Received: 15 April 2023 | Revised: 24 July 2023 | Accepted: 1 August 2023

DOI: 10.1002/vetr.3409

VetRecord

ORIGINAL RESEARCH

Co-occurrence of periodontal lesions and dental wear in incisor and masticatory teeth in two sheep flocks in Brazil

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Funding information
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Grant/Award Number: 001

Abstract

Background: Periodontitis and tooth wear are multifactorial diseases with distinct etiopathogenesis that affect the health, feed efficiency and welfare of sheep.

Methods: This study evaluated the co-occurrence of tooth wear and periodontal lesions in 129 ewes from two Brazilian flocks, clinically classified the lesions and presence of dental calculus, and identified potential pathogens in the dental biofilm of 63 ewes by polymerase chain reaction.

Results: Of the 129 ewes included in the study, 75 presented periodontal lesions, while all animals presented tooth wear and dental calculus. Of the animals with periodontal lesions, 16.2% had lesions in incisor teeth and 52.7% in masticatory teeth. Regarding excessive tooth wear, 38.6% had severe wear on the incisor teeth and 89.1% on the masticatory teeth. Ewes older than 36 months had a higher frequency of periodontal lesions in incisor teeth ($p < 0.001$) and a greater amount of dental calculus ($p < 0.001$), but there was no association between tooth wear and animal age. *Fusobacterium nucleatum*, *Tannerella forsythia* and *Fusobacterium necrophorum* predominated in periodontal lesions.

Limitation: This study is limited by the small sample size and lack of diagnostic imaging to assess periodontal disease.

Conclusion: The co-occurrence of periodontal lesions and excessive dental wear involving both the incisor and masticatory teeth suggests that although the two diseases have different aetiologies, they likely have common risk factors.

KEYWORDS
broken mouth, dental calculus, gingival recession, periodontitis, sheep, tooth wear

INTRODUCTION

Periodontitis and tooth wear are common diseases in sheep that can have severe impacts on both oral and systemic health.[1–6] These conditions can result in low productivity, reduced birth weight and weaker lambs, as well as the premature slaughter of animals that do not reach their genetic potential in terms of longevity.[7] Furthermore, underdiagnosed oral disorders can lead to a high prevalence of maxillary and mandibular disorders in sheep flocks, including mandibular and maxillary osteomyelitis.[8]

Periodontitis is a complex set of polymicrobial and synergistic immunoinflammatory diseases that are associated with dysbiosis of the microbiota in the dental biofilm of sheep.[9] Next-generation sequencing studies of dental biofilm in ruminants have shown that specific groups of microorganisms are involved in healthy and diseased phenotypes.[9–11]

Periodontal diseases in ruminants may be influenced by various environmental, dietary and modifying factors and have a seasonal pattern. These diseases have a progressive and cumulative evolution, with successive episodes of exacerbation and remission of their precursor forms.[12] As the diseases progress to periodontitis, they can cause inflammation, periodontal pocket formation, bone resorption, loss of connective attachment to the periodontal ligament and ultimately, tooth mobility and premature exfoliation.[6,13–16] Such diseases have been reported in various biomes in Brazil[4,17,18] and are likely responsible for significant economic losses in the sheep industry in the country.

Tooth wear, in contrast, is a slow and irreversible loss of tooth structures that occurs without bacterial involvement.[19] Incisors are more frequently affected, and it is common for the entire dental crown to wear away in animals between 3 and 4 years of age.[20–22] Excessive tooth wear in sheep is directly linked to feed efficiency and live weight, with a greater degree of wear resulting in reduced food intake and low body condition score.[23] Studies have shown that sheep with tooth loss may experience a reduction in live weight of 5%–11%.[23] However, many aspects related to the etiopathogenesis, epidemiology and control of tooth wear in sheep are still unknown. Furthermore, the few studies that have evaluated the effects of tooth wear on animal production have generally overlooked the possibility of this disease occurring simultaneously with periodontal diseases, which is likely a common condition in sheep flocks.

It is worth noting that the natural history of these oral health problems in ruminants is ancestral and intensified by domestication. Archaeological and palaeontological studies have provided insight into the occurrence of periodontitis, tooth wear and the presence of dental calculus in ruminants, as well as their relationship with diet and other potential risk factors.[24]

These two complex and often silent diseases can have an acute or chronic course and are typically only diagnosed through active searching during an intraoral clinical examination. However, they can have significant health consequences for sheep flocks, leading to reduced feed efficiency, performance, longevity and animal welfare. In light of this, the present study aimed to investigate the co-occurrence of periodontitis and tooth wear in sheep through intraoral clinical evaluation of incisor and masticatory teeth. Also, the study aimed to characterise the lesions, evaluate the presence of dental calculus and identify potential pathogens in periodontal pockets using polymerase chain reaction (PCR).

MATERIALS AND METHODS

Sheep flocks

A total of 129 breeding ewes from two flocks located in municipalities in the northwest of São Paulo State (Brazil) were examined to determine the prevalence of periodontal lesions, tooth wear and supragingival biofilm. One of the flocks consisted of Suffolk sheep (n = 59) for pedigree breeding, while the other was composed of crossbred animals (n = 70) bred for meat, and all animals from the two flocks were examined. The animals were raised in a semi-extensive system and fed pastures of *Panicum maximum* cv. Massai and *Brachiaria brizantha*, with a commercial mineral mix, provided ad libitum and silage or feed as a winter supplement. It is worth noting that no situational diagnosis or control procedures related to the oral health of the flocks had been carried out in the last 5 years.

Periodontal clinical evaluation, tooth wear and supragingival biofilm

An intraoral clinical examination of animals was conducted using a labial and lingual retractor, mouth opener and flashlight. The results were recorded on an odontogram, and clinical periodontal parameters were evaluated, including gingival recession, periodontal pocket, suppuration, gingival inflammation, presence of calculus and tooth wear. The Williams periodontal probe was used in the partial periodontal evaluation of the dental arch, which was introduced parallel along the dental axis of the labial and lingual surfaces of all incisor teeth and the buccal surface of the masticatory teeth up to the first molar, following the modified Triadan system.[25] Due to limited access, probing of the second and third molars was not carried out. Additionally, clinical attachment loss of the evaluated teeth was not calculated due to the lack of a clear definition of the limits of the enamel–cement junction.

To characterise a periodontal lesion, the presence of a periodontal pocket and/or gingival recession was used as an indicator, following the description of Miller[26] adapted for veterinary medicine, with scores ranging from 0 to 3 (Table 1). Due to the lack of resources for radiological diagnosis and the objectives of the study, the alterations were classified as periodontal lesions without excluding the possibility that they may have originated from endodontic infections. To evaluate tooth wear on both incisor and masticatory teeth, the classification parameters of Hugoson et al.[27] were used, with scores ranging from 0 to 3 (Table 1). Supragingival calculus was assessed by utilising scores from 0 to 3 as parameters (Table 1).

Collection of biofilms from the periodontal pocket and gingival sulcus

Samples of dental biofilm for microbiological evaluation were collected from three sheep flocks located in the State of São Paulo, including the two flocks previously examined. A total of 131 samples were taken from the periodontal pockets of either incisor or masticatory teeth in 47 animals presenting clinical signs of inflammation (spontaneous bleeding or on probing) and clinical probing depth greater than 5 mm. Additionally, biofilm samples from the gingival sulcus of clinically healthy (n = 52) teeth in 16 sheep. The removal of the supragingival bacterial biofilm

TABLE 1 Scores attributed to clinical periodontal parameters evaluated during intraoral clinical examination of sheep.

Score	Gingival recession	Dental biofilm	Dental wear
0	Absence	Clinically not visible	Intact tooth enamel
1	Mild and not extending beyond the mucogingival junction	Slight amount of biofilm adhered to teeth, or less than 25% of teeth with biofilm	Mild tooth enamel wear
2	Moderate or extending beyond the mucogingival junction	Moderate amount of biofilm adhered to teeth, or 25%–50% of teeth with biofilm	Clinical crown wear up to one-third of its length
3	Severe recession or recession extending beyond the mucogingival junction, resulting in the loss of protective tissue in the interdental region and/or poor tooth positioning	Severe amount of biofilm adhered to teeth, or more than 50% of teeth with biofilm	Clinical crown wear greater than one-third of its length

was performed with sterile gauze, and the specimens were collected using a paper cone and preserved in ultrapure water at −80°C until DNA extraction, following the criteria outlined by Gaetti-Jardim et al.[28]

Detection of target microorganisms using PCR

We evaluated the presence of 23 bacterial species: *Aggregatibacter actinomycetemcomitans, Actinomyces israelii, Actinomyces naeslundii, Campylobacter rectus, Dialister pneumosintes, Eikenella corrodens, Enterococcus faecium, Fusobacterium necrophorum, Fusobacterium nucleatum, Porphyromonas asaccharolytica, Porphyromonas endodontalis, Porphyromonas gingivalis, Porphyromonas gulae, Prevotella buccae, Prevotella intermedia, Prevotella loescheii, Prevotella melaninogenica, Prevotella nigrescens, Prevotella oralis, Tannerella forsythia, Treponema amylovorum, Treponema denticola* and *Treponema maltophilum*. Evaluations were made by target DNA amplification using specific primers and amplification conditions for each target microorganism. The bacterial species chosen for analysis were selected based on their ecological and virulence aspects and their potential to cause periodontal disease in various animal species, including ruminants.[4,29,30]

The primers' specificity has been demonstrated in the literature and can be verified through the National Center for Biotechnology Information databases (available at www.ncbi.nlm.nih.gov/). The amplifications were conducted in a PCR machine (Perkin Elmer, GeneAmp PCR System 2400) following a previously described protocol.[4] The programme consisted of one cycle at 94°C for 5 minutes, 30–36 cycles at 94°C for 1 minute, the annealing temperature for each primer for a period varying from 30 seconds to 2 minutes, 72°C for 30 seconds to 2 minutes and one cycle at 72°C for 5 minutes for the final extension of the DNA chain. As a positive control, DNA samples from reference strains were used. PCR amplification products were then subjected to electrophoresis on 1% agarose gel, stained with ethidium bromide (0.5 g/mL) and photographed using a Kodak camera (Electrophoresis Documentation and Analysis System 120) under ultraviolet light transillumination. A 1 kb DNA ladder marker (Gibco) was used as a molecular weight standard.

TABLE 2 Distribution of the occurrence and intensity of periodontal lesions in 129 clinically evaluated sheep, according to age group.

Age range (months)	Number of animals	Gingival recession, *n* (%)			
		0	1	2	3
12–36	41 (32)	26 (63)	4 (10)	9 (22)	2 (5)
>36	88 (68)	28 (32)	15 (17)	29 (33)	16 (18)
Total	129 (100)	54 (42)	19 (15)	38 (29)	18 (14)

Note: '*n*' denotes the number of examples detected.
0: absence of gingival recession; 1: gingival recession in one tooth; 2: gingival recession in two or more teeth.

Statistical analysis

The data collected were analysed using Statistica 7.0 (StatSoft) software. To assess the correlation between animal age (category), presence of gingival recession in incisor and masticatory teeth, supragingival biofilm and tooth wear, contingency tables were organised, and the data were distributed according to frequency for each animal and dental group. The scores for different clinical parameters were also studied. The interrelationships between observed clinical parameters were evaluated using Student's *t*-test. The presence of any association between clinical parameters and the distribution of different microorganisms was assessed through the Spearman correlation test, which has values ranging from −1 (absolute antagonistic association) to +1 (absolute positive association). Animals were grouped by age into two categories: 12–36 months and greater than 36 months. The significance level of the tests was set at 5% ($p < 0.05$).

RESULTS

Prevalence of periodontal lesions, supragingival biofilm and tooth wear

A total of 129 breeding ewes from the two flocks were examined, comprising 41 animals aged between 12 and 36 months and 88 animals aged over 36 months. Of these, 75 (58%) had periodontal lesions, with most ewes ($n = 60$) being older than 36 months. The most common type of lesion observed was a gingival recession (score 2), found in 38 of the affected animals. All animals with periodontal lesions in the incisors (21/129) were older than 36 months, and 86% (18/21) had lesions in more than one incisor. Additionally, 68

TABLE 3 Distribution of the occurrence and intensity of periodontal lesions in the incisor and masticatory teeth of 129 clinically evaluated sheep, according to age group.

Age range (months)	Number of animals	Gingival recession, *n* (%)					
		Incisor tooth[a]			Masticatory tooth[a]		
		0	1	2	0	1	2
12–36	41 (31.8)	41 (100)	0 (0.0)	0 (0.0)	26 (63.4)	10 (24.4)	5 (12.2)
>36	88 (68.2)	67 (76.1)	3 (3.4)	18 (20.5)	35 (39.8)	25 (28.4)	28 (31.8)
Total	129 (100)	108 (83.7)	3 (2.3)	18 (13.9)	61 (47.2)	35 (27.1)	33(25.6)

Note: '*n*' denotes the number of samples detected.
[a]0: absence of gingival recession; 1: gingival recession in one tooth; 2: gingival recession in two or more teeth.

TABLE 4 Distribution of the occurrence and intensity of dental calculus in 129 clinically evaluated sheep, according to age group.

Age range (category) (months)	Number of animals	Dental calculus, *n* (%)			
		0	1	2	3
12–36	41 (31.8)	0 (0)	28 (68.3)	12 (29.3)	1 (2.4)
>36	88 (68.2)	0 (0)	26 (29.5)	46 (52.3)	16 (18.2)
Total	129 (100)	0 (0)	54 (41.9)	58 (44.9)	17 (13.2)

Note: '*n*' denotes the number of samples detected. 0: no visible biofilm; 1: <25% of the tooth with biofilm; 2: 25%–50% of the tooth with biofilm; 3: >50% of the tooth with biofilm.

animals (52.7%) had lesions in their masticatory teeth, with 78% being older than 36 months. Among animals older than 36 months, 32% (28/88) had periodontal lesions in more than one tooth. The frequency and intensity of lesions are described in Tables 2 and 3.

All animals examined had some degree of calculus on the supragingival surface of their teeth. Moderate biofilm (score 2) was present on the incisors and masticatory teeth of 58 sheep (45%), of which 79% were older than 36 months (Table 4). Regarding wear on the dental crown (Table 5), 88% (114/129) of the ewes had some degree of wear on their incisors, with 73% of these (83/114) being over 36 months old. In this group of teeth, grade 1 wear was the most common, affecting 66% (75/114) of the animals. All animals evaluated in the study showed some degree of wear on their masticatory teeth, with grade 2 wear being the most frequent (96/129). The masticatory teeth were more frequently affected and with greater severity than the incisors. Some features of periodontal lesions, tooth wear and dental biofilm are shown in Figure 1.

When evaluating the occurrence of periodontal lesions in animals aged between 12 and 36 months and over 36 months, the older ones had the highest frequency of periodontal lesions (correlation index [CI] = 0.29; p = 0.00001). In the Spearman correlation test and Student's t-test, the degree of significance for the association between periodontal lesions and age was higher in the incisors (CI = 0.29; p = 0.0006) than in masticatory teeth (CI = 0.24; p = 0.0053); therefore, lesions may manifest more clearly in those teeth. Moreover, a positive association was observed between the occurrence of periodontal lesions in the first and second right and left maxillary premolars (CI = 0.32; CI = 0.38), indicating that the lesion tends to be bilateral when present in these teeth.

As for dental calculus, which generally had greater intensity in masticatory teeth, animals over 36 months had a greater amount of biofilm adhered to their dentition (CI = 0.37; p = 0.000015), and a higher occurrence of gingival recession was associated with greater amounts of biofilm (CI = 0.37; p = 0.000002).

No association was found between dental wear and the age of the animal (CI = 0.11). However, an antagonistic association was observed between wear of the masticatory teeth and periodontal injury (CI = −0.20), with the highest degree of periodontal injury in masticators occurring in teeth apparently without crown surface wear. Bonferroni correction was applied, and no new results with statistical significance were identified.

Microbiota associated with ovine periodontitis

The study analysed 131 samples of periodontal pockets and found *F. nucleatum* (77.1%), *T. forsythia* (55%) and *F. necrophorum* (51.1%) to be the most prevalent microorganisms. These microorganisms were significantly more prevalent in periodontitis-affected sites than other clinically healthy periodontal sites (*F. nucleatum*, p = 0.0152; *F. necrophorum*, p = 0.0019; *T. forsythia*, p = 0.0397). Some microorganisms were detected only in samples from periodontal sites affected by bone loss and insertion, but without statistical significance, such as *A. israelii*, *E. corrodens*, *P. asaccharolytica*, *P. endodontalis*, *P. gingivalis*, *P. gulae*, *P. intermedia*, *P. loescheii*, *P. melaninogenica*, *P. nigrescens*, *T. amylovorum*, *T. denticola* and *T. maltophilum*. Other microorganisms, such as *A. actinomycetemcomitans*, *A. naeslundii*, *D. pneumosintes*, *E. faecium* and *P. oralis*, were not detected regardless of the animal's clinical condition (Table 6).

The study also found a statistically significant correlation between the signs of periodontitis in sheep and the occurrences of *T. forsythia*, *F. necrophorum* and *F. nucleatum*, as observed through the Spearman correlation test (CI = 0.36, 0.35 and 0.19, respectively). Additionally, the occurrence of *C. rectus* and *P. nigrescens* was associated with the presence of suppuration (Student's t-test, p = 0.015 and 0.028, respectively), while *F. necrophorum* and *F. nucleatum* were more frequent

TABLE 5 Distribution of tooth wear in 129 clinically evaluated sheep, according to age group (category) and its frequency in incisor and masticatory teeth.

Age range (months)	Number of animals	Dental wear, *n* (%) Incisor tooth[a] 0	1	2	3	Masticatory tooth[a] 0	1	2	3
12–36	41 (31.8)	10 (24.4)	16 (39)	15 (36.6)	0 (0)	0 (0)	4 (9.8)	31 (75.6)	6 (14.6)
>36	88 (68.2)	5 (5.7)	59 (67)	22 (25)	2 (2.3)	0 (0)	10 (11.3)	65 (73.9)	13 (14.8)
Total	129 (100)	15 (11.6)	75 (58.1)	37 (28.7)	2 (1.6)	0 (0)	14 (10.9)	96 (74.4)	19 (14.7)

Note: '*n*' denotes the number of samples detected.
[a]0: no wear; 1: enamel wear; 2: wear of up to one-third of clinical crown length; 3: wear greater than one-third of clinical crown length.

FIGURE 1 Broken mouth: periodontitis and tooth wear in ewes. (a) This ewe presented gingival recession in first (score 2) and second (score 3) incisor teeth and biofilm score of 1 in tooth roots. (b) This ewe lost four incisor teeth and presented gingival recession score 2 in the second right incisor and in first left incisor, periodontal pocket in the third left incisor assessed by probing and biofilm score 1. (c) Severe wear of the dental crown of the incisors with exposed dentine (score 2). (d) This ewe lost five incisor teeth and presented severe tooth wear with crown loss (score 3). (e) Presence of severe amount of brown biofilm on the crown of deciduous incisors (score 3). (f) Accumulation of black pigmented dental calculus on masticatory teeth (score 3).

TABLE 6 Microorganisms detected in the dental biofilm of clinically healthy sheep ($n = 52$ samples) and with periodontitis ($n = 131$ samples) by polymerase chain reaction.

Microorganism	Healthy sites, *n* (%)	Affected sites, *n* (%)	*p*-Value
Actinomyces israelii	0 (0.0)	2 (1.5)	0.5315
Campylobacter rectus	9 (17.3)	40 (30.8)	0.6648
Eikenella corrodens	0 (0.0)	12 (9.2)	0.1137
Fusobacterium necrophorum	6 (11.5)	67 (51.1)[a]	0.0019[a]
Fusobacterium nucleatum	30 (57.7)	101 (77.1)[a]	0.0152[a]
Porphyromonas gingivalis	0 (0.0)	12 (9.2)	0.1137
Porphyromonas gulae	0 (0.0)	9 (6.9)	0.1749
Prevotella buccae	2 (3.9)	7 (5.3)	0.6627
Prevotella intermedia	0 (0.0)	16 (12.2)	0.0642
Prevotella loescheii	0 (0.0)	13 (10)	0.0986
Prevotella melaninogenica	0 (0.0)	7 (5.3)	0.2345
Prevotella nigrescens	0 (0.0)	12 (9.2)	0.1137
Tannerella forsythia	8 (15.4)	72 (55)[a]	0.0397[a]
Treponema denticola	0 (0.0)	16 (12.2)	0.0642

Note: '*n*' denotes the number of samples detected.
[a]Significant *p*-values by Student's *t*-test.

in sites with periodontal lesions without a gingival recession (Student's *t*-test, $p = 0.011$ and 0.027, respectively). Bonferroni correction was applied, and no new results with statistical significance were identified.

DISCUSSION

This cross-sectional study in two commercial sheep flocks under conventional husbandry practices reports the simultaneous occurrence of periodontal lesions and tooth wear – two multifactorial and complex conditions with distinct aetiopathogenesis. Although the study has limitations due to the small sample size, the lack of evaluation of body condition score and the absence of diagnostic imaging techniques, which are common for humans, dogs, cats and occasionally horses, it provides useful information regarding sheep flock performance and economics. The study uncovered unique aspects of sheep oral health through periodontal probing, which identified correlations between periodontal lesions and dental wear on both incisor and masticatory teeth, as well as the presence of dental calculus and microorganisms considered potential periodontal pathogens.

In diphyodont and heterodont ruminants, incisor and masticatory teeth represent different anatomies and functionalities. The study found a high prevalence of periodontal lesions in incisor (58%) and masticatory (52.7%) teeth, especially in ewes aged over 36 months (Table 2). This condition may result from bone loss and periodontal pocket formation in periodontitis, followed by gingival recession, root/furcation exposure or food accumulation. In cattle, periodontal lesions also tend to increase with age. Still, they should not be considered inevitable consequences of ageing but rather cumulative effects of true risk factors.[31] However, it is worth emphasising that the present study did not assess whether lesions were more frequent in juvenile or adult teeth. Thus, this information should be addressed in future studies.

Periodontal probing is the universal procedure to diagnose periodontitis and pockets with a depth of more than 3–4 mm, and followed by bleeding or suppuration it reveals an active process.[9] The term 'periodontal lesion' used in this study does not exclude the possibility that it originates from a primary endoperiodontal infection, which occurs when periodontal disease and endodontic infection exist in the same dental element simultaneously.[32]

In the present study, all evaluated animals had some degree of dental wear on their masticatory teeth, with greater severity than on their incisor teeth. However, there was no observed association between tooth wear and animal age. Although dental wear is a natural ageing process, the intensity and number of affected ewes in both flocks do not reflect a normal natural condition of tooth loss.

Contemporary measures for the diagnosis and control of periodontal disease and tooth wear in sheep are based on the acceptance that both are naturally occurring problems and that eliminating animals based on the evaluation of incisor teeth (broken mouth) would be the only plausible measure. However, a critical discussion is necessary to address why these two health problems are underdiagnosed or neglected and without rational solutions despite the recognition that they cause economic losses in sheep farming and animal welfare problems.[6]

Historically, broken mouth has been the subject of scientific investigation due to the high costs associated with replacing animals removed from the flock before the end of their reproductive life.[7,33] The disease has been clinically characterised by the presence of periodontal infection, bone resorption, loosening of teeth and consequent dental exfoliation.[1,3,34] However, McGregor[23] considered a 'broken mouth' as tooth wear, separating it from periodontal disease. Although, it is not rare to find reports of the occurrence of tooth wear in animals with 'broken mouth',[1] which indicates the possible co-occurrence of both diseases, as observed in the present study.

Tooth wear in sheep has been reported to vary across different countries and may present with varying degrees of severity.[20–22,33,35] In goats, excessive tooth wear and the prevalence of periodontal diseases have been reported for dairy herds in Brazil,[14] but few reports on the occurrence of tooth wear have been made for sheep to date. In this study, more than half of the evaluated ewes had some periodontal disease, and the group of animals older than 36 months was the most affected ($p < 0.001$). A high prevalence of tooth mobility and/or loss (57% and 72.9%, respectively) has also been reported in slaughtered sheep in the UK, suggesting an association between periodontal disease and advancing age.[33,36]

The lack of specific clinical parameters presents difficulty in assessing the establishment and progression of periodontal diseases in sheep. Therefore, the adaptation of visual parameters was used for the clinical diagnosis of dental and endoperiodontal lesions in the 129 ewes. The anatomical particularity of the sheep oral cavity, which does not allow sufficient opening angles for detailed periodontal examination of the last molars, makes a complete evaluation of the periodontium of live animals unfeasible.

Morris et al.[2] reported the occurrence of periodontal lesions in approximately 52.5% of animals aged between 33 and 43 months when evaluating permanent incisor teeth of sheep of different ages. In the present study, injuries to incisors were less frequent, observed in 21 (16.28%) of the 129 animals examined. However, attachment and bone losses in the clinically evaluated posterior teeth were quite pronounced. Similarly, Campello et al.[14] reported a higher occurrence of periodontal lesions in masticatory teeth (62%) of lactating goats than in incisor teeth (28%).

The eating habits and oral cavity pH of sheep are different from those of humans, resulting in a pigmented and adhered deposit on the tooth surface, consisting of a thin layer of mineralised biofilm with some incorporated pigments.[37] Saraiva et al.[15] identified correlations between the presence of carbon, iron and manganese with pigmented supragingival biofilm in cattle and iron and magnesium with periodontitis. These results indicate that dental calculus may be associated with the onset of the disease, and iron deposited on the surface of the supragingival biofilm, as a form of dark pigmentation, may be due to the metabolism of microorganisms possibly involved in the etiopathogenesis of bovine periodontitis. In the present study, the occurrence of gingival recession was observed to increase with the amount of biofilm adhered to the dentition ($p < 0.001$).

In the past, little was known about the role of biofilms in periodontitis development in ruminants despite existing epidemiological data.[38] Recently, Campello et al.[14] reported that 62% of 150 dairy goats examined had a moderate to severe level of supragingival biofilm. In our study, nearly half of the ewes had a moderate amount of supragingival biofilm on both incisor and masticatory teeth, indicating a risk condition for the development of periodontal diseases.

In a recent study, Borsanelli et al.[9] used next-generation sequencing to describe the dental microbiome of sheep with periodontitis and clinically healthy animals. Differences between the microbiomes of the two groups of animals were observed, with the microbiota of animals with periodontitis being richer and more diverse. Genera such as *Fusobacterium*, *Porphyromonas*, *Treponema* and *Tannerella* showed high abundance in sites with periodontal lesions. However, the technique used did not allow for the evaluation of the difference between the two groups at the species level. In the present study, PCR was used to detect important species of periodontal pathogens, such as *P. gingivalis*, *T. forsythia* and *T. denticola*, which are representatives of Socransky's red complex,[39] a reference in studies on the composition of the potentially pathogenic microbiota in humans.

F. nucleatum, *F. necrophorum* and *T. forsythia* were associated with ovine periodontitis. These Gram-negative anaerobes are known for their cytotoxicity and ability to induce intense inflammatory responses,[40] and for inducing bone resorption[41] and profoundly influencing the structure of the supragingival and subgingival biofilm.[42] The 'conjugated' species function of *F. nucleatum* facilitates coaggregation between microorganisms and is considered the most important characteristic of this species in the subgingival ecosystem.[39] This microorganism is responsible for the inflammatory process, the onset of periodontitis development and the recruitment of other periodontal pathogens present in the lesions.[41] In extraoral infections, the species becomes an evident pathogen responsible for complications such as premature birth, stillbirth and neonatal sepsis.[43] In a recent study, the genus *Fusobacterium* showed high relative abundance in the microbiome of sheep with periodontitis.[9]

Tannerella forsythia was initially considered a relatively uncommon subgingival species. However, studies have suggested that this microorganism is more common than previously observed in culture studies and that its frequency is strongly related to increased pocket depth,[44] besides being more prevalent in diseased sites than in healthy sites.[45,46] Recent studies have shown that periodontopathogens produce proteases responsible for inhibiting the action of the host's complement system.[47] Both *F. nucleatum* and *T. forsythia* are related to the progression of periodontal attachment loss.[48,49] Although the species *F. necrophorum* has rarely been reported in periodontal disease in humans, we observed a high prevalence in the subgingival biofilm of sheep with periodontal lesions.

The evidence from the present study suggests that periodontal lesions are a common occurrence in sheep, directly affecting their body score, leading to reduced productivity and quality of life. Excessive dentition wear, which is observed in most sheep, is another oral condition that interferes with an animal's performance by causing dentin sensitivity during feeding, further reducing productivity. This study provides information about the microbiota associated with ovine periodontitis and the simultaneous occurrence of periodontal lesions, excessive tooth wear and dental calculus, suggesting that, although the two diseases have different aetiologies, they share some risk factors. It also indicates that the occurrence and intensity of what is commonly referred to as a broken mouth is the result of a combination of periodontitis, gingival recession, tooth wear and changes in the oral ecosystem of sheep.

AUTHOR CONTRIBUTIONS

Sabrina Donatoni Agostinho, Ana Carolina Borsanelli, Paula Letícia Campello, Júlia Rebecca Saraiva and Iveraldo dos Santos Dutra were responsible for the sample collection and initial data analysis. Sabrina Donatoni

Agostinho conducted the laboratory analysis. Christiane Marie Schweitzer and Elerson Gaetti-Jardim Jr conducted statistical analyses. Sabrina Donatoni Agostinho, Ana Carolina Borsanelli, Iveraldo dos Santos Dutra and Elerson Gaetti-Jardim Jr interpreted the data and prepared the final manuscript. Tamires Ataides Silva assisted with data interpretation as well as preparation of the manuscript. All authors have reviewed and approved the final manuscript.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Brazil).

CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved by the Ethics Committee on Animal Experimentation of São Paulo State University (UNESP), Araçatuba Campus (Protocol FOA No. 00280/15).

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How to cite this article: Agostinho SD, Borsanelli AC, Campello PL, Saraiva JR, Silva TA, Schweitzer CM, et al. Co-occurrence of periodontal lesions and dental wear in incisor and masticatory teeth in two sheep flocks in Brazil. Vet Rec. 2023;e3409. https://doi.org/10.1002/vetr.3409