool with alteration of the yolk into a yellow, sticky, wax-like substance was investigated. The condition was associated with hyperaemia and cellular infiltration into the dermis in the affected areas. Three bacterial species, viz. *Enterobacter aerogenes*, *E. agglomerans* and *Hafnia alvei*, which could grow on the water-extractable component of wool-yolk, were incriminated as the cause of the condition.

INTRODUCTION

Merino rams from 2 farms in the Kokstad and Graaff-Reinet areas respectively were submitted to the Veterinary Research Institute, Onderstepoort, for the investigation of an abnormal condition of their wool.

The manifestations of the condition were investigated on the farms from which the rams were submitted. The epidemiologic features of the condition were similar on both farms.

Farmers purposely select their sheep for the high yolk content of their wool, since a high yolk improves the quality of the wool. The condition occurred frequently in the genetic line of rams with a higher wool yolk content and virtually not at all in the family of rams with a drier fleece with a low yolk content.

The yolk content of wool is influenced to a large extent by the level of nutrition of the sheep. In both studs the rams were kept on a highly nutritious diet consisting of concentrated pellets and lucerne to increase the yolk content of their wool and stimulate their breeding ability. The ewes, on the other hand, were left to graze on open range, with the result that they had a markedly lower yolk content in their wool. The rams, even after the retardation condition had been noticed among them, freely contacted the ewes during the breeding season and thus there would have been ample opportunity for transmitting any infectious agent. No cases of the condition under investigation occurred, however, among the ewes.

The condition closely resembled "Bolo" disease, described in monthly reports of the Division of Veterinary Services during 1982/83, and since it detracted considerably from the value of the fleece, a systematic search for the cause was undertaken.

MATERIALS AND METHODS

Bacteriological investigation

Wool specimens, collected from the affected and normal areas of the fleece, were suspended for 5 min in sterile 0,15 N NaCl in McCartney bottles which were repeatedly shaken. Cultures were subsequently prepared from the saline and the wool fibres were rubbed over the surface of the medium. Ten per cent horse blood-tryptose agar in Petri dishes was used and the cultures were incubated for 48 h at 37 °C in a 10 % CO_2 atmosphere.

To test the action of antibacterial substances on the bacteria isolated, the substances were included in the horse blood tryptose agar in the appropriate dilutions.

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Histological examination of skin biopsies

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

Extraction of wool yolk components

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

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 was 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, filtered through cheesecloth and the ether 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.

Preparation of bacterial cell extracts

The bacteria under test were thickly seeded onto 10 % horse blood tryptose-agar in Petri dishes and incubated for 48 h at 37 °C in a 10 % CO_2 atmosphere to obtain a confluent growth. The bacterial growth from 10 Petri dishes was harvested, using 10 mℓ of physiological saline. This produced a milky white suspension. The suspension was subjected to ultrasonic vibration at 6 micron amplitude for 60 s on 5 consecutive occasions, the resulting suspension constituting the final extract.

Testing the lipolytic activity of bacterial extracts

A mixture was made of 0,5 g of ether extract of wool grease plus 0,1 mℓ of bacterial cell extract and incubated for 18 h at 37 °C. The control consisted of the 0,5 g

of ether extract plus 0,1 mℓ of saline. The free fatty acid content of the extract and control was subsequently determined according to the method of De Villiers, Van der Walt & Procos (1977).

Testing the effects of bacterial cell extracts on the skin of sheep

Cell extracts from the bacteria isolated from affected wool were applied to the skin of sheep once a day for 10 days. After 10 days biopsy specimens were collected from the separate points and examined for histological changes.

Testing the effect of live bacterial suspensions on wooled skin of Merino sheep

Live culture suspensions of the different bacteria isolated from affected wool were applied in separate spots to unshorn skin of sheep. The application was repeated every 2nd day for 10 days. After this the wool was examined visually for changes and biopsy specimens were collected from the skin.

RESULTS

Gross lesions

The affected sheep showed areas of depression on the surface of their fleeces. The number, size and shape of the depressed areas varied and they were irregularly distributed over the body. The largest area was 150 × 70 mm. The normal staple formation of the fleece was changed to smaller, pointed staples, giving the surface a rough, dirty appearance. Inspection of the opened fleece in the depressed areas revealed that wool fibres were glued together by a yellowish, sticky material. Dry flakes of white material were present on the skin and in the wool close to the skin. The skin appeared hyperaemic. In Fig. 1, the difference in length and appearance between wool shorn from an affected spot and from an area adjacent to it can be seen.

When the seemingly normal parts of the fleeces of other rams in the flocks of origin were opened, areas of discoloured, sticky wool, showing white flakes on the skin and in the part of the wool immediately over it were revealed. In these lesions the entire length of the fibres was not affected and the surface of the fleece over them was not depressed.

Bacteria isolated from affected wool

A large variety of bacteria was isolated from the unaffected wool on the sheep under investigation. It would not have served a purpose to identify the individual species in these mixed cultures. On the affected wool, however, 3 different bacteria were consistently present, mostly to the exclusion of all other bacteria: *Enterobacter aerogenes, Enterobacter agglomerans* (syn. *Erwinia herbicola*) and *Hafnia alvei* (syn. *Enterobacter alvei*).

Cultural studies on causative organisms

All 3 organisms grew luxuriantly on blood tryptose-agar. The pH of the medium was changed from 7,06 to the following for the organisms indicated:

E. aerogenes: pH 8,86

E. agglomerans: pH 8,81

H. alvei: pH 8,82

The colonies of *E. agglomerans* turned yellow on culture as a result of the formation of a pigment which is in accord with Buchanan & Gibbon's (1974) description of this organism.

All 3 organisms grew well on the medium prepared from the water-soluble component of the skin gland secretion, while the ether extract did not support any growth.

The lipolytic effect of bacterial extracts

The bacterial extracts of the 3 organisms did not bring about any increase in the fatty acid content of the ether extract of the wool grease. No lipolytic activity was therefore detected.

Histological changes

The section from an affected area of the skin (Fig. 2) showed desquamation of the keratinized layers of the epidermis. The blood vessels in the dermis were dilated. There were accumulations of neutrophils and lymphocytes in the dermis that were more intense in the superficial layers (Fig. 2 & 3). Some neutrophils were scattered around the hair roots and among the dermal glands (Fig. 4). Although there were some neutrophils among the cells of the root sheath of some of the hair follicles (Fig. 5), the hair roots were nowhere sufficiently affected to cause shedding of the wool fibres.

The sections from the areas on the skin to which the live cultures were applied showed the same histological changes for the 3 organisms as the naturally affected skin. The lesions were possibly less severe in the sense that there were fewer neutrophils in the deeper layers among the fibre roots. Fig. 6 shows the changes brought about by *E. agglomerans*. In this section the hyperaemia together with the presence of lymphocytes and some neutrophils in the upper layers of the dermis is evident.

The histological changes brought about by the cell extracts of the 3 bacteria were similar in nature and degree to those brought about by the live organisms.

Quality of wool on diseased areas

Scanning electronmicrographs showed that there was no structural difference between wool fibres derived from affected and those from normal areas of the skin.

Tests performed by the Wool Testing Laboratory of the South African Wool Board showed that the bundle-breaking strength of the wool from affected areas was normal, and the yellow discoloration was easily removed by washing.

Effect of antibacterial agents

The inclusion of Anabac* (1:200) and phenol (1:1000) in horse blood tryptose agar completely prevented the growth of all 3 organisms.

Treatment of affected sheep

Affected sheep were shorn and thoroughly soaked with a 1/200 solution of Anabac. The subsequent wool growth on the affected parts was normal in appearance in all respects. Sections from treated skin were histologically normal 2 weeks after treatment.

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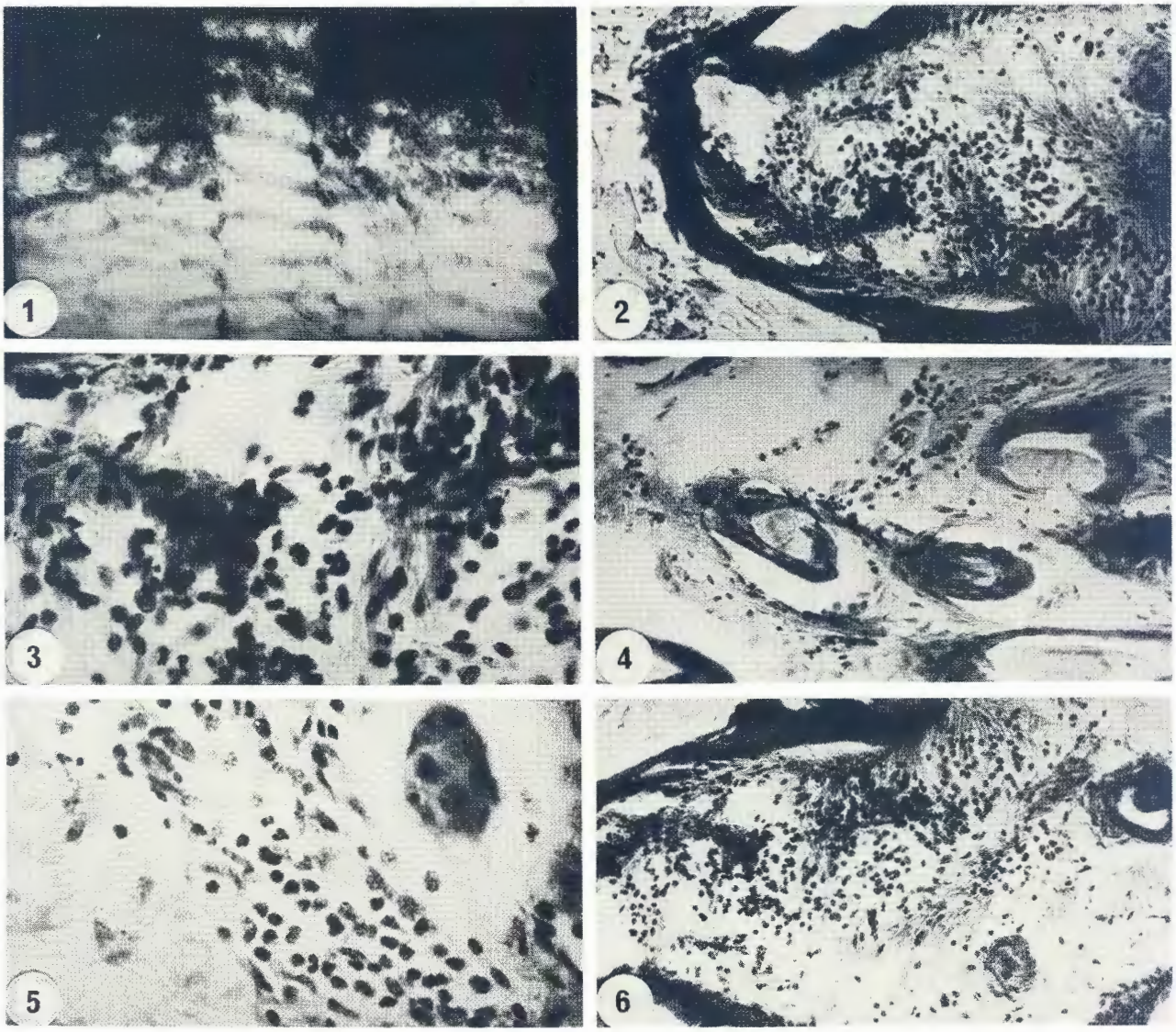

FIG. 1 Difference in length between affected wool and non-affected wool (in centre) from adjacent areas on the same sheep

FIG. 2 Desquamation of surface layers of epidermis and cell infiltration into dermis: HE × 100

FIG. 3 Neutrophils and lymphocytes in dermis: HE × 500

FIG. 4 Cell-infiltration into tissue surrounding wool-fibre roots. HE × 100

FIG. 5 Neutrophils in root sheath of follicles: HE × 500

FIG. 6 Cell-infiltration into dermis after experimental application of live cultures: HE × 100

DISCUSSION

It was found that the problem under investigation was essentially a condition where 3 bacterial species were able to grow on the water-extractable component of wool yolk and that the skin affected by their products altered the growth of the wool fibres. The change towards alkalinity in the blood tryptose agar brought about by the growth of the organisms and the failure of an extract of the organisms to effect an increase in the free fatty acid content of the ether extract of wool yolk, support the finding that the organisms grow on the water-extractable part of wool yolk. An extract of these bacteria could produce pathological changes in the dermis similar to those produced by the live bacteria experimentally applied to the skin and in the condition as it occurs in nature. This condition consisted of hyperaemia of the dermis with infiltration of neutrophils and lymphocytes into the tissue. Although the cellular infiltration was more marked in the upper layers of the dermis, some cells were scattered around the hair roots and among the dermal glands. There were even some neutrophils among the cells of the root sheath of some of the hair follicles. The overall effect of the histological changes in the dermis was a retardation of the growth of the wool fibres, hyperaemic appearance of and the desquamation of flakes from the skin surface.

The similarity of the lesions produced with the extracts to those of the condition as it occurs in nature and the possibility of clearing the lesions within a short while with antibacterial agents incriminate the bacteria as the cause.

The appearance of the wool over the affected areas could also be attributed to the activities of the bacteria isolated. The shortness of the fibres is due to the interference with the physiological function of the fibre root as evidenced by the cellular infiltration around the roots. The tacky substance gluing the fibres together is the wax remaining after the water-extractable component of the yolk has been metabolized by the organisms. The yellow discoloration of the wool is the result of the yellow pigment produced by *E. agglomerans* (Buchanan & Gibbons, 1974).

According to Buchanan & Gibbons (1974), *E. aerogenes* and *H. alvei* are found in the faeces of man and animals, sewage, soil, water and dairy products. *E. agglomerans* exists on plant surfaces. These 3 organisms are, therefore, virtually ubiquitous, and all that is required for their multiplication is a suitable environment. The yolk contained in the fleece of Merino sheep provides a suitable growth medium, but it is not yet known whether other factors, such as moisture, play a decisive role.

Since these bacteria are susceptible to the action of bactericidal substances, to control the infection it seems feasible to develop a system whereby sufficient of an antibacterial substance can be introduced into the fleece of rams selected for a high yolk content of their wool and kept on a high level of nutrition. A large scale experiment is currently being conducted to investigate this possibility.

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