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Analysis of Gingival Pocket Microflora and Biochemical Blood Parameters in Dogs Suffering from Periodontal Disease

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Abstract. *Background/Aim: Periodontal diseases in dogs are caused by bacteria colonising the oral cavity. The presence of plaque comprising accumulations of aerobic and anaerobic bacteria leads to the development of periodontitis. Due to the fact that in a large percentage of cases periodontal diseases remain undiagnosed, and consequently untreated, they tend to acquire a chronic character, lead to bacteraemia and negatively impact the health of internal organs. The aim of the present study was to perform a qualitative microbiological analysis of gingival pockets and determine the correlations between selected morphological and biochemical blood parameters and the extent periodontal diseases. Patients and Methods: Twenty-one dogs treated for periodontal diseases were qualified for the study and subsequently divided into two groups: with 3rd and 4th stage of periodontal disease. Swabs from the patients' gingival pockets were taken for bacteriological testing. Blood was tested for parameters including erythrocyte count, haemoglobin concentration, haematocrit values and leukocyte count. Blood serum was analyzed with respect to the concentrations of alanine transaminase (ALT), aspartate transaminase (AspAT/AST) and urea. Results: The microbiological analysis of gingival pockets indicated the presence of numerous pathogens with a growth tendency in bacterial cultures observed in dogs with advanced-stage periodontal disease. The concentration of biochemical blood markers was significantly higher in dogs with 4th stage of periodontal disease, to compared to the 3rd-stage group. Morphological parameters were not significantly different with the exception of haemoglobin concentration, which was lower in dogs with 4th stage disease. In both groups, elevated leukocyte counts were observed. Conclusion: By conducting a detailed microbiological examination, it is possible to provide a better prognosis, plan adequate treatment and monitor dogs treated for peridontopathy.*

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Periodontal diseases in carnivorous animals are fairly common infections caused by a closely cooperating group of bacteria.

Inflammations in the oral cavity are among diseases most frequently diagnosed in veterinary dentistry. Inflammatory processes in this region are most commonly caused by bacterial colonisations and less often by viral or fungal infections. In fact, canine oral cavities are an ideal environment for microbial colonisation as they provide stable temperatures and humidity, as well as plentiful immune recesses to self-purification (1, 2).

In the oral cavity, more often than in any other part of the body, the natural protective barrier can be mechanically breached for reasons, such as the occurrence of deep gingival pockets, incidence of periapical abscesses or the performance of dental procedures (3, 4).

Bacteria colonising the oral cavity take advantage of their own mechanisms of adhesion and congregation. Bacterial colonies present on tooth surfaces form dental plaque. The term "plaque" may be defined as the result of a complex process of accumulation of aerobic and anaerobic bacteria, which produces a viscous sediment on tooth surfaces. Dental plaque consists of: 50-60% bacteria and fungi, 30-40% glycoproteins produced by salivary glands or as a result of bacterial decay and approximately 10% bacterial polysaccharides. The presence of plaque conditions the development of periodontal diseases (5-7). As it calcifies, plaque forms tartar. A porous tartar surface provides ideal conditions for colonisation and proliferation of pathogenic microorganisms, which are the key factor damaging the periodontium and causing degeneration of elements constituting the gingival connective tissue (8). A healthy organism has defensive mechanisms in place to protect itself from the bacteria responsible for plaque formation. As long as it is able to neutralise the toxins, inhibit the activity of bacterial enzymes and eliminate bacteria as such, the periodontium will remain healthy (9).

Destruction of the periodontal ligament and bone resorption of the dental progress oftentimes lead to increased tooth mobility, even tooth loss (10, 11).

Given the fact that in many dogs gingivitis and periodontitis can remain untreated for most of the animal's life, chronic inflammations within the oral cavity facilitate incessant bacteremia.

The aim of this study was to analyse the microorganisms responsible for periodontal diseases and verify the existence of a significant correlation between advanced stages of periodontal diseases and the biochemical blood serum parameters in the sick animals.

Patients and Methods

The research material was provided by 21 dogs treated for periodontal diseases at the Department and Clinic of Veterinary Surgery at the University of Life Sciences in Lublin. The age of the animals was between 6 and 15 years. Animals suffering from diseases of the endocrine system or cancer were excluded from the study.

Dental examinations were performed on all of the animals, including a general evaluation of gingival and periodontal health, measurements of gingival recession, tooth mobility, tendency for bleeding and depth of gingival pockets in four locations around each tooth using the Williams probe. Swabs from gingival pockets were also taken for bacteriological examinations.

Based on the classification of periodontal diseases, groups of dogs in the 3rd and 4th stage of disease were identified and submitted to specialist dental treatment.

Stage 3 of the disease was characterised by general inflammation of the periodontium, presence of gingival pockets (4-6 mm), tartar deposits on all teeth and early stages of resorption of the dental progress bone.

Animals in the 4th stage of a periodontal disease suffered from acute, general gingivitis, had deep gingival pockets (5-8 mm) with purulent exudate and advanced bone resorption of the dental progress.

Blood was drawn from all the animals for haematological examination with the view of determining the erythrocyte count, haemoglobin concentration, haematocrit values and the leukocyte count. Biochemical examinations helped determine the activity level of alanine transaminase (ALT), the activity of aspartate transaminase (AspAT/AST) and urea concentration in the blood serum of the tested animals. The microbiological examinations were performed at the Diagnostic Laboratory of the Department and Clinic of Epizootiology at the University of Life Sciences in Lublin. Swabs were taken in accordance with the following practice: The dogs were fasted for 24 h before the procedure. Prior to the swab, the oral cavity was washed three times with warm, boiled water. The material was collected from gingival pockets with the use of a periodontal probe introduced parallel to the tooth's long axis, at the depth of 4 to 8 mm. A portion of the probed material was placed in Sheadler liquid medium (BIOCORP, Warsaw, Poland) with the view of culturing anaerobic bacteria. The remaining portion of the material was placed in GHI medium (BIOCORP, Warsaw, Poland) to culture aerobic bacteria. The collected material was delivered immediately to the diagnostic laboratory.

Microbiological identification was performed with classic methodology. The bacteriological examination involved: - preparation of a bacterioscopic specimen; - culture examination and isolation of microorganisms.

The isolation of the cultured bacteria was based on identification of colony morphology and morphology of the bacterial cell. The isolated strains underwent: - identification under aerobic conditions; - identification under anaerobic conditions.

Furthermore, all microorganisms isolated under anaerobic conditions were controlled by observing their growth under aerobic conditions (Columbia agar + 5% mutton blood).

Mycological tests were performed with the use of solid medium - Sabouraud Dextrose Agar (BIOCORP, Warsaw, Poland).

Microorganisms were identified with the use of kits supplied by bioMerieux (Warsaw, Poland). In the analysis of the obtained results, the following statistical tests were used: - the Kolmogorov-Smirnov test as improved by Lilliefors; - the Mann-Whitney test; - the χ^2 concordance test.

The presented results are predominantly quantitative and, therefore, appropriate tests were necessary. For this reason, normal distribution was analysed with the use of the Kolmogorov-Smirnov test. As no normal distribution was identified in the obtained results, the non-parametric Mann-Whitney test was employed in the comparison of parameters, characteristic for the analysed disease, *i.e.* the 3rd and 4th stage of periodontopathy. The test allows the determination of whether the medians vary between the analysed groups. With the use of non-parametric tests, the proposed hypotheses were that the compared groups did not vary, against the alternative hypothesis that they were different.

Results

Bacteriological examinations on the material collected from gingival pockets of dogs in the 3rd and 4th stage of periodontopathy indicated the presence of numerous pathogens. In the case of swabs taken from dogs with advanced stage periodontal inflammation, significant growth of the bacterial cultures was observed (Table I).

Among pathogens related to the disease process most commonly isolated in the 3rd stage of periodontopathy we can list: *Streptococcus sanguis* (15.59% of isolated strains), *Peptostreptococcus* spp. (15.59 %), *Escherichia coli* (12.84 %), *Proteus mirabilit* (5.50 %), *Veilionella* spp. (4.58 %) and *Staphylococcus aureus* (4.58 %). Fungi comprised 0.91% of the isolated strains (Figure 1).

In the 4th stage of priodontal diseases, the most often isolated strains included *Pepto-streptococcus* spp. (13.69 %), *Streptococcus salivarius* (13.01%), *Veilionella* spp. (7.53 %), *Actinomyces* spp (6.16 %) *Actinomyces viscosus* (4.10 %) and *Staphylococcus aureus* (4.79%). Fungi comprised 2.05 % of the isolated strains (Figure 2).

Analysis of laboratory results indicated the activity of ALT to be significantly higher in the 4th stage of periodontopathy, compared to stage 3. AST was also higher in dogs in the 4th stage of the disease. Urea levels were four-times higher in stage 4 dogs and the leukocyte counts were also statistically significantly higher in these animals.

The erythrocyte count did not statistically significantly vary between the two groups and neither did hematocrite values. The haemoglobin concentrations in stage 4 patients were lower than those in stage 3 periodontopathy patients (Table II).

Table I. *The number and percentage of microbial strains isolated from the gingival pockets of dogs treated for periodontal diseases (stage 3 and 4).*

Cultured microorganisms	Number (and percentage) of isolated strains in dogs in		Isolated strains in total	
	Stage 3 periodontal disease	Stage 4 periodontal disease	Number	%
Bacteria				
Escherichia coli	14 (12.84%)	5 (3.42%)	19	7.45
Streptococcus sanguis	17 (15.59%)	19 (13.01%)	36	14.11
Streptococcus pyogenes	1 (0.91%)	7 (4.79%)	8	3.13
Streptococcus equi	0 (0%)	2 (1.36%)	2	0.78
Streptococcus salivarius	4 (3.66%)	9 (6.16%)	13	5.09
Staphylococcus aureus	5 (4.58%)	7 (4.79%)	12	4.70
Staphylococcus epidermidis	3 (2.75%)	5 (3.42%)	8	3.13
Staphylococcus spp. (coagulase-negative)	2 (1.83%)	5 (3.42%)	7	2.74
Staphylococcus intermedius	2 (1.83%)	3 (2.05%)	5	1.96
Actinomyces spp.	3 (2.75%)	9 (6.16%)	12	4.70
Actinomyces viscosus	1 (0.91%)	6 (4.10%)	7	2.74
Pseudomonas aeruginosa	3 (2.75%)	2 (1.36%)	5	1.96
Proteus mirabilis	6 (5.50%)	2 (1.36%)	8	3.13
Proteus vulgaris	2 (1.83%)	4 (2.73%)	6	2.35
Corynebacterium spp.	1 (0.91%)	0 (0%)	1	0.39
Corynebacterium pyogenes	1 (0.91%)	1 (0.68%)	2	0.78
Bacillus spp.	3 (2.75%)	1 (0.68%)	4	1.56
Peptostreptococcus spp.	17 (15.59%)	20 (13.69%)	37	14.5
Eubacterium spp.	4 (3.66%)	6 (4.10%)	10	3.92
Fusobacterium spp.	1 (0.91%)	0 (0%)	1	0.39
Veillonella spp.	5 (4.58%)	11 (7.53%)	16	6,27
Prevotella spp.	3 (2.75%)	2 (1.36%)	5	1.96
Propionibacterium spp	2 (1.83%)	4 (2.73%)	6	2.35
Lactobacillus spp	4 (3.66%)	6 (4.10%)	10	3.92
Porphyromonas spp.	1 (0.91%)	2 (1.36%)	3	1.17
Capnocytophaga spp	1 (0.91%)	1 (0.68%)	2	0.78
Actinobacillus actinomycetemcomitans	2 (1.83%)	4 (2.73%)	6	2.35
Bacteria in total	108 (99.08%)	143 (97.94%)	251	98.43
Fungi				
Candida non albicans	1 (0.91%)	1 (0.68%)	2	0.78
Candida albicans	0 (0%)	2 (1.36%)	2	0.78
Fungi in total	1 (0.91%)	3 (2.05%)	4	1.56
Bacteria and Fungi in total	109 (100%)	146 (100%)	255	100

Statistical analysis of laboratory results in dogs depending on the stage of the periodontal disease are presented in Table III.

Discussion

Veterinary dentistry is enjoying a period of intensive advancement due to its close relationship with human dentistry. Its growth owes much to the knowledge, experience, as well as proper equipment and patience of doctors. Due to the changing expectations of animal owners and the growing offer of available materials and instruments, significant advances are observed particularly in the dentistry of small animals.

Periodontal diseases are the most widespread chronic bacterial infections in both humans and dogs. Gingivitis is an initial and reversible condition but, if untreated, it can lead to periodontitis and consequently to destruction of the dental progress. Identification of the microorganisms responsible for periodontopathy and evidence of their relation to both periodontal inflammation and diseases in general are some of the goals of current dental, as well as general medical research.

According to literature, periodontitis is a disease caused by the presence of plaque that is the main source of pathogenic bacterial flora. Out of the great number of bacterial species inhabiting the oral cavity, a group of

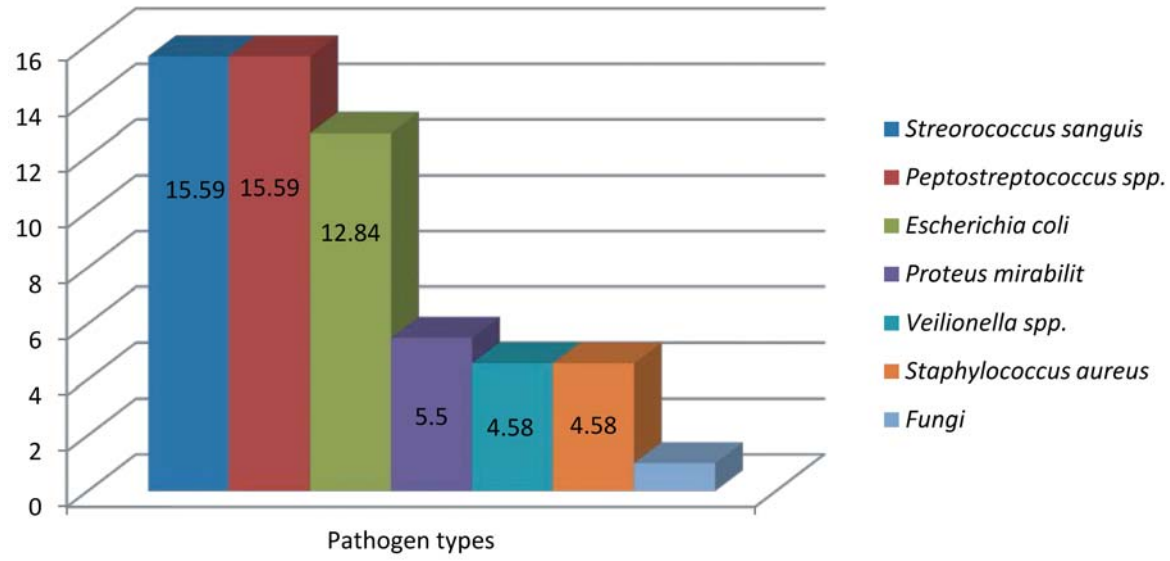

Figure 1. *Pathogens most often isolated in the 3rd stage of periodontopathy.*

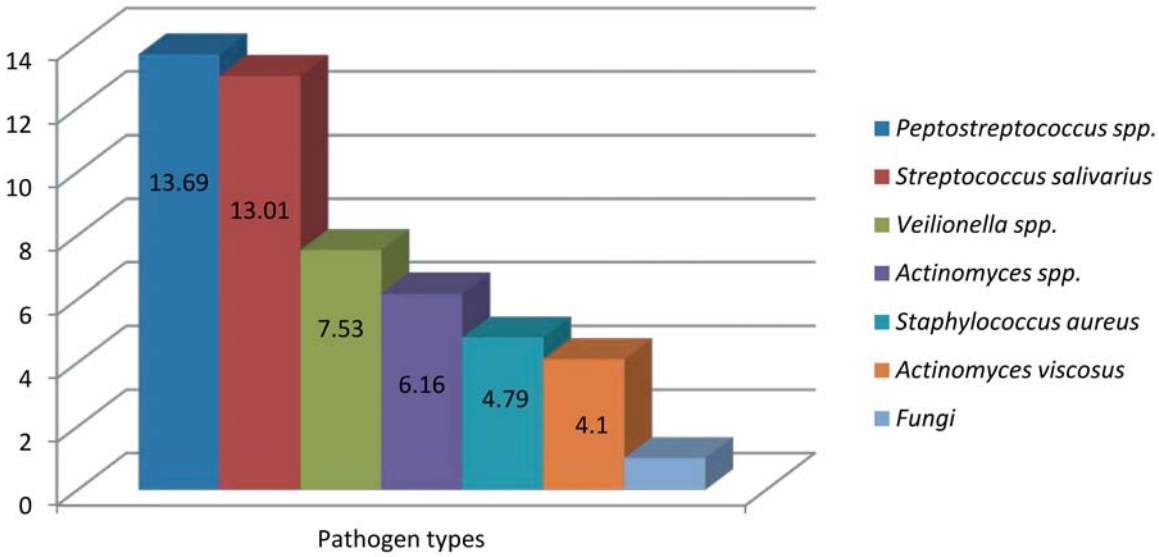

Figure 2. *Pathogens most often isolated in the 4th stage of periodontopathy.*

pathogens can be identified, which contribute, both directly and indirectly, to the destruction of periodontal structures. The strains most commonly responsible for causing periodontal diseases include *Actinobacillus actinomyces*, *Porphyromonas*, *Bacteroides* (12, 13).

Microbiological studies on humans suggest that the most pathogenic microorganism in the context of peoriodontopathy is *Haemophilus actinomycetemcomitans* (14).

The authors' own research indicates that in dogs suffering from periodontal diseases and qualified as stage 4 patients, *i.e.* those with the most clinically advanced disease, the inflammatory process involves Streptococcus spp., *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylo-coccus epidermidis*, *Actinomyces* spp., *Proteus* spp. and *Proteus vulgaris*.

Microbiological studies can facilitate prognosis, help plan the best course of treatment and monitor the dogs treated for periodontal diseases.

Periodontopathy is considered a focus of infection from which bacterial pathogens enter the blood stream and reach internal organs and tissues, where they can cause local lesions and damage. Earlier research has revealed how dangerous this disease can be, especially in older dogs, and what related dangers must be considered before undertaking any dental surgery involving general anaesthesia.

Table II. *Descriptive parameters of the basic haematological and biochemical tests in dogs in the 3rd or 4th stage of periodontal disease.*

Stage of periodontal lesions	Variable	N	Mean	SD	Min	Max	Me
3rd	ALT	8	23.21	15.28	6.80	46.00	18.00
4th	ALT	13	42.74	17.12	17.00	62.00	50.00
3rd	AST	8	12.34	4.36	6.00	17.30	13.00
4th	AST	13	59.14	35.97	10.50	105	67.20
3rd	Urea	8	6.89	4.17	2.80	14.20	5.80
4th	Urea	13	24.75	15.28	7.30	52.00	24.20
3rd	WBC	8	11.13	4.79	7.10	22.30	9.85
4th	WBC	13	32.92	18.99	9.50	67.90	28.60
3rd	RBC	8	5.22	0.88	4.16	6.21	5.42
4th	RBC	13	4.68	0.98	3.17	5.93	4.59
3rd	HCT	8	39.10	3.83	32.50	44.40	39.65
4th	HCT	13	33.47	7.98	21.10	48.20	31.10
3rd	HGB	8	13.16	1.34	10.20	14.10	13.70
4th	HGB	13	10.50	2.44	7.40	14.60	9.50

ALT, Alanine transaminase; AST, aspartate transaminase; WBC, white blood cells; RBC, red blood cells; HCT, hematocrit; HGB, hemoglobin.

Periodontitis caused by pathogenic bacterial flora damages not only the oral cavity but the entire organism. For this reason, biochemical examinations were performed on the blood to determine whether bacterial processes in the particular animal's mouth will indeed influence the blood parameters of the dogs.

Laboratory tests indicated elevated concentrations of urea (24.75 mmol/l), increased activity of alanine (42.72 U/l) and aspartate transaminase (59.14 U/l), as well as increased leukocyte levels in blood sera (32.92 thou/mm^3). In the 21 animals assigned to the group of dogs with stage 3 or 4 periodontal diseases, radiological examinations revealed 8 cases of cardiomegaly accompanied by significantly elevated levels of urea (30.6-52.0 mmol/l) and liver function test results (ALT 58.0-62.0 U/l, AST 67.2-105.0 U/l). In such cases, given the unfavourable results of supplementary tests, the dental procedure was postponed due to the danger that general anaesthesia could pose to the patient's life. Clinical diagnosis of a potentially life-threatening condition requires administering specialist internist treatment before a dental procedure becomes viable (15).

Diseases of the periodontium are classed in the group of non-specific bacterial diseases connected with particular bacterial strains whose presence may have a certain diagnostic value, *e.g. Staphylococcus aureus*, *Streptococcus viridans*, *Streptococcus pyogenes*, *Actinobacillus actinamycetemcomitans* (16, 17).

In 5 dogs in the group of dogs with 4th stage of periodontal lesions in which bacteria from the *Actinomyces spp.* family were isolated in the gingival pockets, blood test results indicated significantly elevated levels of urea (30.6 mmol/l) and AST (91 U/l); after conservative periodontal treatment administered locally the observed therapeutic effects were unsatisfactory. Within a short period of time (approximately a month) acute relapse of periodontitis would occur. In these animals, the inflammatory process could only be conclusively stopped by extracting teeth from the area of periodontal lesions. *Actinomyces spp.* belongs to the group of bacteria which can overcome the leukocyte barrier and easily penetrate into the bloodstream (14, 18, 19).

Table III. *Statistical analysis of laboratory results in dogs depending on the stage of the periodontal disease.*

Stage of the periodontal disease	N	Mean	SD	Me	Test function
			ALT		
3rd	8	23.21	15.28	18.00	Z=2.46
4th	13	42.74	17.12	50.00	$p<0.05$
			AST		
3rd	8	12.34	4.36	13.00	Z=3.34
4th	13	59.14	35.97	67.20	$p<0.001$
			UREA		
3rd	8	6.89	4.17	5.80	Z=2.97
4th	13	24.75	15.28	24.20	$p<0.05$
			WBC		
3rd	8	11.13	4.79	9.85	Z=3.01
4th	13	32.92	18.99	28.60	$p<0.05$
			RBC		
3rd	8	5.22	0.88	5.42	Z=1.38
4th	13	4.68	0.98	4.59	$p>0.05$
			HTC		
3rd	8	39.10	3.83	39.65	Z=1.81
4th	13	33.47	7.98	31.10	$p>0.05$
			HGB		
3rd	8	13.16	1.34	13.70	Z=2.46
4th	13	10.50	2.44	9.50	$p<0.05$

ALT, Alanine transaminase; AST, aspartate transaminase; WBC, white blood cells; RBC, red blood cells; HCT, hematocrit; HGB, hemoglobin.

Following dental procedures, such as tooth extraction or tartar removal, temporary bacteremia occurs, which can pose a threat to patients in the higher risk group (elevated urea, AST, ALT, changes observed in x-ray imaging). Preventing the dangerous consequences of bacteremia requires taking adequate periprocedural measures. Before the procedure, it is recommended to locally administer antibacterial agents in the oral cavity, such as 0.15% chlorhexidine, accompanied by general antibiotic cover, most commonly clindamycin dosed at 20 mg/kg of body mass for 3 days prior to the planned procedure (20-24).

Conclusion

Numerous types of bacteria contribute to the development and progression of periodontal diseases. Elevated concentrations of blood markers suggest that not only does the course of periodontopathy involve the emergence of inflammatory lesions within the oral cavity but, also more importantly, that periodontal diseases have a systemic impact, which leads to damage and dysfunction of distant organs.

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