ORIGINAL ARTICLE

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Clinical laboratory profiling and microbiological analysis of the oral cavity of dogs (*Canis familiaris*) with dental disorders

Perfil clínico, laboratorial e análise microbiológica da cavidade oral de cães (Canis familiaris) *portadores de afecções odontológicas*

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ABSTRACT: Periodontal disease (PD) is caused by the accumulation of biofilm in the oral cavity. Biofilm is a viscous, yellowish film that is initially composed of non-pathogenic bacteria; the biofilm creates an environment that is conducive to the proliferation of pathogenic Gram-negative bacteria if it is not removed, leading to periodontitis. This study identified the bacteria present in oral cavity samples from 22 dogs with dental disorders and related them to clinical and laboratory findings. To identify these microorganisms, samples were collected and cultivated in a variety of culture media and bacterial identification was carried out using biochemical tests. We also performed antimicrobial susceptibility tests to select the most appropriate treatment for each case. The results showed that the dogs in the study presented with anemia, leukocytosis, and increased plasma protein, which were possibly associated with PD. The most severe periodontal changes were observed in dogs between 6 and 12 years old that consumed moist and/or soft foods. We identified eight species of bacteria, including *Klebsiella aerogenes*, *Pantoea agglomerans*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and bacteria from the genus *Clostridium* sp. These results highlight the importance of the antibiogram and of restricting the use of broad-spectrum antibiotics as complementary treatment for PD.

KEYWORDS: veterinary dentistry; dogs; periodontal pathogenic bacteria.

RESUMO: A doença periodontal (DP) é causada pelo acúmulo de biofilme na cavidade oral. O biofilme é viscoso e amarelado, inicialmente composto por bactérias não patogênicas; o biofilme cria um ambiente propício à proliferação de bactérias Gram-negativas patogênicas se não for removido, levando à periodontite. Este estudo identificou as bactérias presentes em amostras da cavidade oral de 22 cães com alterações dentárias e relacionou-as aos achados clínicos e laboratoriais. Para identificar esses microrganismos, amostras foram coletadas e cultivadas em diversos meios de cultura e a identificação bacteriana foi realizada por meio de testes bioquímicos. Também foram realizados testes de suscetibilidade antimicrobiana para selecionar o tratamento mais adequado para cada caso. Os resultados mostraram que os cães do estudo apresentavam anemia, leucocitose e aumento de proteínas plasmáticas, possivelmente associados à DP. As alterações periodontais mais graves foram observadas em cães entre 6 e 12 anos que consumiam alimentos úmidos e/ou moles. Foram identificadas oito espécies de bactérias, incluindo *Klebsiella aerogenes*, *Pantoea agglomerans*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa* e bactérias do gênero *Clostridium* sp. Esses resultados destacam a importância do antibiograma e da restrição do uso de antibióticos de amplo espectro como tratamento complementar da DP.

PALAVRAS-CHAVE: odontologia veterinária; cães; bactérias periodontopatogênicas.

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INTRODUCTION

The periodontium consists of four types of tissues: (a) gingiva, (b) alveolar bone, (c) cementum, and (d) periodontal ligaments. This anatomical structure plays a crucial role in supporting and protecting teeth. The progressive destruction of periodontium tissues that is mediated by the interaction between dysbiotic microbial communities and aberrant immune responses is termed periodontal disease (PD). The etiology of this disease is multifactorial, although the presence of bacterial plaque has been identified as the preponderant factor. PD can result in injuries that range from moderate to severe and are particularly prevalent in small animals. Furthermore, it is often found in association with serious systemic diseases (De Campos *et al.*, 2019; Sedghi *et al.*, 2021).

Dental biofilm, also known as bacterial plaque, is a dense, amorphous and non-calcified mass. It is composed of bacteria enclosed in a matrix that is rich in bacterial extracellular polysaccharides and salivary glycoproteins. The permanent location of these bacteria on the tooth surface favors the adherence and subsequent proliferation of Gram-negative, anaerobic bacteria, which are considered to be pathogenic periodontal bacteria as they play significant roles in the pathogenesis of PD (Gioso, 2007; Harvey, 2022).

Gums are an essential component of the periodontium, and they form the initial defense barrier against periodontal pathogenic bacteria—gum inflammation (gingivitis) represents the first clinical manifestation of PD. If bacteria persist in the oral cavity, thereby stimulating the animal's immune response, then the condition can progress and result in new local and systemic clinical signs (Baia *et al.*, 2017; Roza, 2004).

Severe local consequences of PD include the formation of oral fistulas, pathological fractures, and dental abscesses (Niemiec, 2008). In addition, systemic infection resulting from the hematogenous dissemination of bacteria is a common concern in cases of severe PD (Fernandes *et al.*, 2012).

PD is most prevalence in small and senile animals, and is the most common disease of the oral cavity in dogs (Debowes *et al.*, 2014; Marshall *et al.*, 2014). However, there is a lack of information about the specific oral microorganisms that can lead to periodontitis in these animals. This fact is concerning given that identification of the bacteria that cause local and systemic complications can facilitate the choice of the best method of bacterial control and, consequently, determine the success of treatments for this condition. Therefore, the present study aimed to analyze the oral microbiota of dogs with dental disorders and to correlate it with clinical and laboratory findings.

MATERIAL AND METHODS

This research was carried out at the Veterinary Dentistry Sector of the University Veterinary Hospital of the State University of Maranhão (HVU-UEMA) and included only dogs that were diagnosed with PD in the absence of any other oral conditions. This study was approved by the Ethics and Animal Experimentation Committee of the State University of Maranhão (Protocol no. 26/2020).

138 dogs with PD were included in this study. These animals underwent anamnesis and physical examination with an emphasis on the oral cavity in order to evaluate oral lesions. Patients diagnosed with PD were classified according to the American College of Veterinary Dental Medicine's classification criteria as follows: mild PD, including gingivitis and dental calculus; moderate PD, including some teeth with gingival retraction and bone resorption; and severe PD, in which there were numerous teeth with dental mobility, in addition to furcation exposure and purulent secretion (Niemiec, 2013; Bellows, *et al.*, 2019). After completion of the clinical form the following measures were obtained prior to carrying out periodontal treatment: a blood count; liver, kidney and heart evaluations; and an imaging examination comprising intra- and extra-oral radiography.

To enable microbiological analyses, we used absorbent paper cones to collect samples containing biofilm from the subgingival groove and pulp cavity of canine patients undergoing periodontal treatment; such treatment eliminates dental calculus, degenerative tissue, and periodontal pockets, including through tooth extraction where necessary (Lee *et al.*, 2016). Following sample collection, the paper cones were immersed in Brain Heart Infusion (BHI) broth in sterile tubes before being transported in thermal boxes to the location where the microbiological tests were performed.

While avoiding a bias for collecting samples from patients with severe PD, we randomly selected 22 animals to provide samples for microbiological cultivation; this was performed on 5% sheep blood agar and MacConkey agar, with the plated samples incubated for 24 to 48 hours at 37 °C. After the colonies grew, biochemical tests were carried out to identify bacteria from the *Enterobacteriaceae* family. To do this, we used Triple Sugar Iron (TSI) agar, SIM (sulfide, indole and motility) testing, EMB (Eosin-methylene blue) agar, Simmons Citrate agar, and *Salmonella Shigella* agar. We also performed antimicrobial sensitivity tests using a disk-diffusion method on Mueller-Hinton agar plates (Bauer *et al.*, 1966); this assay used nine antibiotic disks containing the main antimicrobials of choice in veterinary medicine (Pignone and Gioso, 2008; Duarte *et al.*, 2020): amikacin (AMI), amoxicillin with clavulanate (AMC), ciprofloxacin (CIP), clarithromycin (CLA), chloramphenicol (CHL), enrofloxacin (EN), gentamicin (GEN), levofloxacin (LVX), and tetracycline (TET).

RESULTS

The 138 animals with PD that participated in this study were divided into groups according to their PD severity: mild PD made up 28% of all study cases (n=39), moderate PD made up 27.5% (n=38) of the cases, and severe PD made up 44.5% (n=61) of the cases. This classification was made following the interpretation of complementary exams and clinical analysis. Halitosis was the main clinical sign that was reported. A specific physical examination of the oral cavity of these patients also revealed the presence of bacterial plaque, gingivitis, dental calculus, gingival retraction, root exposure, and tooth mobility.

Severe PD was the most common (n=61) type of PD in the study participants, and this was most prevalent in dogs aged 6–12 years old (prevalence of 53.16% in this age group). Furthermore, the animals over 12 years old (n=16) only presented with moderate PD (n=4) or severe PD (n=12) (Figure 1). PD was also more prevalent in female animals (n=80) than in male animals, with females comprising up to 58% of the PD cases included in the study.

In this study, the breeds most commonly affected by PD were Poodle (n=52), Mixed Breed (SRD) (n=27), Pinscher (n=20), Yorkshire (n=17), Shih-tzu (n =10), Dachshund (n=5), Maltese (n=2), Akita (n=1), Cocker (n=1), Dalmatian (n=1), Golden Retriever (n=1), and West Terrier (n=1). We observed that 77.6% of the animals with PD were small breeds.

In the blood count analysis, we identified cases with anemia (n=34), increased plasma protein (n=49), and leukocytosis (n=7) in accordance with the reference values outlined by Feldman et al. (2000). We also observed that anemia was present in 36.1% of patients with severe PD, but was not present in any of the animals with mild PD. Furthermore, biochemical tests indicated the presence of liver (n=26) and kidney (n=11) lesions in some animals in addition to PD.

The dogs in this study were more likely to have severe PD if they were fed with wet food; 80% (n=8/11) of the PD cases among dogs fed this way presented were classified as severe. In contrast, severe PD was diagnosed in only 40% (n=2/5) of the dogs fed with natural food and 36% (n=4/11) of the dogs fed with dry food combined with homemade food. Among the dogs fed only with dry food, cases of mild PD were more prevalent, representing 35% of cases (n=19/39) (Figure 2).

In the samples collected from the study cases, we identified the presence of *Clostridium* sp. (n=3 cases), *Klebsiella aerogenes* (n=4), *Pantoea agglomerans* (n=1), *Escherichia coli* (n=7), *Klebsiella oxytoca* (n=1), *Klebsiella pneumoniae* (n=1), *Proteus mirabilis* (n =1), *Proteus vulgaris* (n=1), and *Pseudomonas aeruginosa* (n=3). Table 1 shows the frequency of these agents among samples isolated from the oral cavity, and whether they were isolated from the pulp cavity or the subgingival groove. The antimicrobial resistance pattern of the isolates is shown in Table 2.

DISCUSSION

The largest number of cases of PD was found in dogs aged 6 to 12 years old (57.2% of cases); animals older than 12 years old presented with only moderate (n=4) or severe (n=12) PD

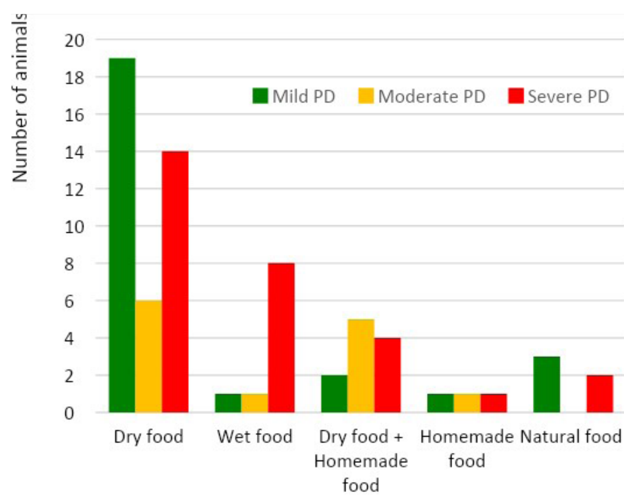

Figure 2. Distribution of cases according to food type provided and degree of PD severity.

Table 1. Distribution of isolates from the oral cavity according to sampling location in the pulp cavity or subgingival groove.

Microorganism	Localization of isolates	
	Pulp cavity	Subgingival groove
Clostridium sp.	1	2
Escherichia coli	3	4
Klebsiella aerogenes	4	0
Klebsiella oxytoca	0	1
Klebsiella pneumoniae	1	0
Pantoea agglomerans	0	1
Proteus mirabilis	1	0
Proteus vulgaris	0	1
Pseudomonas aeruginosa	0	3
Total	**10**	**12**

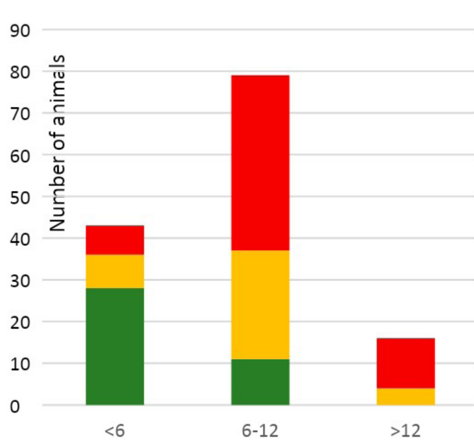

Figure 1. Degrees of severity and distribution of PD cases according to age.

Table 2. Antimicrobial resistance of isolates from the oral cavity of dogs. R—Resistant, IR—Intrinsic Resistance, I—Intermediate, S—Sensitive.

Microorganism	Antibiotic disks								
	AMI	AMC	CIP	CHL	EN	CLA	GEN	LVX	TET
E. coli (A1)	I	I	I	I	S	IR	I	I	R
E. coli (A2)	S	S	I	R	S	IR	S	I	R
E. coli (A3)	S	S	S	S	S	IR	S	S	S
E. coli (A4)	S	S	I	I	S	IR	S	S	S
E. coli (A5)	S	S	R	R	S	IR	R	R	R
E. coli (A6)	R	S	R	R	R	IR	R	R	R
E. coli (A7)	R	S	R	R	S	IR	R	R	R
K. aerogenes (A1)	S	IR	S	S	R	IR	S	R	S
K. aerogenes (A2)	I	IR	R	R	R	IR	R	R	R
K. aerogenes (A3)	R	IR	S	S	S	IR	R	S	S
K. aerogenes (A4)	R	IR	R	S	S	IR	R	R	R
K. oxytoca	R	S	I	I	R	IR	I	I	S
K. pneumoniae	S	S	R	I	S	IR	I	S	I
P. aeruginosa (A1)	S	IR	S	IR	S	IR	S	S	IR
P. aeruginosa (A2)	I	IR	S	IR	I	IR	I	S	IR
P. aeruginosa (A3)	S	IR	S	IR	R	IR	S	S	IR
P. agglomerans	S	IR	S	S	IR	IR	S	IR	R
P. mirabilis	I	I	I	S	I	IR	I	S	S
P. vulgaris	R	S	R	R	R	IR	R	R	S
Clostridium sp. (A1)	S	IR	S	S	S	R	S	IR	S
Clostridium sp. (A2)	S	IR	S	S	S	S	S	IR	S
Clostridium sp. (A3)	S	R	S	S	I	S	S	S	S

in this study. This finding aligns with the conclusions of Rocha and Castro (2018), who identified a greater predisposition to the development of moderate and severe PD in older dogs. We found that female dogs comprised 58% of the PD cases that were analyzed (n=80). However, no significant evidence indicated that sex was a predisposing factor for PD, as has been discussed by Pinto *et al.* (2020). This highlights the multifactorial complexity of PD in dogs and the need for continued investigation to fully understand the factors underlying its development (Pinto *et al.*, 2020)

Freire, Albuquerque and Nascimento (2021) have previously reported that factors such as tooth size, alveolar bone resistance, genetic factors, and malocclusion are related to a greater predisposition of small breed dogs to develop PD. These facts are validated by our results, as 77. 6% of the dogs that presented PD were small breeds.

From the blood count results, we observed that some animals presented with anemia. This finding is in accordance with research by Pinto *et al.* (2020), who reported that the dogs in their study with PD showed signs of anemia. Leukocytosis and hyperproteinemia can be associated with microorganisms from the bacterial plaque reaching the bloodstream, and also with inflammatory processes that are promoted by enzymes and bacterial toxins in plaque (Cunha *et al.*, 2017).

In this study, 11 animals showed signs of kidney damage. Glickman *et al.* (2011) identifies PD as a predisposing factor for chronic kidney diseases and azotemia. Furthermore, toxemia resulting from bacteria present in the biofilm can—due to its ability to alter the proper functioning of the kidney—trigger pyelonephritis and interstitial nephritis. Additionally, microorganisms present in the bloodstream are capable of forming immune complexes in the glomeruli, thereby leading to glomerulonephritis (Yerramilli *et al.*, 2016).

Our results demonstrate that the presence of liver lesions is mainly related to moderate (n=10) and severe (n=14) PD. Whyte *et al.* (2014) observed that bacterial infections can induce liver inflammation and consequently an increase in alanine aminotransferase (ALT), which is suggestive of liver damage.

Although the majority of animals with PD that were analyzed in this study were fed dry food, the animals most commonly presenting with severe PD were those that received wet food as their primary food source. Rocha and Castro (2018) also observed that animals fed exclusively with wet food had bacterial plaque covering a large part of their teeth. Nogueira, Silva, and Ambrosio (2010) reported that dogs fed exclusively with homemade food tended to develop more severe PD. Additionally, Buckley *et al*. (2011) states that the abrasion that takes place during the crushing of dry food (hard food) causes a delay in the formation of bacterial plaque.

Our microbiological results were similar to those of Braga *et al*. (2005), Fonseca *et al*. (2011), and Frias *et al*. (2018). The bacteria found were entirely the same, in the urogenital tract and final portion of the digestive system. Therefore, the presence of these microorganisms in the oral cavity may be related to mechanical deposition caused by licking the genitalia and anus.

In the antimicrobial sensitivity tests, some strains showed resistance to different antibiotics. Furthermore, we observed that strains of *E. coli* and *K. aerogenes* showed resistance to almost all of the antibiotics that were tested. Bacterial resistance is a problem resulting from the indiscriminate and recurrent use of antimicrobial agents (Mcewen; Collignon, 2018).

CONCLUSIONS

The animals in this study presented with varying degrees of PD severity. However, senile, female and small animals were those most likely to be affected by the severe form of the disease. We also observed that animals with severe PD showed anemia, increased plasma protein, and leukocytosis in addition to biochemical changes that indicated liver and kidney damage.

We identified *Klebsiella aerogenes*, *Pantoea agglomerans*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa,* and some strains of *Clostridium* sp. in samples from the oral cavities of dogs. Furthermore, we performed antimicrobial sensitivity tests in order to achieve success in dental procedures. This study therefore increases our understanding of the biological profile of the agents involved in PD; some strains of *E. coli* and *Klebsiella aerogenes* were resistant to several antibiotics that are routinely used in the clinic. Therefore, antimicrobial sensitivity tests are extremely important for the success of dental treatments.

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REFERENCES

BAIA, J. D. *et al*. Doença periodontal em cães: revisão de literatura. **Scientific Electronic Archives**, Mato Grosso, v. 10, n. 5, p. 150-162, 2017.

BAUER, A. W. *et al*. Antibiotic susceptibility testing by a standardized single disk method. **Journal of Clinical Microbiology**, v. 40, p. 2413-2415, 1966.

BELLOWS, J. *et al*. AAHA dental care guidelines for dogs and cats. **Journal of the American Animal Hospital Association**, v. 55, n. 2, p. 49-69, 2019.

BRAGA, C. A. S. B. *et al*. Isolamento e identificação da microbiota periodontal de cães da raça Pastor Alemão. **Ciência Rural**, v. 35, n. 2, p. 385–390, 2005.

BUCKLEY, C. *et al.* The impact of home-prepared diets and home oral hygiene on oral health in cats and dogs. **British journal of nutrition**, v. 106, n. 1, p. 124-S127, 2011.

CUNHA, E. *et al*. Canine periodontal disease and its systemic implications – a review. **Revista Portuguesa de Ciências Veterinárias**, n. 113, p. 12-22, 2017.

DEBOWES, L. J. *et al*. Odontologia: aspectos periodontais. **Tratado de medicina veterinária interna**: doenças do cão e do gato. 5 ed. Rio de Janeiro: Guanabara Koogan. v. 2. cap. 133. p. 1189-1197, 2014.

DE CAMPOS, M. *et al*. Doença periodontal em cães–uma revisão. **Revista Científica Unilago**, v. 1, n. 1, 2019.

DUARTE, L. F. *et al*. Diagnóstico e tratamento de doença periodontal grave em cão idoso: relato de caso. **Brazilian Journal of Development**, v. 6, n. 7, p. 45742-45761, 2020.

FELDMAN, J. G. *et al*. **Jain Schalm's Veterinary Hematology** (5th ed.), Lippincott Williams and Wilkins, New York, 2000.

FERNANDES, N. A. *et al*. Prevalence of periodontal disease in dogs and owners' level of awareness-a prospective clinical trial. **Revista Ceres**, v. 59, n. 4, p. 446-451, 2012.

FONSECA, S. A. *et al*. Microbiological analysis of bacterial plaque of periodontal disease on dogs and effects of antibioticotherapy on it. **Ciência Rural**, v. 41, n. 8, p. 1424-1429, 2011.

FREIRE, S. C. D. S.; ALBUQUERQUE, J. M. D. O.; NASCIMENTO, H. B. Tipo de crânio, raça e idade como fatores predisponentes da doença periodontal em cães. **Revista Multidisciplinar em Saúde**, v. 2, n. 3, p. 06-06, 2021.

FRIAS, D. F. R. *et al*. Identificação da microbiota gengival de cães aparentemente hígidos. **Nucleus**, v. 15, n. 1, p. 129-136, 2018.

GIOSO, M. A. **Odontologia veterinária:** para o clínico de pequenos animais. 2 ed. São Paulo: Minha Editora, p. 7-24, 2007.

GLICKMAN, L. T. *et al*. Association between chronic azotemic kidney disease and the severity of periodontal disease in dogs. **Preventive Veterinary Medicine**, v. 99, n. 2-4, p. 193-200, 2011.

HARVEY, C. The Relationship Between Periodontal Infection and Systemic and Distant Organ Disease in Dogs. **Veterinary Clinics of North America**: Small Animal Practice, v. 52, n. 1, p. 121-137, 2022.

LEE, B. *et al*. Controlledrelease of tetracycline and lovastatin by poly (D,L-lactide-co-glycolide acid)-chitosan nanoparticles enhances periodontal regeneration in dogs. **Internal Journal of Nanomedicine**, v. 18, n. 11, p. 285-297, 2016.

MARSHALL, M. D. *et al*. Uma avaliação longitudinal da doença periodontal em 52 schnauzers miniatura. **BMC Veterinary Research**, v. 10, p. 166, 2014.

MCEWEN, S. A.; COLLIGNON, P. J. Antimicrobial resistance: a one health perspective. **Microbiology Spectrum**, v. 6, n. 2, p. 6.2. 10, 2018.

NIEMIEC, B. A. Periodontal Disease. **Topics in Companion Animal Medicine**, v. 23, n. 2, p. 72–80, 2008.

NIEMIEC B. Home plaque control in veterinary periodontology. In: **Veterinary periodontology**. Ames:Wiley-Blackwell Inc, 2013. pp. 175–185.

NOGUEIRA, J.L.; SILVA, M.V.M.; AMBRÓSIO, C.E. Doença Periodontal em Cães: Métodos Preventivos. **Nosso Clínico**, n. 73, p. 4-14, 2010.

PIGNONE, V. N.; GIOSO, M. A. Antimicrobianos utilizados no tratamento da doença periodontal em cães e gatos. MEDVEP. **Revista Científica de Medicina Veterinária. Pequenos Animais e Animais de Estimação**, v. 6, n. 18, p. 145-149, 2008.

PINTO, B. A. M. *et al*. Estudo retrospectivo das doenças da cavidade oral de cães em um serviço de odontologia veterinária. **Revista Brasileira de Ciência Veterinária**, v. 27, n. 4, 2020.

ROCHA, S. A.; CASTRO, S. V. Prevalência de placa bacteriana em cães submetidos à alimentação sólida e/ou macia. **Revista científica eletrônica de Medicina Veterinária**, 2018.

ROZA, M. R. **Odontologia em pequenos animais**. 1. ed. Rio de Janeiro: Livraria LF - Livros de Veterinária LTDA. p. 361, 2004.

SEDGHI, L. *et al*. The oral microbiome: Role of key organisms and complex networks in oral health and disease. **Periodontology 2000**, v. 87, n. 1, p. 107-131, 2021.

WHYTE, A. *et al*. Canine stage 1 periodontal disease: a latent pathology. **The Veterinary Journal**, v. 201, n. 1, p. 118-120, 2014.

YERRAMILLI, M. *et al*. Kidney disease and the nexus of chronic kidney disease and acute kidney injury: the role of novel biomarkers as early and accurate diagnostics. **Veterinary Clinics**: Small Animal Practice, v. 46, n. 6, p. 961-993, 2016.