



Pathology of chronic ovine periodontitis¹

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Periodontitis is an inflammatory process of infectious origin affecting the teeth and their supporting structures, causing significant economic losses and reducing animal welfare. Bacteria in the gingival biofilm are one of the main factors in initiating inflammatory lesions. Bacteria act directly on tissues or indirectly through substances that cause tissue damage. Studies on the etiopathogenesis of periodontitis in Brazilian sheep herds are scarce. The present study aimed to characterize histologically periodontal lesions of culled sheep from the Brazilian breed, Santa Inês. Periodontal lesions, such as periodontal pockets containing plant tissue and bacteria, replacement of the periodontal ligament by connective tissue and inflammatory cells, superficial pustules, hydropic epithelial degeneration, and epithelium hyperplasia, were observed. Submucosal changes were characterized by granulation tissue, edema, swelling of the endothelial cells, bacteria, and predominantly perivascular lymphoplasmacytic inflammatory infiltrate. In the alveolar bone, osteoclastic resorption and bone apposition were observed. This study revealed subacute to chronic inflammation, alveolar bone resorption, and cortical bone apposition in ovine periodontitis. Thus, these findings can contribute to the evolution of knowledge about the etiopathogenesis of ovine periodontitis and, possibly, the development of measures to control the disease.

INDEX TERMS: Sheep diseases, pathology, ovine, sheep, periodontitis, periodontal disease, anaerobic, histopathology.

RESUMO.- [Patologia da periodontite crônica em ovinos.]

A periodontite é um processo inflamatório de origem infecciosa que afeta os dentes e suas estruturas de suporte, causando perdas econômicas significativas e redução do bem-estar animal. As bactérias do biofilme gengival são um dos principais fatores envolvidos no início das lesões inflamatórias. As bactérias agem diretamente nos tecidos ou indiretamente por meio de substâncias que causam dano tecidual. Estudos sobre a etiopatogenia da periodontite em rebanhos ovinos

brasileiros são escassos. O presente estudo teve como objetivo caracterizar histologicamente as lesões periodontais de ovinos de descarte da raça brasileira Santa Inês. Foram observadas lesões periodontais, como bolsas periodontais contendo tecido vegetal e bactérias, substituição do ligamento periodontal por tecido conjuntivo e células inflamatórias, pústulas superficiais, degeneração hidrópica epitelial e hiperplasia do epitélio. As alterações da submucosa foram caracterizadas por tecido de granulação, edema, tumefação das células endoteliais, bactérias e infiltrado inflamatório linfoplasmocitário predominantemente perivascular. No osso alveolar, observou-se reabsorção osteoclástica e aposição óssea. Este estudo revelou inflamação subaguda a crônica, reabsorção óssea alveolar e aposição de osso cortical na periodontite ovina. Assim, esses achados podem contribuir para a evolução do conhecimento sobre a etiopatogenia da periodontite ovina e para o desenvolvimento de medidas de controle da doença.

TERMOS DE INDEXAÇÃO: Doenças de ovinos, patologia, ovinos, periodontite, doença periodontal, anaeróbica, histopatologia.

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INTRODUCTION

Periodontitis is a polymicrobial and multifactorial disease that affects teeth protection structures (Arcaute et al. 2020a) and represents one of the most common oral disorders in sheep (Arcaute et al. 2020b, Wicpolt et al. 2022). This disease has been reported in sheep (Cutress & Schroeder 1982, Spence et al. 1988, Borsanelli et al. 2016, 2021), goats (Campello et al. 2019, Borsanelli et al. 2022) and cattle (Borsanelli et al. 2015a) in several countries. In Brazil, periodontitis was first observed in cattle in the late 1960s in Mato Grosso (Döbereiner et al. 1974, Dutra et al. 2019). Periodontitis affecting sheep was firstly reported in sheep by Döbereiner et al. (1974). Currently, it was described in the North (Silva et al. 2016, 2019), Southeast (Borsanelli et al. 2017) and Northeast (Wicpolt et al. 2022) Brazilian regions.

Some studies were carried out to understand the disease's occurrence. Periodontitis is associated with a multispecies biofilm, in which bacteria are essential but insufficient for developing the inflammatory process (West & Spence 2000). Among the bacteria most reported as potential periodontal agents in sheep, members of *Fusobacterium*, *Treponema*, *Tannerella*, *Porphyromonas*, *Prevotella* and *Petrimonas* genera were identified by culture-independent methods (Borsanelli et al. 2017, 2021, Silva et al. 2019). The imbalance and polymicrobial synergism affect the biofilm ecology and result in loss of oral homeostasis (Hajishengallis 2015). Consequently, it leads to an inflammatory response that destroys periodontal structures. Inflammation starts with gingivitis, ulceration, resorption, necrosis of the alveolar bone, dental mobility, periodontal pocket formation, abscess, and culminates with tooth loss (Kumar et al. 2003). However, these lesions are noticed only when facial swelling is due to chronic inflammation (Cutress & Ludwig 1969) or if a clinical periodontal examination is part of the health herd routine.

In several countries, dental affections stand out among the main reasons for sheep slaughter and early disposal (Gunn 1970, Aitchison & Spence 1984, Dutra & Borsanelli 2022). These conditions affect animal food consumption and lead to reduced weight gain and milk and wool production (West & Spence 2000, McGregor 2011). In addition, ewes with periodontitis tend to give birth to smaller and weaker lambs, causing low productivity in the herds (West & Spence 2000).

In Brazil, studies on the etiopathogenesis of ovine periodontitis are scarce, and the sheep farming sector presents growth, including the creation of Brazilian breeds. Describing tissue lesions associated with ovine periodontitis could help develop control and prophylaxis measures for this disease. In this sense, we describe the gross and histological changes observed in Santa Inês sheep with periodontitis.

MATERIALS AND METHODS

The Ethics Committee on Animal Experimentation of "Universidade Estadual Paulista 'Júlio de Mesquita Filho'" (Unesp), Jaboticabal Campus (Protocol FCAV No. 018976/17), approved this study. Ten heads presenting periodontal lesions were obtained at the abattoir from a flock of adult Santa Inês sheep in Nhandeara/SP, Brazil. The sheep were raised in a semi-extensive system, fed with *Panicum maximum* cv. Massai and *Urochloa (=Brachiaria) brizantha* pasture, commercial mineral mixture, and silage or hay.

The clinical status of sheep was established through oral examination with a Williams periodontal probe. The criteria for diagnosing periodontitis were the presence of gingival recession (i.e., the tooth root was visible at the gum margin) and the existence of periodontal pockets (the distance from the gingival margin to the base of the periodontal pocket) greater than 5 mm in depth. As this was a *post-mortem* examination, it was impossible to assess the presence of gingivitis and to bleed on probing. Other information such as tooth loss, facial bulging or fistula was recorded in a specific odontogram. Two adult sheep that died of unrelated causes were selected as the control group. The oral examination revealed no evidence of gingival recession, no periodontal pockets, or other oral diseases. Histologically, the two control sheep presented mild multifocal lymphoplasmacytic infiltrate at the superficial layer of connective tissue, and the junctional epithelium raised mild *rete ridges*. Both control sheep were considered healthy.

The pathological examination was carried out at the "Setor de Anatomia Patológica" (SAP) from the "Universidade Federal do Rio de Janeiro" (UFRRJ). The dental arches of sheep were evaluated, and dental, mandibular, and maxillary changes were described. Two fragments of vestibular periodontal tissues of each sheep were sampled at areas with significant lesions and fixed in 10% buffered formalin. The soft tissues of 10 sheep were routinely processed for histopathology and stained with hematoxylin and eosin (HE), Gram Histological (GH), and Warthin-Starry silver impregnation (WS). For histology, we also sampled the incisor teeth, the mandibles and the maxilla at the premolars and molars. The bone and dental tissue fragments were decalcified with a 10% nitric acid solution for eight days. During this period, the acid solution was twice replaced, and the samples were observed daily to evaluate the chemical process. The decalcified bones were stored for seven days, 70% alcohol, and underwent routine histopathology processing and staining.

RESULTS

Out of 10 sheep, all presented a periodontal pocket with a depth greater than 5mm in at least one tooth, nine (90%) presented some degree of gingival recession in at least one tooth, seven (70%) showed loss of at least one tooth element, one (10%) presented bulging facial with the presence of fistula and one (10%) animal was prognathous.

Gingival recession and deep periodontal pockets were more prevalent in masticatory teeth than incisors. Four animals lost only masticatory teeth, one sheep lost only incisor teeth, and two animals lost masticatory and incisor teeth. The loss of masticatory teeth was more frequent in the third premolar (57.1%) (Fig.1) than in the first molar (42.9%) and the second molar (42.9%). The most lost incisor tooth was the first incisor (28.6%).

All ten culled sheep showed typical periodontal lesions, such as gingival recession (Fig.2) with tooth root exposition, periodontal pockets over 5mm in depth, absence of the premolars and upper and lower molars, teeth mobility, and irregular occlusion. In two sheep (Sheep 3 and 10), we observed abscess formation and the presence of fistulas that drained suppurative exudate.

Histological examination of the ovine periodontal soft tissues is presented in Table 1. Microscopic lesions were demonstrated by replacement of the periodontal ligament by connective tissue and inflammatory cells, multifocal subacute to chronic lymphoplasmacytic and histiocytic periodontitis and gingivitis with granulation tissue, epithelial hyperplasia,

hydropic degeneration, and edema. Submucosal edema was seen in 100% of the evaluated sheep. Plant material within a periodontal pocket in conjunction with a colony of basophilic

bacteria was detected only in the Sheep 9 (10%), which also had pustules in the gingival epithelium. The gingiva had an epithelial fissure filled with bacteria and cell debris (Fig.3).

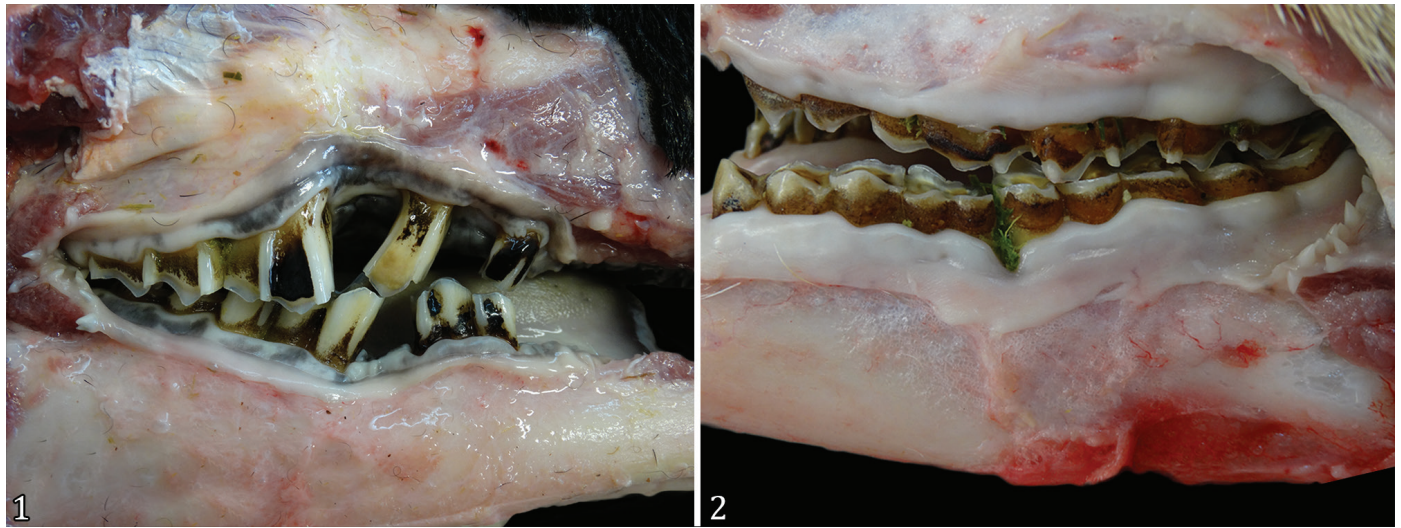


Fig.1-2. Gross lesions of ovine periodontitis. (1) Severe gingival retraction of maxillary and mandibular premolars, loss of maxillary second premolar and first molar, loss of mandibular third premolar. (2) Gingival retraction and impacted food between mandibular premolars.

Table 1. Histological changes of periodontitis in sheep

Sheep	Hydropic degeneration	Epithelial hyperplasia	Edema	Leucocyte profile	Inflammatory location	Bacteria	Endothelial cell tumefaction	Other changes
1	(+)	(++++)	(+)	Lymphocytes (++) Plasma cells (++++) Macrophages (++)	Submucosal (Perivascular)	Absent	Absent	Absent
2	(+)	(+++)	(+++)	Lymphocytes (++) Plasma cells (++) Macrophages (++)	Submucosal (Perivascular multifocal to coalescent)	Absent	Presence	Absent
3	(+)	(++)	(+++)	Lymphocytes (+++) Plasma cells (+++) Macrophage (+++)	Submucosal (Perivascular)	Absent	Presence	Granulation tissue (++)
4	(+)	(+)	(+)	Lymphocytes (+) Plasma cells (+++) Macrophage (+)	Submucosal (Perivascular)	Absent	Presence	Absent
5	(++)	(+)	(++)	Lymphocytes (++) Plasma cells (+++) Macrophage (++)	Epithelial and submucosal (Perivascular)	Absent	Presence	Granulation tissue (+)
6	(++++)	(++)	(+++)	Lymphocytes (+++) Plasma cells (++++) Macrophages (+++)	Epithelial and submucosal (Perivascular)/ Interface	Absent	Presence	Granulation tissue (+++)
7	(++)	(+++)	(++)	Lymphocytes (++) Plasma cells (++) Macrophage (++) Neutrophils (+) Eosinophils (+)	Submucosal (Perivascular)/ Epithelial interface	Absent	Presence	Granulation tissue (+++)
8	(+)	(+)	(++)	Lymphocytes (++) Plasma cells (++) Macrophages (++)	Epithelium, Interface Epithelium/ Lamina propria and submucosa (Multifocal)	Absent	Absent	Absents
9	(+++)	(++)	(+++)	Lymphocytes (+++) Plasma cells (+++) Macrophages (+)	Submucosal (Perivascular)/ Epithelial interface	(++++)	Presence	Plant material/ Epithelial pustule
10	Absent	(+)	(++)	Lymphocytes (+) Plasma cells (+)	Submucosal (Perivascular)	Absent	Presence	Granulation tissue (++)/ Epithelial pustule

(+) Discreet, (++) mild, (+++) moderate, (++++) severe.

Epithelial hyperplasia (*rete ridges*) (Fig.4) was also observed to varying degrees in 100% of cases (10/10). The hydropic degeneration was observed in several degrees, ranging from mild to severe in 90% (9/10) of the evaluated sheep. The inflammatory infiltrates predominantly lymphoplasmacytic (100% of cases) (Fig.5), with neutrophils and eosinophils present only in the Sheep 7. The leukocyte infiltrations were mainly perivascular in the submucosa, 80% (8/10) of the cases (Fig.6). In 70% of cases (7/10), we detected endothelial cell swelling; of these, 50% (5/10) presented the formation of granulation tissue.

Osteoclastic resorption was observed in the alveolar bone tissue and bone apposition in the cortical bone. An enlarged space between the root and the bone (periodontal pocket) was observed in all sheep. Periodontal pocket adjacent tissue

presented infiltrated with a few degenerated neutrophils and a moderate amount of lymphocytes, plasma cells and macrophages.

In tissues stained by Gram Histology (Table 2), bacteria were present in 100% (10/10) samples. Morphologically noting the predominance of coccus in 100% (10/10) of the cases, compared to rods in 30% (3/10). Gram-negative was 60% (6/10) of the cases and Gram-positive 40% (4/10). Rods were viewed as Gram-negative in 66.6% (2/3) of the cases and Gram-positive in 33.3% (1/3). The bacteria were seen more frequently in the perivascular infiltrate, and its intensity ranged from mild to moderate. Bacteria were also detected in superficial biofilms in 20% (2/10) of sheep, between superficial epithelial cells in 10% (1/10), in the vascular

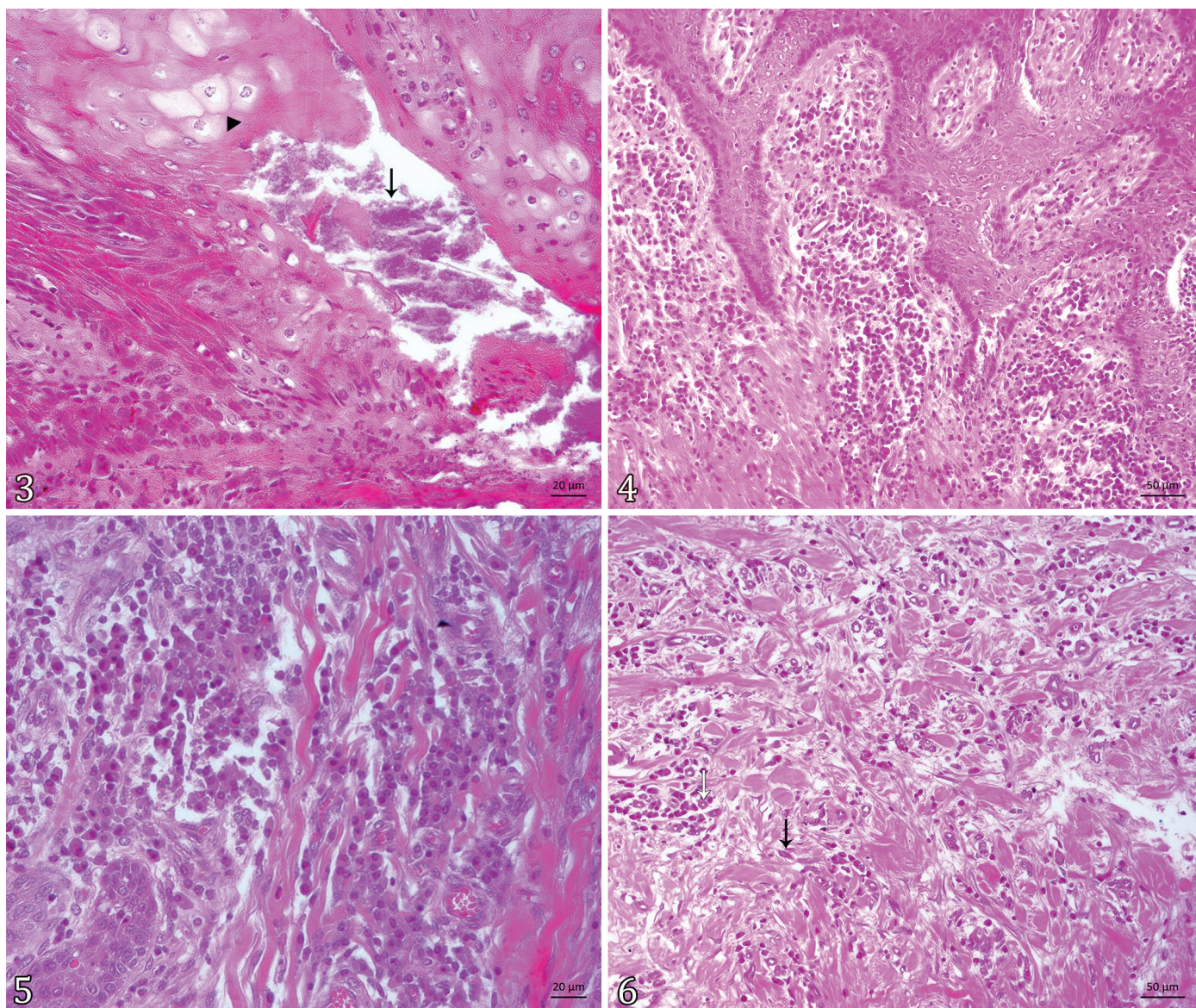


Fig.3-6. Histopathology of ovine periodontitis. (3) Gingival epithelium with a fissure filled with bacteria (black arrow) and cell debris (white arrow). The epithelial cells show hydropic degeneration (arrowhead). HE, obj.40x. (4) Marked epithelial thickening (hyperplasia) and marked submucosal lymphoplasmacytic inflammation. HE, obj.20x. (5) Marked lymphoplasmacytic infiltrate dissecting collagen bundles at gingival submucosa. HE, obj.40x. (6) The gingival submucosa shows numerous fibroblasts (black arrow) and newly formed blood vessels (white arrow) with collagen bundles spacing (edema) in an area of chronic inflammation and healing. HE, obj.20x.

endothelium, intravascular, and the epithelial pustule area in 10% (1/10) each.

In the silver impregnation (Warthin-Starry), bacteria were seen in two samples. In Sheep 8, rods were observed on the lamina propria; in Sheep 6, spirochetes were present in the inflammatory infiltrate. In both cases, the intensity of the marking was mild.

DISCUSSION

Results of pathological examination showed that sheep cases of chronic periodontitis were characterized by loss of attachment, mobility and loss of teeth, gingival retraction, pocket formation, collagen and bone loss, infiltration of lymphocytes, plasma cells and macrophages. These findings are similar to periodontitis in human patients (Hasan & Palmer 2014). Pain, difficulty chewing, and emaciation are limits of livestock production associated with periodontitis (Silva et al. 2016). Clinical signs and progression of lesions are related to the direct effects of bacterial virulence factors on host tissues and the self-destructive inflammatory responses of the host to bacteria (Borsanelli et al. 2018).

Histological changes of spontaneous periodontitis in miniature goats included gingival epithelial hyperplasia and food accumulation between teeth (Suzuki et al. 2006). Döbereiner et al. (1974) characterized cattle periodontitis, including changes to the epithelium, forming small periodontal pockets, firstly without apparent inflammation, followed by accumulation of ingesta and sequent pocket enlargement. The alveolar bone was initially unaffected, followed by an inflammatory process and resorption. With this process, from suppurative alveolar periostitis, the teeth roots become exposed, and teeth loosen. Our results and literature reveal that ovine periodontitis pathogenesis has similarities to miniature goat and cattle periodontitis (Döbereiner et al. 1974, Suzuki et al. 2006, Silva et al. 2016, 2019).

In this study, the ovine lymphoplasmacytic and histiocytic periodontitis with granulation tissue and epithelial hyperplasia reveals subacute to chronic changes. The leukocyte infiltrations were predominantly in the perivascular region of the submucosa, in the lamina propria-epithelium interface. The response of men and other animals against gingival bacterial biofilms comprises a series of acute inflammatory and immunopathological phenomena. The emigration of

neutrophils is one of the most important (Page & Schroeder 1982). Based on the leukocyte profile, the studied sheep presented subacutely to chronic periodontitis. Döbereiner et al. (1974) reported subacute to chronic cattle periodontitis, including infiltration of neutrophils, lymphocytes, and plasma cells. These leukocytes were subepithelial next to the lymphatic vessels. In turn, mild neutrophil infiltrations were near the epithelium, while severe neutrophil infiltrations were adjacent to the periodontal pocket.

Plant material was noted in a periodontal pocket in this study. This critical change is caused by the possibility of occupying the physical space between the tooth and marginal gingiva, which means the formation of a periodontal pocket. Consequently, plant tissue as a foreign body aggravates the inflammatory process. Previously, plant tissue was also identified in bovine periodontal lesions (Döbereiner et al. 1974). Silva et al. (2016) also observed an accumulation of grass stalks in the masticatory teeth during the post-mortem examination of sheep with periodontitis.

Several edema degrees cause submucosal spacing between collagen fibers in all sheep evaluated due to inflammation. Edema was a clinical sign that Ramos et al. (2019) observed in calves with gingivitis. Döbereiner et al. (1974) also observed this change in their study in cattle, in which they noticed that the collagen fibers were disorganized in mild to moderate intensity in the periodontal region.

The hydropic degeneration, sometimes called ballooning degeneration, reveals injury to the epithelial cell. The resulting cell water and sodium infiltration in the cytoplasm are due to the reduction of adenosine triphosphate (ATP) and alteration of the sodium-potassium pump (Hasan & Palmer 2014). The epithelial surfaces with hydropic degeneration could evolve and form epithelial vesicles. The vesicle rupture contaminates itself with pyogenic bacteria and forms pustules. Pustules were detected in two sheep in this study with intralesional bacteria.

Epithelial hyperplasia was present in varying degrees of intensity in all sheep gingival epithelium evaluated. There is a relationship between the lesion's inflammatory process and the epithelial proliferation of the spinous layer into the deep submucosa (Hasan & Palmer 2014). The sheep presented epithelial hyperplasia and inflammation, although this alteration's intensity was not directly proportional to the intensity of the inflammatory infiltrate in all cases.

Table 2. Morphotintorial characteristics and location of bacteria in sheep with periodontitis, Gram Histological technique

Sheep	Morphology	Tintorial feature	Marking location	Marking intensity	Result
1	Coccus	Negative	Perivascular inflammatory infiltrate	(+)	Positive
2	Coccus	Positive/Negative	Perivascular inflammatory infiltrate	(+)	Positive
3	Coccus	Negative	Perivascular inflammatory infiltrate	(+)	Positive
4	Coccus	Negative	Perivascular inflammatory infiltrate	(+)	Positive
5	Coccus	Positive	Perivascular inflammatory infiltrate	(+)	Positive
6	Coccus/Rods	Positive/Negative	Perivascular inflammatory infiltrate and superficial biofilm (leucocytes migrating to biofilm)	(++)	Positive
7	Coccus	Negative	Perivascular inflammatory infiltrate	(+)	Positive
8	Coccus	Negative	Perivascular inflammatory infiltrate	(++)	Positive
9	Coccus/Rods	Negative/Positive	Perivascular inflammatory infiltrate, endothelial, superficial biofilm, epithelial pustule	(+++)	Positive
10	Coccus/Rods	Positive/Negative	Perivascular inflammatory infiltrate and superficial epithelial cells	(+)	Positive

(+) Discreet, (++) mild, (+++) moderate, (+++++) severe.

The infectious nature of periodontitis has been well established in studies in cattle (Blobel et al. 1984, Kopp et al. 1996), sheep (Bogdan-Cătălin et al. 2020), and man (Socransky et al. 1998, Faveri et al. 2008) that used conventional identification methods. More sensitive and specific techniques have been consolidated, favoring discovering pathogenic microorganisms involved in periodontitis (Borsanelli et al. 2015a, 2015b, 2017). Molecular studies amplified the DNA of several bacteria species characterized mainly as Gram-negative rods (Borsanelli et al. 2018, 2021, Silva et al. 2019). In this study, morphological examination of periodontal tissue and bacteria were detected in only one sheep by hematoxylin and eosin staining. In the histological Gram, bacteria were visualized in all samples, with a morphology predominantly of Gram-negative cocci. Rods and spirochetes were found less frequently. Our findings differ from those of Döbereiner et al. (1974), who identified the presence of Gram-positive coconuts in cattle tissues with periodontitis. Bogdan-Cătălin et al. (2020) observed coconuts by Giemsa staining; in the Gram, bacteria were characterized as Gram-positive.

The microorganisms observed by histochemical techniques in this study were in different tissue regions such as vascular endothelium, intravascular, epithelial pustule, between superficial epithelial cells and superficial biofilms, especially in the perivascular region inflammatory infiltrate. The location of these microorganisms in the inflammatory infiltrate is due to the macrophages' phagocytosis and inflammatory mediators that attract more cells to the aggression site (Kumar et al. 2003).

The predominantly perivascular inflammatory infiltrate of the sheep in this study means marked diapedesis. The vascular endothelium contributes to the development of the inflammatory process, as stated by Döbereiner et al. (1974). They found cell swelling or dilation of vascular endothelial cells in sheep and cattle with periodontitis.

Osteoclastic resorption of the alveolar bone of sheep was also observed. Cattle with periodontitis showed the exact morphological change (Döbereiner et al. 1974). They also observed replacing collagenous connective tissue and alveolar bone with granulation tissue with marked neutrophil infiltration. Seifert et al. (1983) also observed cattle's osteoclastic processes in the alveolar septum's central region. Ionel et al. (2017) noted osteoclasts and evident bone resorption in rats with periodontitis. Kocaman et al. (2019) observed that the cementum and the alveolar bone surfaces were irregular, with zones of osteoclastic activity of various sizes. Bogdan-Cătălin et al. (2020) verified sheep's bone resorption, cementum dentin, and tooth resorption. All these results differ from the findings by Cutress & Schroeder (1982), which showed the regular distribution of the adjacent alveolar bone in the apical third of all teeth radiographically, without alveolar bone resorption present in sheep. Mild cortical bone apposition was found herein. Döbereiner et al. (1974) also observed cattle with bone apposition but significant intensity, which causes swelling and justifies the disease's former popular name, "swollen face".

CONCLUSIONS

The gross and histological findings contribute to the knowledge about the aetiopathogenesis of ovine periodontitis. This research demonstrated that histological changes of

culled Santa Inês sheep with periodontitis included chronic inflammatory response, resorption of the alveolar bone, and cortical bone apposition.

Considering the economic relevance of periodontitis, the oral cavity physical examination and adoption of prophylaxis strategies on sheep farms are crucial.

Data availability.- The datasets used and analyzed during the current study are available from the corresponding author upon request.

Authors' contributions.- Ana Carolina Borsanelli: conceptualization, methodology, data curation, formal analysis, investigation, writing- reviewing and editing. Daniel Ubiali: conceptualization, methodology, data curation, formal analysis, investigation, supervision, project administration, writing- reviewing and editing. Iveraldo Dutra: conceptualization, methodology, data curation, funding acquisition, writing- reviewing and editing. Márcia Jansen: writing-original draft preparation, data curation, investigation, and formal analysis.

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