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Research Article

The Impact of Periodontal Disease on the Heart and Kidneys in Dogs

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Abstract

Periodontal disease is one of the most common canine diseases that can have serious systemic consequences. The aim of the study was the identification of the bacterial microflora causing periodontitis as well as its influence on the pathological changes in heart and kidneys. The study was performed on the group of 19 dogs (10 males and 9 females, aged between 6-15 years) sectioned at the Pathological Anatomy Department in Lublin, Poland. In all dogs the periodontal disease (third stage in 5 dogs and the fourth stage in 14 dogs) was diagnosed. A culture of aerobic bacteria from clinical material (swab of gingival, heart and kidneys) and histopathological examination on heart and kidney were performed. In the material the presence of bacteries such as: *Escherichia coli, Streptococcus* spp., *Streptococcus pyogenes, Streptococcus equi, Staphylococcus epidermidis, Staphylococcus* spp. and *Corynebacterium* spp. The results of this study showed the advanced pathological changes in kidney glomeruli and the anterior wall of the left heart in all animals, especially in the dogs with the fourth stage of periodontitis. In histopathological examination of preparations from the anterior wall of the left heart ventricle the intense interstitial cardiac oedema and interstitial fibrosis was diagnosed. Additionally, subepicardially between the muscle fibres and around the vessels single adipocytes were found. The study reveals the important correlations between chronic inflammatory lesions within periodontal tissues and heart and kidneys.

Keywords: *Periodontal disease, Dog, Bacteriological culture, Heart, Histopathological examination, Kidney*

Köpeklerde Kalp ve Böbrekler Üzerine Periodontal Hastalığın Etkisi

Öz

Periodontal hastalık sistemik sonuçları olabilen en yaygın köpek hastalıklarından biridir. Bu çalışmanın amacı periodontitise neden olan bakteriyel mikrofloranın tespiti ve aynı zamanda kalp ve böbreklerdeki patolojik değişiklikler üzerine etkilerini araştırmaktır. Çalışma Lublin, Polanya'daki Patolojik Anatomi Departmanında disekte edilen 19 köpek (10 erkek ve 9 dişi, 6-15 yaşlı) üzerinde gerçekleştirildi. Tüm köpeklerde (5 köpekte 3. seviyede ve 14 köpekte 4. seviyede) periodontal hastalık tespit edildi. Klinik materyalden (diş eti, kalp ve böbreklerden svab) aerobik bakteri kültürü yapıldı ve kalp ile böbreklerde histopatolojik muayene gerçekleştirildi. Materyallerde tespit edilen bakteriler: *Escherichia coli, Streptococcus* spp., *Streptococcus pyogenes, Streptococcus equi, Staphylococcus epidermidis, Staphylococcus* spp. ve *Corynebacterium* spp. Çalışmanın sonuçları tüm köpeklerin, özellikle dördüncü seviye periodontitisli olan, böbrek glomeruluslarında ve sol kalbin anterior duvarında şiddetli patolojik değişiklikler bulunduğunu gösterdi. Kalbin sol ventrikülünden hazırlanan preparatların histopatolojik muayenesinde şiddetli interstisyel kardiyak ödem ve interstisyel fibrozis tespit edildi. Kas fibrilleri arasında ve damar çevresinde tek adipositler gözlemlendi. Bu çalışma periodontal dokulardaki kronik yangısal lezyonlar ile kalp ve böbreklerdeki lezyonlar arasında önemli bağlantı olduğunu göstermektedir.

Anahtar sözcükler: *Periodontal hastalık, Köpek, Bakteriyolojik kültür, Kalp, Histopatolojik muayene, Böbrek*

INTRODUCTION

The oral cavity in human and animal is a reservoir of pathogenic bacteria that cause not only local infections, but also systemic diseases [1]. A number of human and canine disease affecting organs located outside the oral cavity may be initiated by inflammatory mediators, toxaemia or recurring bacteraemia caused by chronic periodontal diseases-PD [2]. Chronic periodontal disease is one of the most dangerous chronic infection in dogs. It has been observed that 85% of dogs over three years of age suffer from periodontopathic lesions of varying

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intensity [3-5]. Peridonthopathy or chronic periodontal disease is described as an inflammatory condition that affects tissues surrounding and supporting the teeth. The underlying cause of this disease is bacterial infection.

It is generally accepted that the mouth of a dog, with its steady temperature, humidity and recesses that cannot be self-cleaned, offers good conditions for the proliferation of microorganisms. The inflammatory process results in damage to the gingival attachment and the penetration of bacteria and their toxins into the circulatory system. In many dogs, periodontal disease remains untreated for many years, and the resulting chronic inflammation may be conductive to permanent bacteraemia [6]. The inflammatory process taking place in the mouth, and especially in the periodontium, is the same as in other body parts and causes an acute or chronic inflammatory response [7].

Research conducted in the field of dentistry yields substantial information about the cause and effect relationship between periodontopathies and the risk factors for cardiovascular diseases, renal diseases and diabetes [8,9]. Until the early 20th century, focal diseases of dental origin were not a particular point of interest to dentists and general practitioners. It was only after the death of President Theodore Roosevelt in 1919 due to odontogenic sepsis that researchers became interested in the topic [10]. Currently it is generally accepted that the mechanism of inflammatory-allergic reactions in the endocardium, kidneys, skin and joints involves tissue sensitization by bacterial antigens from inflammatory foci in the mouth [11].

Microbiological examinations allow us to determine the microbiological profile of odontogenic infections, which are usually of a multibacterial character, with mixed aerobic-anaerobic flora. The pathogens which usually initiate the infectious process are aerobic bacteria, mainly streptococci, which penetrate into the deeper layers of the tissues and initiate the inflammatory process [12].

The immune system of the animal can prevent these bacterial infections thanks to local barriers as well as humoral and cellular immune responses. Undamaged layers of the skin and mucosa prevent the penetration of bacteria deeper into the tissues. In the mouth, the protective barrier is broken more often than in other places of the body due to periodontal disease, tooth extraction, periapical abscess or mechanical injuries in this area [13]. It has been shown that pathogenic periodontal bacteria may penetrate to the periodontal tissues and then to the blood stream, through which they may reach distant organs [14].

The aim of the study was the identification of the bacterial microflora causing periodontitis as well as its influence on the pathological changes in heart and kidneys

MATERIAL and METHODS

Among 210 dogs treated dentally for periodontal disease between 2006 and 2010, at the Department and Clinic of Animal Surgery of the Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Poland, fifty-six dogs were diagnosed with stage 3 or 4 periodontal disease. The age of these 56 dogs ranged between 6 and 15 years (37 male, 19 female).

All study protocols were approved by the Local Ethics Committee of the University of Life Sciences in Lublin (No. 15/2009, 10 II 2009). Dogs qualified for the study were selected relative to systemic diseases based on medical history and clinical examination. Patients suffering from diseases of the endocrine system, cancer or generalized infectious diseases were excluded from the study.

The periodontal health status of each dog was determined following the Wiggs & Lobprise scoring system [15]. The diagnosis of the stage of the disease was made on the basis of clinical and radiological examinations. The following parameters were assessed: connective tissue attachment (the distance from the cemento-enamel-junction to the bottom of the pocket) and periodontal pocket depth. The examination of these indicators was performed with the use of a calibrated periodontal probe.

All the patients were provided with periodontal treatment appropriate for their respective stage of periodontal disease. During the 4-year observation and ongoing treatment period, a study group *(post mortem* group) was selected, comprising 19 dogs (10 males and 9 females) which underwent euthanasia due to poor general condition. The group consisted of 5 dogs in the third stage and 14 dogs in the fourth stage of the disease. From the gingival margin of each dog (targeting the teeth believed to be most often affected by PD: upper 103,104, 108 at right side and lower 404, 408 and 409 at right side) swabs were taken to perform microbiological examinations. In order to specify what kind of bacterial flora is responsible for periodontitis in the selected group of 56 dogs material was collected from the gingival margin by sterile swab friction and scraping with a sterile curette.

The pathomorphological and bacteriological examination was performed on the dogs condemned to euthanasia for medical reasons.

During the necropsy, swab material was collected once more from the gingival margin additionally from heart and kidney of the dogs. The samples were inoculated on solid media (Columbia Agar with Sheepblood), McConkey Agar, Enterococci Bile Azide Agar, Sabouraund Glucose Selective Agar, Mannitol Salt Agar (Chapman). The plates were incubated at 35-36ºC for 24-48 h. Bacteria were at first identified by colony morphology, haemolytic pattern and Gram staining (Color Gram 2 kit, bioMérieux, F-69280

Marcy-l'Etoile, France). For Gram-positive cocci, a catalase test was carried out to differentiate catalase-negative streptococci from catalase-positive staphylococci. A CAMP (Christie, Atkins, Munch-Petersen) test were used to differentiate *Str. agalactiae* from other streptococci. Enterococci were initially identified with the use of bile esculin agar (Biocorp, Parc technologique Lavaur la Béchade 63500 Issoire, France). Gram-negative bacilli were identified based on the cultural and morphological characteristics, growth on MacConkey agar (Oxoid, Wade Road Basingstoke Hampshire RG24 8PW United Kingdom), indole production and oxidase test (bioMérieux, Marcy-l'Etoile, France) [16]. Coryneform bacteria were initially identified based on colony morphology and microscopic view. When pure cultures were isolated, the further identification was performed using the diagnostic tests GP and AST -P644, and in the case of *Streptococcus pyogenes/ equi* GP and AST- ST03 with the use of bioMerieux Poland sets (VITEK® MS microbial identification system) [17,18].

During the post mortem examination, samples were also taken from the anterior wall of the left ventricle (a section through the whole thickness of the ventricle) and kidney. Collected tissues were fixed in 10% buffered formalin (pH 7.2) for 24 (48 h) and embedded in paraffin blocks which were used to obtain 4 μm thick samples. Standard hematoxylin and eosin (HE) stain was used, as well as a specific staining: periodic acid-Schiff reaction (PAS) and Van Gieson stain, Masson's Trichrome staining, silver staining of reticular fibres [19] and the Nielsen-Selye staining method were used to evaluate recent cardiomyocyte necrosis [20]. The histopathological examination was performed at the Department of Clinical Pathomorphology of the Medical University of Lublin.

Statistical Analysis

The χ^2 test was used to determine whether the aerobic bacteria counts in the heart, kidneys and gingiva were the same or different in patients in the third and fourth stages of periodontal disease.

With a statistical significance of $P<0.05$, the differences between the studied groups were significant. The authors used the STATISTICA 8.0 software for calculations.

RESULTS

Culture

Bacteria most commonly isolated from the gingival margin in dogs from study group dignosed with third stage of PD included *Streptococcus* spp., *Escherichia coli,* and *Staphylococcus epidermidis*. Bacteria isolated from kidney tissue most commonly included: *Streptococcus* spp., and *Staphylococcus* spp; and from the anterior wall of the left heart ventricle: *Staphylococcus epidermidis*, *Streptococcus spp.*, and *Streptococcus pyogenes (Table 1)*.

Table 1. *Results of the bacteriological examination of the gingival margin, kidney and heart of patients in the third stage of periodontal disease in the study group (post mortem)*

Isolated Microorganisms (CFU/mL)	Gingival Margin	Kidney	Heart
Escherichia coli	14	1	0
Streptococcus spp.	16	3	2
Streptococcus pyogenes	1	1	2
Streptococcus equi	0	1	1
Staphylococcus epidermidis	3	1	3
Staphylococcus spp.	2	2	0
Corynebacterium spp.	1	1	0
Total	37	10	8

Table 2. *Results of the bacteriological examination of material from the gingival margin, kidney and heart in patients in the fourth stage of periodontal disease in the study group (post mortem)*

Isolated Microorganisms (CFU/mL)	Gingival Margin	Kidney	Heart
Escherichia coli	5	2	1
Streptococcus spp.	17	4	6
Streptococcus pyogenes	7	3	5
Streptococcus equi	2	3	4
Staphylococcus epidermidis	5	3	7
Staphylococcus spp.	5	6	0
Total	41	21	23

Table 3. *Number of microorganisms (CFU/mL) isolated from the gingival margin, kidney and heart in the study group (post mortem)*

Sample	Third Stage of Periodontal Disease	Fourth Stage of Periodontal Disease
Gingival margin	37	41
$\chi^2=0.21$ (-)	P>0.05	
Kidney	10	21
$\chi^2=3.84$ (*)	P<0.05	
Heart	8	23
$\chi^2=7.06$ (**)	P<0.01	

Bacteria most commonly isolated from the gingival margin in dogs from study group diagnosed with fourth stage of PD included: *Streptococcus* spp., *Streptococcus pyogenes, Staphylococcus epidermidis*, and *Staphylococcus* spp. Bacteria isolated from the kidneys were *Staphylococcus* spp., *Streptococcus* spp., *Streptococcus pyogenes, Streptococcus equi,* and *Staphylococcus epidermidis;* while bacteria from the anterior wall of the heart ventricle were- *Staphylococcus epidermidis*, *Streptococcus* spp., *Streptococcus pyogenes,* and *Streptococcus equi (Table 2)*.

No significant difference was observed in terms of the number of bacteria isolated from the periodontal pockets in the third and fourth stages of periodontal disease. The

percentages of bacteria in both groups were similar, approx. 43.4% in the third stage and 56.6% in the fourth stage [χ^2=2.76 ; P>0.05].

The number of bacteria isolated from the kidneys was significantly higher in patients in the fourth stage of periodontal disease-65.91%, compared to 34.09 % in the group of third stage animals [χ2=4.45; P<0.05].

The χ^2 test revealed that microorganisms isolated from the dogs' cardiac tissues occurred much more frequently in the group in the fourth stage of periodontal disease-70.59%, compared to 29.41% in patients in the third stage of periodontal disease [χ^2=5.76; P<0.01].

The statistical analysis of bacterial counts in the periodontal pockets in the third and fourth stages of periodontal disease has been shown in *Table 3*. Together with results of number of bacteria in heart and kidneys.

Histopathological Examination on Heart and Kidney Samples Obtained from Dogs in The Study Group

In the standard hematoxylin and eosin (HE) stain of the material collected from the anterior wall of the left ventricle, single cardiomyocytes with a more homogeneous eosinophilic character were observed focally, with the disappearance of transverse striation and hyperchromatic nuclei (third and fourth stage). In fourth stage between the muscular fibres and around vessels mononuclear cells -limfocytes, either single or in small groups, were visualised *(Fig. 1)*. However, the Nielsen-Selye staining method revealed segmental necrosis of single cardiomyocytes in several samples *(Fig. 2)*. The Van Gieson's and Masson's Trichrome stains revealed fragmentation of the cardiac fibres and subepicardial parenchymal fibrosis *(Fig. 3)*. Subepicardially, between the muscle fibres and around the vessels, single adipocytes were found. The lesions were characteristic of endocarditis.

In the material collected from the kidneys, focal infiltrations of mononuclear cells (located near renal tubules and glomeruli) were observed *(Fig. 4)*. Foci of intensified interstitial hyalinization, dilated tubules with homogeneous fluid, tubule atrophy (the so-called "thyroid-like area"), intense infiltrations of mononuclear cells (usually in clusters), numerous solidifying and hyalinising glomeruli with clearly marked periglomerular fibrosis were also observed *(Fig. 5)*. The identified pathomorphological lesions were characteristic of pyelonephritis.

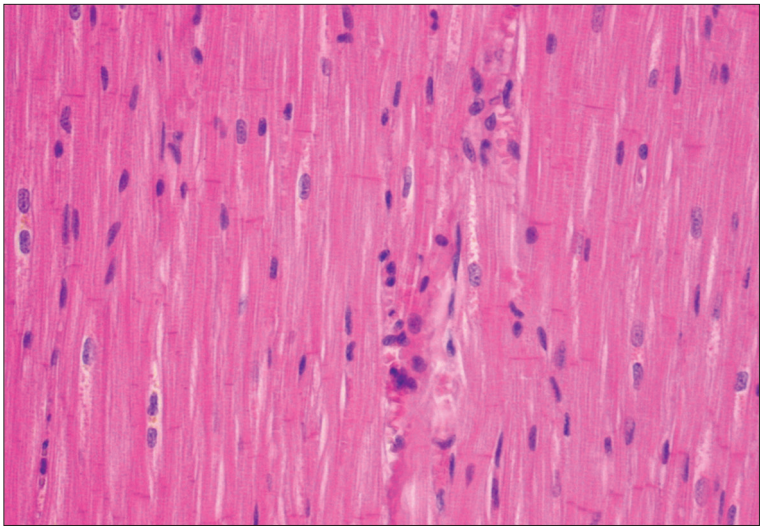

Fig 1. Mononuclear cells between cardiomyocytes. HE staining (20X)

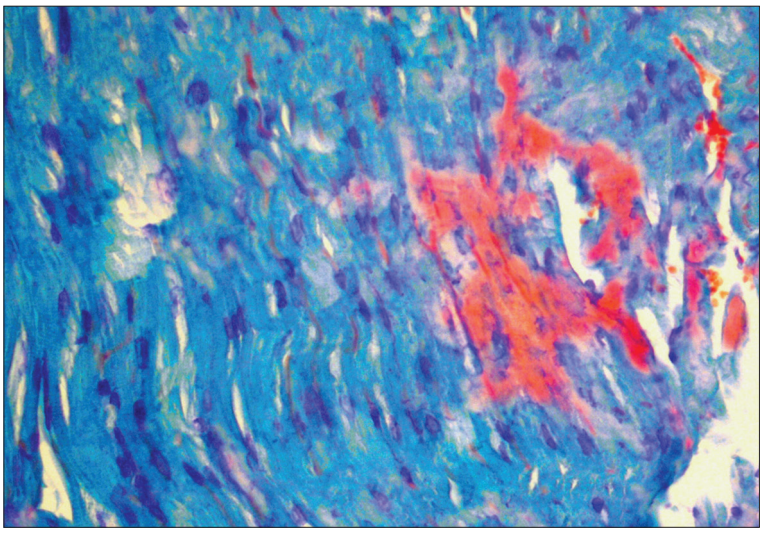

Fig 2. Cardiomyocyte necrosis. Nielsen- Selye's staining (20X)

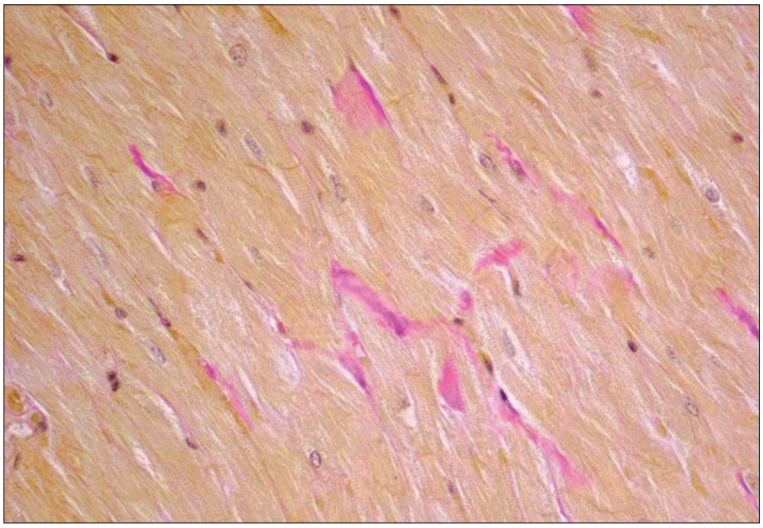

Fig 3. Connective tissue - red-stained between cardiomyocytes. Interstitial fibrosis. Periodic acid-Schiff reaction (PAS) and Van Gieson stain (20X)

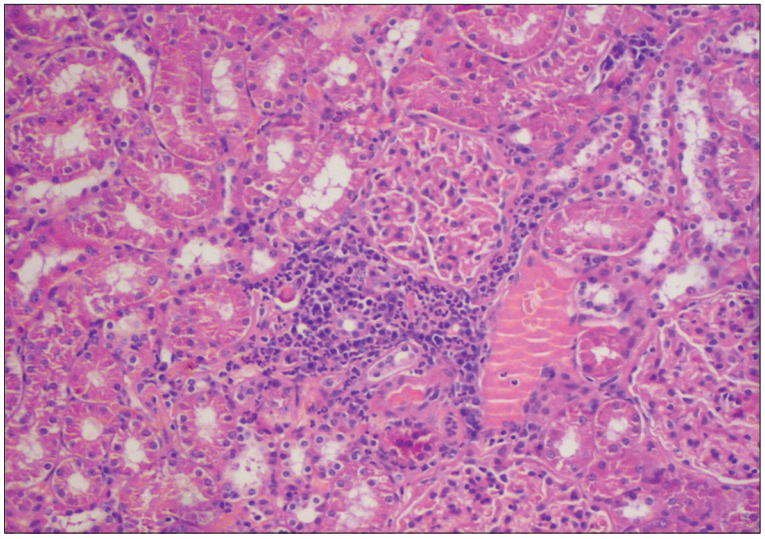

Fig 4. Focal infiltrations of mononuclear cells. HE staining (20X)

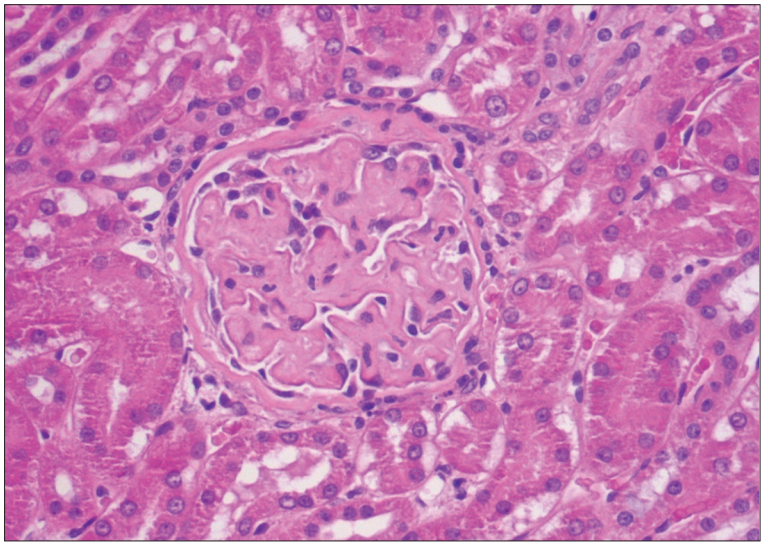

Fig 5. Glomerular hyalinization and periglomerular fibrosis. HE staining (20X)

DISCUSSION

The most common chronic bacterial infection, both in humans and dogs is periodontal disease. *Gingivitis* is an initial and reversible form of periodontopathy which, when untreated, leads to *periodontitis* and, in consequence, to the destruction of the alveolar bone. Identification of bacteria associated with periodontopathy and seeking evidence for this association, as well as with generic diseases, is a subject of both dental and general medical research. There are some premises allowing the possibility to associate periodontitis with the pathophysiology of chronic systemic inflammatory diseases. Ongoing research indicates that long-term untreated periodontopathies negatively affect animal health [6,21].

Nowadays, periodontopathies are not considered a problem related solely to the oral cavity. Recent research results indicate that there is an association between periodontitis and systemic diseases, such as cardiovascular diseases, kidney diseases, atherosclerosis, and respiratory system diseases in dogs [22,23]. Immunological kidney diseases, especially glomerulonephritis, are considered a potential result of chronic bacteraemia and toxaemia associated with periodontal disease in humans and dogs [22-24]. Pavlica, in his study on 44 poodles, identified a correlation between periodontal disease and renal impairment, indicating the impact of periodontopathies on occurring lesions, probably caused by repeated or chronic infections [6]. Other researchers also confirmed these results in their studies in canines [25,26]. The 4-year results of the authors' own studies performed on a group of dogs with periodontal disease confirm Pavlica's observations stating that glomerulonephritis was associated with a sustained inflammatory factor in the oral cavity. It could be concluded that, in the studied dogs, frequent bacteremia occurred due to repeated minor injuries in the area of the affected periodontium. In our own research the lesions identified by anatomopathological examination prove the development of a chronic glomerulonephritis associated with an oral cavity infection. The studies show important correlations between chronic inflammatory lesions in periodontium and increases in the number of microorganisms in the kidney and heart tissues. These results may be a confirmation that periodontopathies affect not only the teeth but also can have a negative influence on the whole organism, leading to systemic diseases.

It was indicated that in risk group humans, repeated bacteraemia in the oral cavity may constitute an important causal factor of infective endocarditis or pyelonephritis [27-29]. Increasingly often, periodontopathies are regarded as an independent risk factor for ischemic heart disease [30]. Studies have showed that in a group of people with chronic periodontitis, the risk of developing ischemic heart disease is twice as high as in healthy people [31].

In dogs statistical analysis of our results revealed that the microorganism counts in the periodontal pockets in the third and fourth stages of periodontal disease did not differ significantly. However, a significant increase in the number of microorganisms was noted with exacerbation of inflammatory processes in the material collected from the hearts and kidneys. The results show that the development and advancement of periodontal disease significantly increases the number of bacteria in distant organs, such as the heart and kidneys.

In conclusion, the results gained from the above study confirm the association between periodontopathies and the pathology of organs; however, other factors should also be taken into account, such as: age of the animal, nutritional problems and compromised immune system, which may at the same time affect both the development of periodontopathy and the pathology of remote organs. Despite credible traces, irrefutable evidence for the direct impact of peridontopathologies on the development of diseases of remote organs is still being sought.

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