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The bacteriology of sheep periodontitis with special reference to *Actinobacillus actinomycetemcomitans*

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SUMMARY

Some clinical features of periodontitis in sheep from the Karoo are similar to those of juvenile periodontitis in man. The aim of this study was to investigate whether the bacteriology of this disease had any resemblance to that of human juvenile periodontitis, with particular reference to ***Actinobacillus actinomycetemcomitans.*** *Twenty-five sheep were used: 11 were diseased animals and 6 were healthy animals from the same flock. A further 8 healthy controls were selected from a different breed and flock. Darkfield microscopy revealed that the subgingival plaque of both diseased and healthy animals was sparse and with few motile organisms and spirochaetes. On culture* ***A. actinomycetemcomitans*** *was not recovered from any of the sheep. Serum antibody titres, however, revealed raised titres against this organism in two of the diseased sheep but the mean values for diseased and control animals did not differ significantly. The titres against* ***Bacteroides intermedius*** *and* ***B. gingivalis*** *were raised in the diseased sheep in comparison to their flock controls ($p<0,05$). It appears as if* ***A. actinomycetemcomitans*** *plays an insignificant role in sheep periodontitis but that the* ***Bacteroides*** *species may be a more productive organism to investigate.*

OPSOMMING

Sekere kliniese kenmerke van periodontitis in Karoo-skape toon 'n ooreenkoms met menslike juveniele periodontitis. Die doel van hierdie studie was om die bakteriologie van skaapperiodontitis te ondersoek vir moontlike ooreenkomste met dié van juveniele periodontitis en met spesiale verwysing na ***Actinobacillus actinomycetemcomitans.*** *Vyf-en-twintig skape is ondersoek: 11 was aangetaste Karoo-skape en 6 was gesonde skape vanuit dieselfde kudde. 'n Verdere 8 diere is as kontroles uitgesoek uit 'n ander kudde en ras. Die donkerveld-mikroskopiese studies het getoon dat die subgingivale plaak van aangetaste en gesonde skape weining verskil het en dat motiele organisme- en spirogeettellings in beide laag was. Met kweking is* ***A. actinomycetemcomitans*** *nie gevind nie. Die antiliggaamvlakke tenoor dié organisme was egter redelik hoog in twee aangetaste skape maar statisties is nie betekenisvolle verskille tussen die gemiddelde waardes van aangetaste en kontrole diere gevind nie. Die waardes teenoor* ***Bacteroides intermedius*** *en* ***B. gingivalis*** *was egter beduidend in die aangetaste skape ($p<0,05$) verhoog. Dit blyk dus dat* ***A. actinomycetemcomitans*** *waarskynlik nie 'n belangrike rol in skaapperiodontitis speel nie. Daarenteen mag studies toegespits op die* ***Bacteroides*** *spesies in hierdie diere meer vrugbaar wees.*

INTRODUCTION

Periodontitis in sheep, or 'broken-mouth' as it is frequently referred to colloquially, is a condition which has been reported from several countries and has been observed in different breeds (Hitchin, 1957; Hitchin and Walker-Love, 1959; Armstrong, 1960; Dalgarno and Hill, 1961; Duckworth *et al,* 1962; Markham and Lyle-Stewart, 1962; Lyle-Stewart, Hatt and Cresswell, 1966: Quarterman and Delgarno, 1968; Cutress and Ludwig, 1969; Hatt, Lyle-Stewart and Cresswell, 1968; Cutress *et al,* 1972; Suckling *et al,* 1974; Cutress, 1976; Steele and Henderson, 1977; Spence, Aitchinson and Sykes, 1980). Although the disease has also been observed in South African flocks (Dreyer *et al,* 1982) no further information about sheep periodontitis in this country is available.

The pathogenesis of the condition has been studied (Cutress, 1976; Spence *et al,* 1980) and it is generally accepted that it is a bacterially induced inflammatory disease similar to that found in man (Page and Schroeder, 1982). A number of other causative factors have, however, been considered. Excessive occlusal stress (Hitchin and Walker-Love, 1959; Markham and Lyle-Stewart, 1962; Duckworth *et al,* 1962), insufficient dental excercise (Porter, Scott and Manktelow 1970), genetic factors (Hitchin, 1957) selenium deficiency (Cutress and Ludwig, 1969), low calcium and phosphorus intake (Gunn, 1969; Cutress *et al,* 1972; Suckling *et al,* 1972; Suckling *et al,* 1974) and grazing offering poor

mechanical cleansing (Dreyer *et al,* 1982) are some of the factors that have been reported. Convincing experimental evidence supporting their role as primary factors is, however, lacking. In light of the accepted role of bacteria in periodontitis of humans and animals, it seems probable that periodontopathic bacteria are the major cause of sheep periodontitis (Page and Schroeder, 1982). Qualitative assessment of the bacteriology of the condition has, however, received no attention in the literature.

In sheep periodontitis the clinical features resemble those of juvenile periodontitis in man in as far as the condition appears circumpubertal and presents with an incisor distribution (Page and Schroeder, 1982). The bacteria involved in human juvenile periodontitis have been investigated by Slots (1979) and others and evidence of a causative role for *Actinobacillus actinomycetemcomitans* in this disease has been offered. The aims of this study were three-fold:

(a) to determine the predominant bacterial morphotypes in periodontal pockets of sheep,

(b) to investigate the role of *Actinobacillus actinomycetemcomitans* and the level of antibodies to this organism in sheep periodontitis, and

(c) to determine serum antibody levels to other suspected periodonto-pathogenic microorganisms in sheep.

MATERIALS AND METHODS

Twenty-five sheep were used for the study. Of these 10 showed signs of periodontal breakdown assessed on the basis of gingival bleeding, recession and detachment of the interdental papillae (Fig. 1). The latter animals were

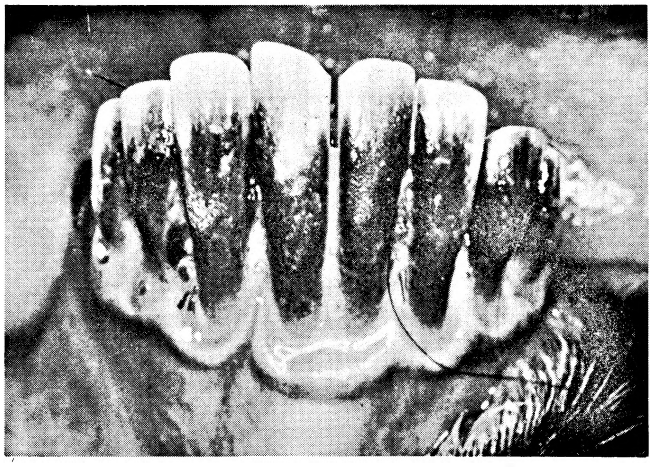

Fig 1: **The diseased sheep from the Karoo all showed signs of interdental gingival destruction, bleeding and recession. Note also the buccal calculus and slight attrition.**

selected from a flock from the semi-arid regions of the Western Cape (Karoo sheep) which was referred to in a previous report (Dreyer *et al,* 1982). All the animals had most of their incisor teeth present and were 2-3 years of age as estimated from the eruption pattern of the fourth permanent incisors and the general physical appearance of the animals. In addition an older animal that had lost all its incisor teeth as a result of periodontal breakdown was included in the sample of diseased animals. Six control animals of similar age were selected from the same flock on the basis of absence of overt clinical signs of periodontitis. Eight periodontally healthy control sheep of a different breed but comparable age were randomly selected from a flock in a different geographical and climatic area near Stellenbosch (Elsenburg sheep, Fig. 2).

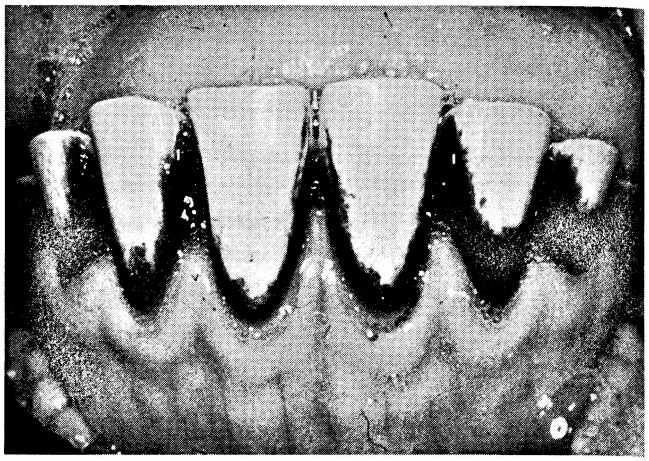

Fig 2: **In contrast to the diseased sheep the healthy animals from Elsenburg showed normal gingival morphology and only moderate attrition.**

Darkfield microscopic studies

Samples of the microbial flora from the pockets or sulci of all the sheep, except the older partially edentulous animal (number 2), were taken for darkfield microscopic study. After removing the supragingival plaque on the distal surface of a central or lateral incisor, a subgingival sample of plaque was removed by inserting the tip of a fine periodontal curette (Gracey pattern 3/4) as deeply into the pocket/sulcus as possible. The specimen was immediately placed in 0,5 ml of 0,85 per cent saline containing 1 per cent gelatine (Listgarten and Helldén, 1978) in screw-cap vials containing 6 glass beads of 4 mm diameter. Within 1 h of removal the samples were dispersed for 10 sec using a vortex mixer. An aliquot was removed for darkfield viewing at 1000x enlargement under a Leitz Laborlux microscope with a darkfield condenser and objective with variable iris diaphragm. One hundred organisms were counted and 4 different morphological forms, namely, spirochaetes, rods, motile rods and cocci, were counted and expressed as a percentage of the total count.

Cultural studies on a selective medium

After removal of the supra-gingival plaque as described above, samples for culture were collected from the contralateral site in the same animals by placing 3 consecutive sterile paper points into the pocket, each for 2-3 sec. The tip of the paper point was cut off with sterile scissors and allowed to drop into a vial containing 0,2 ml of anaerobic Ringer's solution. Within 1 h of removal, the samples were vortexed for 10 sec, ten-fold dilutions in Ringer's solution prepared and 0,1 ml aliquots placed onto a selective media for *A. actinomycetemcomitans* (TSBV medium; Slots, 1982). The plates were incubated in candle jars for 3 days whereafter the total number of

colonies on the appropriate dilution plate was counted and the predominant species identified biochemically.

Enzyme-linked immunosorbent assay (Elisa)

Venous blood was obtained from all 17 sheep from the affected flock and 2 ml aliquots of serum freeze-dried and air-freighted to one of the co-authors (JLE) for determination of antibody titres using the ELISA technique as described by Ebersole *et al* (1980). A battery of organisms was selected for testing mostly on the basis of previously established association with human periodontitis (Taubman, Ebersole and Smith, 1982). These were *Bacteroides intemedius* strain 581, *B. gingivalis* 381, *A. actinomycetemcomitans* Y4, *Eikenella corrodens* 1073, *Campylobacter consisus* 484, *Fusobacterium nucleatum* 364, *Wolinella recta* 371, *Selenomonas sputigena* 1304 and *C. sputorum* S17. With the exception of the latter, a sheep isolate, all the other organisms were human isolates.

The data were expressed as reciprocal titres determined by generating a two-fold dilution curve and assigning the titre as that dilution which showed an OD 405 nm that was two times the background.

Statistical analyses

In light of the relatively small numbers of individuals studied, non-parametric tests were used for the statistical analyses. Pair-wise comparisons were used after the significance levels were adjusted by means of the Bonferroni inequality. In some instances it was necessary to apply logarithmic or root transformations to stabilize the spread. However, where similarity of spread could not be achieved the Wilcoxon signed rank test was applied. A probability level of 0,05 was elected to signify statistical significance.

RESULTS

The mean counts of morphological forms as determined by darkfield microscopy revealed no significant difference in the percentage of spirochaetes between the 10 diseased animals when compared to their flock controls nor the healthy controls from Elsenburg (Table I). Al-

Table I: **The mean percentages of bacterial morphotypes observed in subgingival plaque of periodontally diseased and control sheep using darkfield microscopy.**

Area	N	Spirochaetes	Rods	Motile rods	Cocci
Karoo (diseased)	10	4%	44%	0%	52%
Karoo (normal)	6	0%	17%	0%	83%
Elsenburg (normal)	8	4%	61%	3%	32%

though values for cocci and rods differed significantly between the three groups when using a pair-wise comparison ($p<0,05$) a consistent pattern was not revealed between diseased and control animals of both groups. Very few motile rods were observed and consequently no significant difference between the groups could be established for this morphotype.

The mean total number of bacteria cultured on TSBV medium from the subgingival plaque of the diseased animals did not differ from the controls from the same flock (Table II). Although both were higher than the

Table II: **The mean count of bacteria cultivated on TSBV medium from the subgingival plaque of diseased and normal sheep.**

Area	N	Mean Values
Karoo (diseased)	10	1.5×10^5/ml
Karoo (normal)	6	1.0×10^5/ml
Elsenburg (normal)	8	4.0×10^4/ml

values for the other control group, this difference was not found to be significant in a pair-wise comparison ($p>0,05$). The predominant organism isolated from both experimental and control groups from the affected flock was a gram negative pleomorphic rod and from its metabolic requirements, biochemical reactions, enzyme activities and gas chromatographic profile it most closely fitted the characteristics of *Pasteurella haemolytica* Type A but was not oxidase positive. The predominant organism recovered from the other control group had similar characteristics but with a few exceptions that most closely fitted the characteristics of *Actinobacillus lignieresii* and was also not oxidase positive. In one of the animals from the latter group. *P. haemolytica* Type A was also isolated and from yet another an unidentified assacharolytic gram negative rod was obtained.

The individual antibody titres of the diseased and control animals from the Karoo flock showed varying levels against all the organisms tested (Table III). Statistically significant differences between the two groups of animals were found for *B. intermedius* and *B. gingivalis*. Although a significant difference was not found for the antibody levels against *A. actinomycetemcomitans*, two diseased animals (number 4 and 5) had markedly raised titres against this organism. These animals also had high antibody titres against *E. corrodens*, *C. consisus* or *F. nucleatum*, which were well above the respective mean values (Table III).

In an attempt to demonstrate further trends in these data, the sheep were grouped according to arbitrary high, moderate and low antibody levels (Table IV). This approach revealed that 9 out of the 11 diseased sheep had antibody levels against *B. intermedius* in excess of 3 000 compared to only 2 out of the 6 controls. However, both diseased and healthy animals had low antibody levels against *B. gingivalis*. Antibody levels in excess of 3 000 were measured in 6 of the 11 diseased sheep against *A. actinomycetemcomitans*, in 5 sheep against *C. consisus* and in 3 against *F. nucleatum* whilst practically all the relevant values for control sheep fell into the low category (Table IV). The values for all the other organisms appeared to be similar for both diseased and control animals.

DISCUSSION

The darkfield microscopic features of the plaque harvested from the diseased animals revealed a flora which did not differ from the controls. It was sparse with a paucity of spirochaetes and motile rods. In contrast to other forms of periodontal diseases, where an abundant flora with a high percentage of spirochaetes and motile rods are prevalent (Listgarten and Helldén, 1978), the present results for the diseased animals were consistent

Table III: **The individual antibody titres for the diseased and control Karoo sheep as determined by an ELISA**

	SHEEP NO.	*A. actinomycetemcomitans*	*B. intermedius*	*B. gingivalis*	*E. corrodens*	*C. concisus*	*F. nucleatum*	*W. recta*	*S. sputigena*	*C. sputorum*
DISEASED	1	579	3 146	1 214	1 167	2 890	1 392	2 504	1 590	1 083
	2	3 598	10 635	2 077	1 405	2 214	2 733	3 617	8 456	2 130
	3	1 909	3 134	1 133	1 432	1 938	1 937	1 776	2 666	1 764
	4	13 176	4 285	1 777	1 887	2 416	10 840	2 988	3 894	3 468
	5	11 205	13 851	2 331	8 296	7 282	2 958	4 669	4 024	2 349
	6	1 490	4 749	1 799	2 260	2 333	2 116	3 830	2 072	2 166
	7	1 824	5 041	1 932	1 667	3 304	2 141	1 874	1 445	2 524
	8	1 527	2 324	2 040	1 445	2 174	1 967	1 778	1 928	1 587
	9	3 380	2 627	2 213	2 409	4 332	2 254	3 411	2 566	1 944
	10	4 709	7 004	2 070	2 427	3 105	4 111	4 908	1 578	3 747
	11	3 383	6 825	2 170	2 370	4 318	3 231	6 976	1 628	1 639
CONTROL	12	1 375	5 474	1 227	953	1 374	2 472	4 193	1 323	1 308
	13	1 891	1 258	531	1 824	2 126	2 535	3 142	2 167	2 099
	14	2 793	2 402	407	1 511	2 034	3 638	3 627	1 515	2 133
	15	1 213	1 920	1 307	827	2 248	2 424	3 551	1 939	1 637
	16	1 360	1 366	1 158	1 064	1 404	2 178	3 304	2 431	1 095
	17	1 975	3 560	1 483	3 171	2 521	2 391	8 716	2 578	2 496
	P=	0,417	0,039*	0,010*	0,175	0,073	0,862	0,393	0,862	0,339

* statistically significant

Table IV: **The numbers of diseased and control Karoo sheep grouped according to the antibody titres**

Organism	Reciprocal titres					
	>5000		3000-5000		<3000	
	Dis	Nor	Dis	Nor	Dis	Nor
A. actinomycetemcomitans	2	0	4	0	5	6
B. intermedius	4	1	5	1	2	4
B. gingivalis	0	0	0	0	11	6
E. corrodens	1	0	0	1	10	5
C. consisus	1	0	4	0	6	6
F. nucleatum	1	0	2	1	8	5
W. recta	1	1	5	5	5	0
S. sputigena	1	0	2	0	8	6
C. sputorum	0	0	2	0	9	6

with the findings in human juvenile periodontitis (Listgarten, 1976).

Extensive bacteriological investigation of subgingival microflora is difficult and time-consuming because of the labour-intensive nature of cultural studies. For this reason a selective medium (TSBV) was used on the basis of clinical similarities between this form of sheep periodontitis and human juvenile periodontitis. On cultivation on TSBV medium a gram negative pleomorphic rod, *Pasteurella haemolytica* Type A, was found to be the predominant organism from both the diseased and healthy sheep from the Karoo flock. A morphologically similar gram negative pleomorphic rod, differing on some biochemical criteria and identified as *Actinobacillus lignieresii,* was recovered from the Elsenburg control animals. *P. haemolytica* Type A occurs as a commensal in the nasopharynx of cattle and sheep and as a pathogen causing pneumonia in these animals and septicaemia in lambs (Buchanan and Gibbons, 1974). The same reference quotes that *A. lignieresii* has been recovered from the ruminal contents of cattle and sheep and from the oral mucosa of sheep. This organism has also been found in granulomatous lesions of the upper alimentary tract of cattle and associated with suppurative lesions in the skin and lungs of sheep. The periodontopathogenic potentials of these two organisms are, however, unknown and it is possible that they occupy a commensal niche in subgingival plaque. The fact that no organism with the characteristics of *A. actinomycetemcomitans* was recovered from the diseased sheep is contrary to the hypothesis that sheep periodontitis is an animal equivalent of human juvenile periodontitis. Microbiological studies have shown that *A. actinomycetemcomitans* is an important aetiological agent in human juvenile periodontitis (Zambon, Christersson and Slots, 1983; Eisenmann *et al,* 1983). In sheep the periodontal pockets start on the lingual aspect of the lower incisors and progress in a circumferential fashion around the root (Cutress, 1976; Spence *et al,* 1980; Page and Schroeder, 1982). Consequently it is possible that the failure to recover *A. actinomycetemcomitans* may have been because the deepest part of the pocket was inaccessible for sampling.

The use of the ELISA technique for the determination of antibody titres against *A. actinomycetemcomitans* has been well documented (Ebersole *et al,* 1980; Ebersole *et al,* 1982). The results of the present study did not reveal significantly raised levels of antibodies against this organism in the diseased sheep compared to the flock controls. This supports the contention that the organism is of minor consequence in this form of periodontitis. High levels of antibodies to this bacterium were, however, found in two of the diseased animals. Interestingly these same animals had markedly raised titres against a few other organisms as well. The distinction between diseased and healthy sheep was made on the basis of subjective clinical criteria; if more defined criteria were possible, they may have revealed clearer trends because the antibody data could perhaps then have been related to the level of activity or clinical variant of periodontal disease.

Antibody titres against *B. intermedius* and *B. gingivalis* were significantly raised in the diseased sheep in comparison to the flock controls. In addition the levels against *B. intermedius* in particular were found to be generally high, that is, 9 of the 11 diseased sheep had titres in excess of 3 000 but for *B. gingivalis* all the sheep had low levels. These observations were based on arbitrary levels and although the presence of such trends does not necessarily indicate a pathogenic role for these organisms, they have been implicated in human periodontal disease (Slots, 1979; Spiegel *et al*, 1979; Tanner *et al*, 1979; White and Mayrand, 1981). Consequently further studies directed at isolating the oral *Bacteroides* species in periodontitis-affected sheep should be undertaken. The antibody levels to the other organisms did not reveal any statistically significant patterns. There was, however, a suggestive trend in the results for *F. nucleatum* and *C. consisus* and further studies should include investigation of these bacteria as well.

Unfortunately it was impossible to obtain serum samples from the Elsenburg sheep for antibody determination. Although the values for healthy sheep from a different breed and origin may have been interesting, the healthy sheep from the same flock were deemed to be more valid controls. Control animals from the same flock and breed reduce the number of variables between the animals which is particularly important in a study employing rather small numbers. It is the intention to investigate the antibody levels against suspected periodontopathogens on a larger number of sheep, from various breeds and localities, which may supplement the results of this study.

A few inherent problems were associated with the present study. Disease activity could not be objectively assessed which makes the interpretation of the results speculative. Moreover, in field studies of this nature supportive diagnostic methods, such as radiographic examinations, are impossible. The tortuous nature of periodontal pockets around sheep incisors may prevent adequate sampling from the deepest point in the pocket and because of the inelastic nature of the buccal tissues in these animals it is impossible to study the teeth further back in the arch. Subject to these restrictions, the results of this study suggest that *A. actinomycetemcomitans* plays an insignificant causative role in sheep periodontitis. The most significant conclusion seems to be the raised antibody titres to *B. gingivalis* and *B. intermedius* which may indicate that these two organisms are prevalent in the subgingival plaque of periodontally diseased sheep. Consequently the presence of these organisms should be investigated by cultural studies and the levels of antibodies to them established in a larger sample of animals.

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