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## Small Ruminant Research

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## Occurrence of periodontitis and dental wear in dairy goats

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## ARTICLE INFO

## Keywords:

Periodontitis  
Dental wear  
Dental biofilm  
Gingival recession  
Dairy goat

## ABSTRACT

Periodontitis and excessive tooth wear are considered two of the most important oral diseases affecting the health, performance, and welfare of small ruminants. The present study aimed to describe the occurrence of periodontitis and excessive wear of the incisor and masticatory teeth among a herd of dairy goats, the presence of possible risk factors, and the presence of microorganisms considered potential periodontal pathogens. For this, 150 dairy goats of the Saanen and Pardo Alpina breeds with different periodontal conditions and aged 13 months to 8 years were clinically examined. Animals were fed a commercial ration specific for each age group and supplied at will bulky hay composed of Tifton grass hay (*Cynodon nlemfuensis*) or massai (*Panicum* hybrid vr. Massai) and corn silage. Scores from 0 to 3 were initially attributed to the presence and intensity of gingival recession, presence of supragingival biofilm, and wear of the dental crown. The presence of 23 microorganisms considered, as potential pathogens was evaluated by polymerase chain reaction (PCR) from subgingival biofilm samples obtained from periodontal pockets with clinical probing depth of  $\geq 5$  mm ( $n = 22$ ) and the gingival sulcus of periodontally healthy animals ( $n = 22$ ). The occurrence of periodontal lesions, characterized by gingival recession in at least one tooth, was 70.7% (106/150) of animals for which 28.0% (42/150) had lesions in the incisor teeth and 62.0% (93/150) had lesions in the masticatory teeth. On examining the teeth, 96.0% (144/150) of animals presented levels of wear with a degree of severity of 1 to 3. Among the animals, 40.0% (60/150) had wear on the incisor and masticatory teeth together; 37.3% (56/150) had wear only on the masticatory teeth; and 18.6% (28/150) had wear only on the incisor teeth. Goats older than 36 months were the most had gingival recession, which had a greater frequency and degree of severity in the masticatory teeth ( $p = 0.001$ ). This same group had a greater occurrence of wear and severity of wear in the masticatory teeth ( $p < 0.001$ ; confidence interval [CI] = 0.40). The occurrence of gingival recession and wear on the incisor teeth had an inverse relationship ( $p = 0.03$  and  $p = 0.04$ , respectively). However, in the masticatory teeth, there was statistical significance between the occurrence of gingival recession and wear ( $p = 0.04$  and  $p = 0.04$ , respectively). Goats with higher supragingival biofilm scores had a higher frequency and severity of gingival recession in the masticatory teeth ( $p = 0.001$  and  $p = 0.03$ , respectively), and a higher degree of wear in the incisors ( $p < 0.001$ ). In samples from sites with periodontitis, *Fusobacterium nucleatum* (81.8%), *Tannerella forsythia* (63.0%), and *Fusobacterium necrophorum* (63.0%) were the most prevalent microorganisms. In periodontally healthy animals, the most prevalent microorganisms were *Fusobacterium nucleatum* (68.0%), *Tannerella forsythia* (27.0%), *Actinomyces israelii* (27.0%), *Prevotella nigrescens* (22.7%), *Enterococcus faecium* (22.7%), and *Fusobacterium necrophorum* (18.0%). Significant statistical associations between the presence of identified periodontal pathogens and the occurrence of periodontitis and its clinical signs such as periodontal pocket, gingival recession, suppuration, and mobility of the dental unit were evidenced by the Student's *t*-test and Spearman's correlation test. The Spearman correlation test indicated a statistical significance of favorable ecological interactions in the subgingival biofilm of the animals studied.

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Received 10 October 2018; Received in revised form 5 May 2019; Accepted 5 May 2019

Available online 06 May 2019

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## 1. Introduction

Periodontitis and excessive tooth wear are frequent oral diseases in small ruminant herds and have repercussions on the health, production, and welfare of animals. These two lesions derive from two distinct multifactorial processes, affect food consumption and reduce weight gain (Spence and Aitchison, 1986; West, 2002) and milk and wool production (McGregor, 2011). In humans, the multifactorial nature of these diseases reveals that different risk factors can affect the occurrence of these conditions and the severity with which these processes develop (e.g., aggressive periodontitis); with their own clinical and microbiological characteristics (Haffajee and Socransky, 1994; Osborne-Smith et al., 1999; Hajishengallis, 2015).

Dental wear is the slow and irreversible loss of the structure of the outer surface, which apparently occurs without bacterial involvement. Excessive wear of the dental crown causes a high economic impact on small ruminants (West, 2002). It is a recurrent problem in sheep breeding that hampers grazing, severely reduces the productive efficiency of the animals (Spence and Aitchison, 1986; McGregor, 2011), as well as compromises their longevity and productive life due to the deterioration of the dental and periodontal condition. In goats, the intensity of dental wear generates irreparable losses by affecting ingestion, chewing, and rumination, and by influencing the immune status of the animals (McGregor, 2011).

Periodontitis can be characterized as an infectious and inflammatory disease that affect the tissues of the dental support, being considered a mixed anaerobic infection of synergistic character, but whose ecological and virulence peculiarities show specific characteristics of each host species and its microbial buccal biofilm (Marsh et al., 2011; Riggio et al., 2011; Gaetti-Jardim et al., 2012; Borsanelli et al., 2018). This relationship between the anaerobic microbiota of the buccal biofilm of ruminants and the occurrence and severity of periodontitis has been corroborated in studies conducted in several countries, particularly in cattle in which this condition seems to be associated with a predominantly Gram-negative microbiota in the subgingival biofilm (Blobel et al., 1984; Botteon et al., 1993; Dutra et al., 2000; Borsanelli et al., 2015a, b; Borsanelli et al., 2018).

In sheep, periodontitis has been described in several countries (Spence et al., 1988; West and Spence, 2000; West, 2002; Silva et al., 2016), while in goats, it was reported in Japan and associated with various periodontopathogens (Suzuki et al., 2006). In Australia, the United Kingdom, New Zealand, and European countries, a form of periodontitis in sheep, called “broken mouth”, has microbiological characteristics similar to those of periodontitis in humans and other animals, with frequent identification of *Porphyromonas gingivalis*, *Fusobacterium necrophorum*, *Fusobacterium nucleatum*, *Tannerella forsythia* and *Prevotella intermedia* in samples of periodontal pockets (Friskken et al., 1989; McCourtie et al., 1990; Duncan et al., 2003).

However, despite the existing data on the clinical and microbiological aspects of periodontitis in other animal species, particularly in ruminants, due to the multifactorial nature of these diseases, it is not possible to extrapolate these data to caprine periodontitis, which still lacks adequate characterization about its occurrence, severity, and microbiological aspects as well as the problem of tooth wear, that together form a complex capable of seriously compromising the health of the individual and the herd as a whole. The objective of this study was to describe the occurrence of periodontitis in dairy goats, supragingival dental biofilm, and excessive wear of the dental crown, and to identify the microbiota associated with caprine periodontitis through polymerase chain reaction (PCR) using the primers of 23 species of periodontopathogens Figs. 1–6



Fig. 1. Gingival recession in permanent first and second incisors of a Saanen goat.



Fig. 2. Gingival recession in permanent first and second incisors of a Brown Alpine goat.



Fig. 3. Biofilm score 2 observed on permanent masticatory teeth of a Saanen goat.

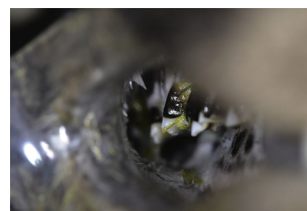


Fig. 4. Biofilm score 3 on permanent masticatory teeth of a Brown Alpine goat.



Fig. 5. Excessive tooth wear score 2 at different intensities on permanent incisor teeth of Brown Alpine goat.



Fig. 6. Excessive tooth wear score 3 on permanent incisor with pulp chamber exposure in a Saanen goat.

## 2. Material and methods

### 2.1. Caprine herd

The study was conducted on a dairy goat farm in the northwest of the state of São Paulo (Brazil). The goats were maintained in an intensive system and confined in suspended facilities. The person responsible for agricultural practices on the farm and the responsible veterinarian provided information on the production system, reforestation of forage production areas, feeding, animal husbandry, and sanitary management. Intra-buccal clinical evaluations were performed in 150 lactating goats of the Saanen and Pardo Alpino breeds of which 79 goats were aged 13–36 months and 71 goats were older than 36 months.

The animals were maintained in a permanent lairage regime. Nearly all of the 48 ha of the production system was intended for the production of fodder, which was supplied in the form of corn silage or hay. The feeding management on the property was based on the animal category with specific commercial ration for each age group, hay comprising Tifton grass (*Cynodon nlemfuensis*) or massai (*Panicum hybrid* vr. Massai), and corn silage provided at will. The food was produced on the property.

### 2.2. Evaluation of the periodontal condition, presence of tooth wear, and presence and extent of supragingival microbial biofilm

The periodontal clinical parameters (i.e., gingival recession, presence of microbial biofilm, suppuration, loss of conjunctive insertion, and gingival inflammation) and the presence of dental wear were evaluated by the exploratory clinical examination of the buccal cavity and adjacent tissues with the aid of a labial and lingual retractor, and mouth and lantern opener. This examination allowed the visualization of the dental crowns, with the exception of the lingual aspect of the lower teeth.

For the periodontal clinical examination, an exploratory and a periodontal probe were used, which were introduced parallel along the dental axis of the labial and lingual faces of all incisor teeth and the vestibular face of the masticatory teeth until the first molar. Owing to the difficulty of access, it was not possible to probe the second and third molars. The presence of gingival recession was used as an indicator to characterize a periodontal lesion, as defined by Miller (1985), and adapted for veterinary medicine. Thus, they were grouped by scores ranging from 0 to 3: score 0 indicated an intact and clinically healthy gingiva with smooth, curved, and rounded edges surrounding the cervical portion of the tooth at the amelocementary border; score 1 indicated the presence of recession that did not overcome the mucogingival junction and occurred without the loss of support or protection tissue in the interdental region; score 2 indicated recession that was beyond the mucogingival junction and occurred without the loss of support or protection tissue in the interdental region; and score 3 indicated recession that went beyond the mucogingival junction and occurred with the loss of support or protection tissue in the interdental region and inadequate dental positioning.

The presence of clinically detectable microbial biofilm on the dental surface was evaluated by adapting the parameters defined by Silness and Løe (1964) in which the scores range from 0–3, as follows: score 0 indicated no visible biofilm or a small amount of biofilm adhered to some teeth; score 1 indicated that a discrete amount of biofilm was adhered to the dentition or a clinically visible amount was adhered to a few teeth; score 2 indicated the biofilm was clinically visible on most of the dentition with partial coverage of the clinical crown of the teeth by a dark brown to black biofilm; and score 3 indicated an abundant biofilm that was adhered to a large part of the dentition, and characterized by the coverage of practically the whole dental biofilm unit with a black coloration.

To evaluate wear of the dental crown on the incisors and

masticatory teeth, the parameters of tooth wear classification suggested by Hugoson et al. (1988) were adapted with scores of 0–3, as follows: score 0 indicated dental enamel integrity or minimal wear; score 1 indicated visible tooth enamel wear or enamel wear with dentin exposure at a single point; score 2 indicated wear of the dentine up to one-third of the crown height; and score 3 indicated dentine wear greater than one-third of the crown height. In the present study, wear of the incisor and masticatory teeth of the upper and lower hemiarcs was considered; therefore, the classification was by group and not by individual teeth.

### 2.3. Collection of clinical specimens of the microbial biofilm of the periodontal pocket and the gingival sulcus

To evaluate the presence of target microorganisms in the subgingival biofilm of goats, twenty-two goats with periodontitis and 22 periodontally healthy goats were selected from the initial group of the selected herd. Subgingival microbial biofilm samples from animals with periodontitis were obtained from at least two periodontal sites with a greater clinical probing depth and clinical evidence of inflammation (e.g., spontaneous bleeding or probing or suppuration).

Only sites with a clinical probing depth of  $\geq 5$  mm were considered, and totaled 92 samples from 22 goats with periodontitis. For periodontally healthy goats ( $n = 22$ ), the subgingival biofilm samples were obtained from animals that did not present gingival recession and inflammation in the periodontal sites, and totaled 48 samples. Clinical specimens were collected and the material was preserved by using the criteria described by Gaetti-Jardim et al. (2012).

### 2.4. Detection of target microorganisms by using PCR

Microbial biofilm samples were transferred to microtubes containing 200  $\mu\text{L}$  of sterile ultrapure water. They were then maintained in a freezer at  $-80^\circ\text{C}$  until the extraction of microbial deoxyribonucleic acid (DNA), which was accomplished by thermal lysis. The microorganisms *Aggregatibacter actinomycetemcomitans*, *Campylobacter rectus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Tannerella forsythia*, *Treponema denticola*, (Ashimoto et al., 1996), *Fusobacterium necrophorum* (Antiabong et al., 2013), *Prevotella buccae*, *Prevotella melaninogenica*, *Prevotella oralis*, *Prevotella loescheii* (Nadkarni et al., 2012), *Porphyromonas endodontalis* (Fouad et al., 2002), *Porphyromonas asaccharolytica* (Tran et al., 1997), *Treponema amylovorum*, *Treponema maltophilum* (Mayanagi et al., 2004), *Actinomyces israelii*, *Actinomyces naeslundii* (Xia and Baumgartner, 2003), *Porphyromonas gulae* (Kato et al., 2011), *Enterococcus faecium* (Cheng et al., 1997) and *Dialister pneumosintes* (Avila-Campos and Velázquez-Meléndez, 2002) were identified by amplifying the target DNA by PCR using specific amplification primers and conditions for each target microorganism. The specificity of these primers has been demonstrated in the aforementioned literature and can be evaluated through the National Center for Biotechnology Information databases at <http://www.ncbi.nlm.nih.gov/>.

The target DNA was amplified by PCR in 25  $\mu\text{L}$  volumes containing 1X PCR/ $\text{Mg}^{2+}$  buffer (Boehringer Mannheim, Indianapolis, IN, USA), 0.2  $\mu\text{L}$  of each deoxyribonucleotide triphosphate (Pharmacia Biotech, Piscataway, NJ, USA), 0.5 U Taq DNA polymerase (Invitrogen do Brasil, São Paulo, SP, Brazil), 0.4  $\mu\text{L}$  of each primer pair (Invitrogen do Brasil), and 10 ng template. The amplifications were conducted in a thermocycler (Perkin Elmer, GeneAmp PCR System 9700, Norwalk, CT, USA), which was programmed at  $94^\circ\text{C}$  (5 min), 30–40 cycles at  $94^\circ\text{C}$  (30–60 s), based on the primer used, specific annealing temperature for each primer pair,  $72^\circ\text{C}$  (30–60 s), followed by 5 min at  $72^\circ\text{C}$  for the final extension of the amplified DNA strands. The PCR amplification products underwent agarose gel electrophoresis in 1% tris/borate/ethylenediaminetetraacetic acid (EDTA) buffer (1 M Tris, 0.9 M boric acid, 0.01 M EDTA, pH 8.4) and were stained with ethidium bromide

(0.5 mg/mL).

DNA samples of reference strains were used for control of detection procedures, as well as clinical samples positive for the target microorganisms (Gaetti-Jardim et al., 2012). Ultrapure water was used as a negative control.

### 2.5. Statistical analysis

The data were analyzed using Statistica 7.0 software (Statsoft, Inc., Tulsa, OK, USA). To evaluate the relationship between gingival recession in the incisor and masticatory teeth, dental wear, and presence of supragingival biofilm, the data were tabulated in contingency worksheets and the data were distributed, according to their frequency, in incisive and masticatory teeth, for each animal, as well as the scores of the different clinical parameters studied (biofilm, dental mobility, suppuration, dental arch, dental wear, gingival recession). Possible interrelationships between the observed clinical parameters were evaluated using the Student's *t*-test and the multiple comparison test. Interrelationships were confirmed through the Spearman correlation test, which presents values ranging from -1 (i.e., negative or antagonistic association) to +1 (i.e., positive association). For the evaluation of the data, the animals were grouped by age: 13–36 months and > 36 months. The value of 5% (i.e.,  $p < 0.05$ ) was adopted as the level of significance.

The results concerning the prevalence of bacterial species in the biofilm samples of goats were tabulated by “animal” and confirmed by analysis using the criterion “periodontal site”. The association between the different clinical parameters of periodontal infection and the occurrence of target microorganisms was evaluated using the Student's *t*-test and Spearman's correlation test.

Spearman's correlation test was also used to evaluate the existence of possible synergistic or antagonistic correlations between the different target microorganisms. The criteria “periodontal status of the animal” and “periodontal condition of the sites collected” were considered simultaneously as a function of the number of clinical samples analyzed per animal and their relation to the total number of animals evaluated. The level of significance adopted in both tests was 5% (i.e.,  $p < 0.05$ ).

### 3. Results

Periodontal lesions were observed in 70.7% (106) of the 150 lactating goats. Periodontal lesions involved the incisor teeth in 28.0% (Fig. 1, Fig. 2) of animals and involved the maxillary and mandibular masticatory teeth in 62.0% (93) of animals. When grouped by age, these alterations occurred more often among the older goats. In the incisors, the occurrence of periodontal lesions ranged from 21.5% (17/79) in goats 13–36 months old to 35.2% (19/71) in goats > 36 months. In the maxillary and mandibular masticatory teeth, the frequency of gingival recession was 52.0% (41/79) in goats 13–36 months old and 74.6% (53/71) in goats > 36 months. The greater occurrence and severity of lesions were observed bilaterally in the third premolar and first maxillary molar. Gingival recession involved two or more incisor teeth in 27.3% of goats, and involved two or more masticatory teeth in 46% of animals analyzed. The frequency of lesions related to periodontitis and the gingival recession scores are presented in Tables 1 and 2, respectively.

All animals had clinically visible supragingival microbial biofilm. Regarding the formation and retention of dental biofilm, 37.3% (56/150) of goats had a score of 1; 44.6% (67/150), a score of 2 (Fig. 3); and 18.0% (27/150), a score of 3 (Fig. 4).

The occurrence of excessive tooth wear was detected in 96.0% (144/150) of animals with the degree of severity ranging from 1 to 3 (Fig 5, Fig. 6). Forty percent (60/150) of animals had wear on the incisor and masticatory teeth; 37.3% (56/150) of animals had wear only on the masticatory teeth, and 18.6% (28/150) of animals had wear only on the incisor teeth. Only 4.0% (6/150) of animals in the study did not

have excessive wear on the dentition. When weighed only in the incisor teeth, goats older than 36 months had a higher frequency of wear (31.0%, 22/71 goats), compared with goats aged 13–36 months (7.6%, 6/79 goats). Animals 13–36 months had a higher occurrence of wear on the masticatory teeth (53.0%, 42/79 goats), compared with older goats (20.0%, 14/71 goats). When the general wear data were analyzed by using the two age groups and dental groups, no significant differences were observed between goats > 36 months (42.0%, 30/71 goats) and goats aged 13–36 months (38.0%, 30/79 goats). The results of the analysis of caprine dentition for dental crown wear are presented in Table 3.

The occurrence and severity of gingival recession, which denote periodontal impairment, were more significant in older animals, particularly in the masticatory teeth (Student's *t*-test,  $p < 0.001$ ). The Spearman correlation test (CI = 0.84) confirmed a correlation between recession gingival occurrence in two or more teeth and the severity of lesions on the masticatory teeth.

Regarding the presence and extent of visible biofilm in the dentition, it was possible to observe that the animals that had a higher biofilm adherence score also had a higher degree of gingival recession in the masticatory teeth ( $p = 0.03$ ) and a higher frequency of recession in one or more teeth in this group ( $p = 0.001$ ). In relation to the incisor teeth, the presence of biofilm was higher in animals with higher levels of wear in this dentition ( $p < 0.001$ ). Based on the Spearman correlation test, it was possible to establish that the presence of biofilm had a positive correlation with the frequency of gingival recession in the incisor teeth (CI = 0.19) and with the severity of wear on the masticatory dentition (CI = 0.23).

However, there was no statistical correlation between the incisor teeth and the masticatory teeth in the occurrence of wear. Compared with younger animals, animals older than 36 months had a higher occurrence of dental wear with a greater severity of lesions in the masticatory teeth ( $p = 0.001$ ). This correlation was confirmed by the Spearman correlation test (CI = 0.40).

Of the 23 microorganisms for which the occurrence was evaluated, *A. naeslundii*, *P. gulae*, *P. asaccharolytica* and *P. oralis* were not detected. In the animals affected by periodontitis, the most prevalent species were *F. nucleatum* (81.8%), *T. forsythia* (63.6%), *F. necrophorum* (63.6%), and *C. rectus* (59.0%). In healthy animals, only *F. nucleatum* had a broader distribution (68.0%). However, on refining the analysis by categorizing by the periodontal condition of the animal, the association of *F. nucleatum* with healthy periodontal sites was not significant (Student's *t*-test;  $p = 0.3$ ; Spearman's correlation test, CI = -0.16) (Table 4).

When the microbiota analysis was conducted comparing healthy goats and goats with periodontitis, a significant association was observed with the presence of *T. forsythia* and *F. necrophorum* ( $p = 0.008$ ). However, when only the condition of each periodontal site was taken into account, the presence of *T. forsythia*, *P. gingivalis*, *F. nucleatum*, *C. rectus*, *E. corrodens*, *F. necrophorum* and *P. buccae* was higher in the sites with periodontitis ( $p < 0.001$ ,  $p = 0.03$ ,  $p = 0.02$ ,  $p = 0.001$ ,  $p = 0.02$ ,  $p < 0.001$ , and  $p = 0.007$ , respectively) and associated with suppuration, dental mobility ( $p = 0.03$ ), gingival recession, and a greater clinical probing depth ( $p < 0.001$ ).

Among the evaluated microorganisms, *T. forsythia* and *F. necrophorum* showed the highest correlation with the presence of a periodontal pocket ( $p < 0.001$ ). Dental mobility was significantly correlated with the clinical probing depth and the presence of *T. forsythia* ( $p = 0.02$ ) and *P. melaninogenica* ( $p = 0.04$ ), as confirmed by the Spearman correlation test (CI = 0.35 and CI = 0.31, respectively). Suppuration was statistically associated with the presence of certain Gram-negative microorganisms, particularly *P. gingivalis* ( $p = 0.002$ ); *P. intermedia* ( $p < 0.01$ ); *P. melaninogenica* ( $p = 0.04$ ); and *P. loescheii*, *D. pneumosintes*, and *A. actinomycetemcomitans* ( $p < 0.01$ ), especially when “suppurated” and “nonsuppurated” animals were compared. However, this association was also observed when sites with

**Table 1**

Distribution of the gingival recession occurrence of gingival recession in one or more incisor and masticatory teeth of 150 clinically evaluated goats, according to the age of the animal (category).

Teeth	Number of teeth				
	Maxilla		Mandible		
	13-36 months (n = 79)	> 36 months (n = 71)	13-36 months (n = 79)	> 36 months (n = 71)	
Incisors	First incisor		17	25	
	Second incisor		0	0	
	Third incisor		0	0	
	Fourth incisor		0	0	
Premolars and molars**	PM1	10	21	0	1
	PM2	19	23	0	1
	PM3	40	52	5	8
	M1	19	38	1	6
	M2	2	9	1	1
	M3	0	0	0	0

\* Number of goats.

\*\* PM1, first premolar; PM2, second premolar; PM3, third premolar; M1, first molar; M2, second molar; M3, third molar.

**Table 2**

Distribution of the presence and intensity of gingival recession in 150 clinically evaluated goats according to age (category) of the animal.

	Teeth			
	Incisors		Masticatory	
	13-36 months (n = 79)	> 36 months (n = 71)	13-36 months (n = 79)	> 36 months (n = 71)
Gingival recession				
0	62 (78.4%)	46 (64.8%)	38 (48%)	19 (26.7%)
1	11 (14%)	19 (20.7%)	5 (6.3%)	13 (18.3%)
2	6 (7.6%)	6 (8.5%)	17 (21.5%)	18 (23.3%)
3	0 (0)	0 (0)	19 (24%)	21 (30%)

suppuration and without suppuration were compared by the Spearman correlation test (CI = 0.31 – 0.69).

Through the Spearman correlation test, we verified significant statistical associations in the occurrence of the different target microorganisms, which suggested favorable interspecific ecological interactions in the animals' microbial biofilm, primarily involving Gram-negative anaerobic bacteria. These associations are beyond the objectives of the present study.

**4. Discussion**

The occurrence of periodontal lesions (70.7%) and tooth wear (96.0%) in a significant proportion of the evaluated goat herd constitutes an original report of importance in the veterinary medicine of small ruminants. Although these two diseases cause irreversible and cumulative lesions and are not classical and linearly progressive diseases they cause considerable impairment to health, welfare, and production because they impact gripping, chewing, and rumination. It likewise should be noted that the occurrence of tooth wear and periodontitis does not imply a causal relationship or a correlation

**Table 3**

Occurrence of dental wear on incisive and masticatory teeth. with a score of 0–3, in 150 clinically evaluated goats according to age (category) of the animal.

Tooth wear	Age (months)				Total (n = 150)	
	13-36 (n = 79)		> 36 (n = 71)		Incisors	Masticatory
	Incisors	Masticatory	Incisors	Masticatory		
0	44 (55.6)	10 (12.6)	17 (24)	22 (31)	61 (40.6)	32 (21.3)
1	23 (29)	24 (30.4)	25 (35)	6 (8.5)	48 (32)	30 (20)
2	10 (12.6)	36 (45.5)	23 (32.4)	36 (50.7)	33 (22)	72 (48)
3	2 (2.5)	9 (11.4)	6 (8.5)	7 (10)	8 (5.3)	16 (10.6)

**Table 4**

Bacteria species detected in periodontal pocket of goats with periodontitis (n = 22) and gingival sulcus of periodontally healthy animals (n = 22) by PCR.

Species	Periodontal pocket n (%)	Gingival sulcus n (%)
<i>Fusobacterium nucleatum</i>	18 (81.8)	15 (68.1)
<i>Tannerella forsythia</i>	14 (63.6) β	6 (27.2)
<i>Fusobacterium necrophorum</i>	14 (63.6) β	4 (18.1)
<i>Campylobacter rectus</i>	13 (59.0) β	3 (13.6)
<i>Eikenella corrodens</i>	10 (45.4) β	2 (9.0)
<i>Prevotella buccae</i>	7 (31.8) β	0 (0.0)
<i>Actinomyces israelii</i>	6 (27.2)	6 (27.2)
<i>Porphyromonas gingivalis</i>	4 (18.1) β	0 (0.0)
<i>Prevotella nigrescens</i>	4 (18.1)	5 (22.7)
<i>Prevotella loescheii</i>	4 (18.1) β	1 (4.5)
<i>Treponema denticola</i>	3 (16.6) β	0 (0.0)
<i>Porphyromonas endodontalis</i>	3 (13.6)	1 (4.5)
<i>Treponema maltophilum</i>	3 (13.6) β	0 (0.0)
<i>Treponema amylovorum</i>	2 (9.0)	1 (4.5)
<i>Prevotella melaninogenica</i>	2 (9.0) β	0 (0.0)
<i>Prevotella intermedia</i>	2 (9.0) β	0 (0.0)
<i>Enterococcus faecium</i>	2 (9.0)	5 (22.7)
<i>Dialister pneumosintes</i>	1 (4.5) β	0 (0.0)
<i>A. actinomycetemcomitans</i>	1 (4.5) β	0 (0.0)
<i>Actinomyces naeslundii</i>	0 (0.0)	0 (0.0)
<i>Porphyromonas gulae</i>	0 (0.0)	0 (0.0)
<i>Prevotella oralis</i>	0 (0.0)	0 (0.0)
<i>Porphyromonas asaccharolytica</i>	0 (0.0)	0 (0.0)

\*n = number of detected samples; % = percentage.

β: Association with clinical condition by Spearman's correlation test.

relationship; however, tooth wear and periodontitis may be associated with common predisposing or risk factors. These aspects are aggravated by the limited knowledge available on the causal and modifying factors involved in the pathogenesis of these clinical conditions.

Dental problems in goat breeding are not part of the common concerns of rural producers and veterinarians. The reasons for this lack of concern are because these conditions present themselves as silent,

chronic, undiagnosed by routine procedures and apparently in the popular culture, have little impact on the profitability of economic activity, which is not real. In New Zealand, Moss (1987) reported that sheep with periodontitis need to receive 30% more fodder and not have to compete for it to have a similar weight gain profile as a periodontally healthy sheep. In sheep breeding, Aitchison and Spence (1984) reported the occurrence of dental problems in 542 animals slaughtered in Scotland: 60.0% of the animals had mobility or absence of one or more teeth and 87.0% of the animals presented periodontal pocket. Ingham (2001) likewise observed evident signs of periodontitis and dental crown wear levels in cows with different scores for biofilm adherence to the dental surface in the arches. Although not quantified, the occurrence of periodontitis interferes with the production and welfare of the animals, since it can be a chronic pain condition leading to difficulty in feeding and the consequent loss of body condition, increased susceptibility to diseases, and reduced food efficiency and productivity.

Periodontitis in ruminants is closely linked to soil management and diet. Outbreaks of the disease in cattle were described by Döbereiner et al. (1990), in pastures formed after the clearing of native forests or Cerrado vegetation and in recently reformed areas (Dutra et al., 1993). Although the animals evaluated in the present study were reared in a feedlot system, the supply of fodder grown in periodically reformed areas suggests a risk factor for the occurrence of periodontitis in the goat herd, as also observed by Dutra et al. (1993) in two bovine herds with severe periodontitis in the state of São Paulo and by Silva et al. (2016) who reported an outbreak of periodontitis in sheep in the state of Pará after pasture reform and forage use in animal feeding. In this context, the practices in the feeding management of goats and the presence of periodontal lesions and dental wear in the proportions and intensities reported in this study suggest a chronic process, which may occur because of one or more unknown risk factors, but are associated with the diet fed to the animals. Kinane (2001) reported periodontitis involves continuous chronic infectious processes with successive aggressive episodes in which the consequence is the loss of clinical insertion and eventual exfoliation of the teeth.

The etiology of periodontitis in humans and several animal species is associated with specific microorganisms and the presence of complex biofilms. Some bacteria are considered periodontopathogenic. The presence of a microbial biofilm of specific composition and its adherence to the dental surface, is considered the etiological factor of periodontitis, whose occurrence is influenced by modifying factors such as age, immunological condition, and endocrine function (Genco and Borgnakke, 2013; Carreira et al., 2015). In this sense, an excessive amount of a black supragingival biofilm adhered to the teeth was observed in a great proportion of the goats involved in the study. Spence and Aitchison (1986) reported that it is relatively common to observe an excess of biofilm on sheep dentition and that this factor is possibly related to the development of periodontitis in these animals. According to Albandar (2000), the presence of a biofilm adhered to the dental surface is the primary etiological factor in the development of gingival recession, which is one of the main clinical signs of periodontitis. In the present study, statistical analysis verified that the animals with the highest scores for a biofilm adhered to the dentition also presented a higher degree of gingival recession and a higher frequency of this lesion on the masticatory teeth. This finding suggested that a bacterial biofilm is an important factor associated with periodontal lesions.

In relation to the occurrence of gingival recession, it was possible to observe that it is associated with the age factor, which older animals have a longer period of exposure to risk factors, as has also been described in humans and dogs (Kinane, 2001; Carreira et al., 2015). When evaluating the occurrence of these lesions in two or more teeth of each affected animal, it was possible to observe that they were more frequent in the masticatory teeth, with a greater severity of lesions and occurrence in older animals. This finding corroborates the results in sheep (Aitchison and Spence, 1984).

Dental wear in small ruminants is a factor of great economic and

animal health importance because dental abnormalities affect the grip and ingestion of food and can cause various digestive and nutritional disorders such as pregnancy toxemia (Spence and Aitchison, 1986). In the present study, the presence of excessive dental wear was quite high. However, no statistical correlation was observed between the occurrence of wear on the incisal dentition and on the masticatory teeth. This finding suggested that tooth wear in each dental group can be triggered by different processes such as wear of the incisor teeth caused, in large part, by abrasion by soft tissues (i.e., tongue and cushion) and wear of the masticatory teeth caused by a different set of factors. The results also revealed a higher occurrence of this condition in older animals and in the masticatory teeth, possibly because of the longer exposure of the dentition to the risk factors for this lesion.

Cases of wear of the dental structure in several instances are caused by habits acquired by humans and animals. Attrition of the teeth is often triggered by stress episodes, and the process of abrasion in goats may be associated with the chewing habit of foreign objects, often observed in breeding such animals (Malafaia et al., 2011). Although the occurrence of tooth crown wear is a physiological factor occasioned by time, the cases reported here avoid the natural conditions of tooth structure wear.

West (2002) reported that premature wear of the incisor teeth of sheep raised in pastures is relatively common in Australia and New Zealand. In the present study, the animals were maintained in a confinement system in suspended bays, without contact with the soil, and present a different epidemiological situation from the cases reported by other authors, suggesting that the etiology of the observed wear is not on account of the presence of sand and soil components in food.

Dental crown wear and periodontitis are two multifactorial diseases with distinct etiologies that were mutually observed in the studied herd. In the statistical analysis it was possible to observe that, in the incisor teeth, the presence of periodontitis had an inverse correlation with the occurrence of wear in this dentition, demonstrating that animals that have periodontal lesions in their incisors are less likely to have wear on these teeth. However, when evaluating the masticatory teeth, it was possible to observe a positive association between the occurrence of periodontitis and wear. In the analysis of the presence of biofilm and dental wear, it was possible to observe that the animals that presented the highest degree of tooth wear on the incisors also had a higher score for biofilm adhered to the teeth. Bruère et al. (1979) similarly reported the occurrence of periodontitis and excessive wear of the dentition, observed mutually in ovine and bovine herds, with a higher occurrence in animals older than 3 years.

Periodontitis results from dysbiosis or loss of the ecological balance of the biofilm, and from its relationship with host defense mechanisms. Different local and systemic factors may contribute to the quantitative and/or qualitative changes in the periodontal microbiota, and thereby cause an increase in virulent microorganisms, which would facilitate loss of equilibrium with the host (Hajishengallis, 2017; Herrero et al., 2018). This dramatic change in the microbial community is responsible for the transition from periodontal health to disease with significant population growth of anaerobes that are adapted to survival in an inflammatory environment (Hajishengallis, 2015).

Microbial biofilm disturbance with specific bacterial proliferation has been associated with periodontal destruction, as evidenced when using molecular techniques or based on the detection of antibodies. Recent studies demonstrate that the pathogenesis of periodontitis is linked to polymicrobial synergism and dysbiosis, which lead to the loss of local homeostasis (Hajishengallis, 2015).

Through statistical analysis, it was possible to observe that *T. forsythia* and *F. necrophorum* were associated with the occurrence of periodontitis when the periodontal condition of the animal was evaluated (i.e., the host as a whole rather than the isolated periodontal site). The relative frequency of *T. forsythia* at different sites is a strong marker of chronic periodontitis in humans. Its presence in diseased sites is associated with increased clinical probing depth (Settem et al., 2012), being

considered a major cause of alveolar bone loss and conjunctive insertion and in the formation of periodontal abscesses and other pyogenic processes (Bird et al., 2001; Yoneda et al., 2001). *Tannerella forsythia* has been detected in various animals with periodontitis such as dogs (Booij-Vrieling et al., 2010), in dogs (Kato et al., 2011; Yamasaki et al., 2012), horses (Sykora et al., 2013), monkeys (Gaetti-Jardim et al., 2012), and sheep (Duncan et al., 2003; Riggio et al., 2013).

*Fusobacterium necrophorum* is part of the innate microbiota of the gastrointestinal tract of ruminants and is in the skin and hull lesions and liver abscess (Venter and Amstel, 1994; Nagaraja et al., 2005). The bacteria has been identified in sites with periodontitis in humans (Flaukler et al., 2000) and sheep (McCourtie et al., 1990), and has been implicated in the development of periodontitis for systemic diseases (Yoneda et al., 2001). The identification of *T. forsythia* and *F. necrophorum* in goat sites with periodontitis corroborates the results by Suzuki et al. (2006), who identified the microorganisms in most samples they studied.

When considering only the condition of the periodontal site, the following microorganisms were associated with the diseased sites: *Tannerella forsythia*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Campylobacter rectus*, *Eikenella corrodens*, *Fusobacterium necrophorum*, and *Prevotella buccae*. *Porphyromonas gingivalis* is involved in a large proportion of severe periodontal infections, which may be associated with its wide range of virulence factors such as its notorious proteolytic capacity and ability to induce inflammation (Hajishengallis, 2015). Its participation in periodontal infections is a consensus in cases of periodontitis in humans (Socransky and Haffajee, 2005), and it is frequently identified in periodontitis lesions in sheep (McCourtie et al., 1990; Duncan et al., 2003; Borsanelli et al., 2017). For goats, this study constitutes an unprecedented account of the importance of this Gram-negative anaerobic in the etiology of this disease in dairy goats.

The association of *C. rectus* and *E. corrodens* in sites with periodontitis in the studied animals deserves new evaluations because they have an “S” layer on their surface that can exacerbate the process and that facilitates the survival of these species in the infectious site. In addition, *C. rectus* may assist in the development of periodontitis by increasing the expression of proinflammatory cytokines, which destroy the periodontal tissues (Suda et al., 2004).

*Fusobacterium nucleatum* was the most identified bacterium in diseased and healthy sites of the studied animals, which suggests that this microorganism is part of the resident buccal microbiota in this species. However, the statistical analysis revealed that the presence of the bacterium is associated with sites with periodontitis. This finding suggests that *F. nucleatum* is involved in oral microbiota dysbiosis and in bacterial consortia that can favor the increase of the pathogenicity and the progression of disease caused by the proinflammatory and deleterious properties of this Gram-negative anaerobic microorganism, which has the most active and toxic endotoxin among oral microorganisms (Gaetti-Jardim et al., 2010). To form the bacterial biofilm, *F. nucleatum* works synergistically with several species such as *T. forsythia* and *Prevotella* species with coaggregation between species (Settem et al., 2012; Tamaki et al., 2012a,b).

In humans, the presence of *P. buccae* is usually reported in cases of deep periodontal pockets (Maestre et al., 2007). In recent studies, the occurrence of *P. buccae* has been associated with the development of ovine and bovine periodontitis (Borsanelli et al., 2015a, 2017). In the present study, this microorganism had a statistical association with caprine periodontitis.

Through statistical analysis, it was possible to verify the existence of significant associations between the different microorganisms studied, which suggests favorable interspecific ecological interactions in the microbial biofilm of the studied animals. Early studies on the pathogenic microbiota involved in periodontitis advocated the specific microbiota theory for lesion development. However, recent studies have shown that classical periodontopathogens such as *P. gingivalis* are incapable of causing periodontitis without the presence of commensal

microorganisms in the oral cavity. In this context, *P. gingivalis*, even when present in low amounts, is an important potential pathogen in periodontitis because it induces microbial communities to act as accessory pathogens (Hajishengallis et al., 2011; Hajishengallis and Lamont, 2012). In the present study, several species such as *P. gingivalis*, *T. denticola*, *P. intermedia*, *A. actinomycetemcomitans*, and *T. forsythia*, which are potential periodontal pathogens, had positive associations with opportunistic microorganisms in the biofilm of the studied goats, suggesting a bacterial consortium favorable to the survival and multiplication of pathogenic microorganisms and accessory microorganisms in the lesions of periodontitis.

The results suggest that the progression of healthy periodontal sites to sites compromised by infectious and inflammatory conditions is associated with a significant increase in the frequency of important periodontopathogens such as *T. forsythia* and Gram-negative anaerobic bacteria, especially those producers of black pigment of *Porphyromonas* and *Prevotella* genera and spirochetes. Some species such as the *Fusobacterium* species, *C. rectus*, *E. corrodens*, *A. actinomycetemcomitans*, and *D. pneumosintes* have a positive ecological relationship with these anaerobes, which suggests a bacterial consortium that is important for maintaining the ecosystem and the pathogenicity of these microorganisms.

The evidence suggests that periodontitis in goats is an inflammatory, multifactorial, infectious disease that affects animals, based on the length of exposure to risk factors. Despite its peculiarities, with regard to the microbiota involved in the lesions, it is similar to cases of periodontitis described in humans and in ruminants.

The occurrence of excessive dental wear in the studied animals ratifies the findings of studies that have reported the frequent presence of excessive wear in the dentition of small ruminant animals and a correlation of lesions in the dentition with the age of the animals. The findings in the present study indicate that diseases that affect the teeth and their supporting tissues may be present in all breeding systems and in the most technified systems. Thus, periodontal pathogens that are frequently involved in periodontal lesions in humans, cattle, sheep, and other animal species, and detected in samples from periodontal pockets of goats is an important report for the elucidation of the etiopathogenesis of periodontitis in these animals. The results suggesting a favorable interspecific bacterial consortium in the biofilm demonstrate possible ecological relations within the biofilm in the species investigated. These findings reinforce the hypothesis of the occurrence of dysbiosis in the microbiota for the progression of the disease.

## 5. Conclusion

The occurrence of periodontitis and excess supragingival biofilm formation and the presence of excessive wear on the dentition of the animals studied demonstrated common risk factors exist for its development within the herd. The large variability in the progression of periodontitis in a sample of an apparently homogeneous population suggested that other variables, besides age and presence of biofilm adhered to the dentition, may be important for the onset and progression of the disease over time.

In goats, periodontitis and its different clinical signs are associated with an increase in the population of predominantly Gram-negative microorganisms. The presence of potentially pathogenic microorganisms in the subgingival microbiota of goats with periodontitis was similar to the biofilm ecology in humans and other animal species with periodontitis.

The correlations between the studied bacteria demonstrated favorable interspecific ecological interactions in the subgingival biofilm, which suggest bacterial synergism in the occurrence of the disease.

## Declarations of interest

None

## Acknowledgements

To Coordination for the Improvement of Higher Education Personnel (CAPES) for the scholarship.

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