ECRONICON OPEN ACCESS

EC VETERINARY SCIENCE
Research Article

Effect of Diet on the Induction of Pathogens in the Oral Cavity of Humans and Animals

Ilona Rowińska[1], Rafał Lizut[2], Piotr Zariczny[3], Robert Pasławski[4], Karol Kramkowski[5], Grzegorz Skiba[6], Stanisława Raj[6] and Paweł Kowalczyk[6]*

[1]*Medical and Social Center for Vocational and Continuing Education in Toruń, Poland*

[2]*The John Paul II Catholic University of Lublin, Institute of Mathematics, Informatics and Landscape Architecture ul. Konstantynów 1 H, Lublin, Poland*

[3]*Toruń City Hall, Business Support Center in Toruń, Toruń, Poland*

[4]*Veterinary Institute, Nicolaus Copernicus University in Toruń, Poland, Toruń, Poland*

[5]*Department of Physical Chemistry, Medical University of Bialystok, Kilińskiego, Bialystok, Poland*

[6]*Department of Animal Nutrition, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Instytucka, Jabłonna, Poland*

***Corresponding Author:** Paweł Kowalczyk, Department of Animal Nutrition, The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Instytucka, Jabłonna, Poland.

Received: July 07, 2021

Abstract

Disturbances in homeostasis in the oral bacterial flora can cause various diseases, chronic periodontitis and systemic diseases. Contemporary eating habits influence the formation of a specific microbiome of the mouth and intestines, which increases the risk of inflammatory bowel disease, obesity, type 2 diabetes, cardiovascular disease and cancer. The studies determined the effect of various components of four diets (proteins, carbohydrates, fats, vegetables) on inflammation induced by bacterial biofilms in the human oral cavity. The study was conducted on 40 volunteers (divided into four groups) who consumed traditional meals (control diet, F) or experimental diets (diet B, W and T). In addition, it was investigated whether the occurrence of zoonoses is related to human contact with domestic animals, using the methods of microbiology and molecular biology. The analysis of sequences supporting the evaluation of dental indicators was performed. The influence of certain nutrients contained in certain types of diets labeled F, B, W and T on the development of periodontitis in the oral cavity in the presence of biofilm in the interdental spaces was investigated. Biological material was collected non-invasively from volunteers in the form of a bacterial inoculum and grown on complete and selective media for specific strains of all bacterial complexes. The chosen research methodology is an ideal screening test for the analysis of the oral bacterial flora. The obtained results suggest that a specific diet can be an effective prophylaxis in the treatment of inflammation of periodontal tissues and gingival pockets.

Keywords: *Analysis of Sequence; Bacterial Biofilms; Diet; Inflammation; Statistical Tukey Test; Stomatological Indicators; Oral Cavity; Periodontitis*

Abbreviations

A. actinomycetemcomitans: Aggregatibacter actinomycetemcomitans; P. gingivalis: Porphyromonas gingivalis; T. forsythia: Tannerella forsythia; T. denticola: Treponema denticola; F. nucleatum: Fusobacterium nucleatum; P. intermedia: Prevotella intermedia; L. salivarius:

Ligilactobacillus salivarius; S. pyogenes: Streptococcus pyogenes; S. sanguinis: Streptococcus sanguinis; S. mutans: Streptococcus mutans; L. delbrueckii: Ligilactobacillus delbrueckii; E. corrodens: Eikenella corrodens; C. concisus: Campylobacter concisus; V. parvula: Veillonella parvula; A. odontolyticus: Actinomyces odontolyticus; P. micros: Peptostreptococcus micros; E. nodatum: Eubacterium nodatum; S. constellatus: Streptococcus constellatus

Introduction

The physiological flora of the oral cavity

Inflammation in the mouth can trigger the development of serious diseases in humans, such as chronic pharyngitis and upper respiratory tract inflammation, endocarditis, and the formation of abscesses in the liver or kidneys. The development of gingivitis and periodontitis occurs as a result of an imbalance between the interaction of the bacterial plaque and the host's immune system [1]. Bacteria and their products present in the biofilm stimulate immunocompetent cells to produce and release inflammatory mediators that cause the destruction of periodontal tissues and the development of inflammatory processes in the body [2]. The host's inflammatory immune response may be influenced by genetic determinants and environmental factors, such as stress, smoking, and it may also be modified by some systemic diseases and as a result of their treatment (e.g. immunosuppression, radiotherapy) [3-6]. Other factors may be a specific improper diet inducing oxidative stress in the oral cavity and the formation of pathogenic bacterial biofilms [7,8]. Still other "external factors" of inflammation in the human oral cavity can be zoonoses. Zoonoses are spread by animals or are transmitted to humans through direct contact with animals or through raw materials of animal origin.

Biophilic bacteria and possiblitlity of its transmition from animals to humans as a result of long-term exposure as well as its zoonoses causing potential

In order to prevent infection with bacterial biofilms of zoonotic origin, in the case of animals kept at home, e.g. dogs, it is necessary to regularly check the condition of the dog's teeth at the vet, especially for the build-up of plaque and tartar, and cleaning the gingival pockets of deposits. In the case of very advanced disease, the animal should undergo gingival surgery and removal of rickety teeth [19-22]. It is also necessary to carry out grooming treatments and give the appropriate food. The basis of the diet should be dry food, but not more than 1 - 2 times a day, and not soft, homemade food eaten almost continuously. The dog cleans the smooth surfaces of the teeth by chewing on hard food. These should be products containing a large amount of carbohydrates, such as rice, porridge or pasta [23]. Vets distinguish between three main stages of periodontal disease: gingivitis and periodontitis. They mainly concern small breed dogs weighing less than 10 kg. This is due to the specific structure of the oral cavity of these animals. Because they have relatively large teeth in relation to the size of the mouth, the process of tartar deposition is more intense [19-23]. Dental plaque in small dogs can start to build up at a young age. This process may be additionally accelerated by factors such as malocclusion, weakened immune system, hormonal disorders and vitamin deficiencies [19-23]. Owners are often unaware that their pets are sick and can be a potential source of infection for them. Symptoms that the owner should notice immediately include mouth odor, increased salivation, cavities in the teeth, gum swelling and bleeding, and severe yellowing of the teeth due to plaque build-up [19-23]. Prophylaxis of animal research should mainly include regular dental check-ups by a veterinarian. Every owner of a dog over the age of three should visit the veterinary office at least once every six months, out of concern for their own health.

People working with pets who live in inadequate zoohygienic and nutritional conditions are at risk of bacterial zoonoses [24]. These include actinomycetes, gram-positive saprophytes, non-spore and non-acid-resistant bacteria, occurring in the form of pleomorphic sticks (0.2 - 0.4 × 0.5 - 1.0 **µm**), cocci or branched threads (1 x 50 **µm**), mycelium-like appearance. They live on the skin and mucous membranes of the mouth, throat and digestive tract of animals. They usually cause infections and disrupt the integrity of the shell penetrating the body, causing inflammation and lesions [25-31]. Viscose found on dogs' skin causes a disease called actinomycosis, which is common in pets and less commonly in other species of animals and humans. It is characterized by the presence of abscesses, fistulas and connective

tissue hypertrophy [25-31]. Actinomycosis is a zoonotic disease and is included in the list of infectious diseases and infections in humans in the Act on preventing and combating human infections and infectious diseases [25-33]. As a result of human-animal contact, there is a possibility of transmission of infection from a sick animal [33].

Another source of infection may be common feeds during food intake or animal housing, according to the European Union classification, actinomycetes constitute the second group of risk [34]. In humans, the only natural reservoirs inhabited by actinomycetes are the mucous membranes of the larynx, pharynx, and gastrointestinal tract, and the female reproductive system. The result of infection in humans is inflammation, the appearance of nodules (acinomycoma), damage to the affected tissues, the formation of abscesses and fistulas. After phagocytosis, some pathogens are spread to adjacent tissues, spongy bones, e.g. the mandible, where they multiply under anaerobic or microaerophilic conditions and through the production of toxins and enzymes (initiate) periostitis (periostitis ossificans) and softening of the internal bone structure (osteomyelitis rarefaciens) [35]. Chronic purulent-granulomatous inflammation of the mandible or maxilla lasts weeks or even months, and its result is the formation of atypical granulation tissue interspersed with abscesses, the so-called radiation bodies. The lumps may break down or calcify due to the accumulation of mineral salts. Additional purulent fistulas may also form. The pus contains small, mineralized grains with bacterial clusters in the center, on the periphery gram-negative pear-shaped bodies, constituting a protein complex of antigen - antibody and calcium phosphate, causing mineralization. Depending on the advancement of the disease process, depending on the individual stiffness of the organism and pathogen virulence, micro- and macroscopic changes may be productive or exudative-purulent. There are four clinical forms of the disease: abdominal, cervical, pulmonary and generalized. Primary skin actinomycosis caused by *A. meyeri* may be one of the first clinical symptoms of HIV infection [35]. The abdominal form is found in 20% of patients and is characterized by inflammation of the appendix, cecum and peritoneum. Sometimes abscesses are formed that penetrate the abdominal wall. There is abdominal pain, fever, diarrhea, vomiting and weight loss. In the facial-cervical form, observed in 55% of patients, clearly delimited painful tumors appear in the mouth, on the skin of the neck and under the mandible, giving rise to fistulas from which pus is secreted. Infection can spread to the pharynx, tongue, salivary glands, skull bones, brain and meninges [36-43]. The diagnosis of actinomycosis is made on the basis of clinical symptoms, pathological changes and the results of histopathological and bacteriological examinations. Clinical symptoms are not pathognomonic for actinomycosis and only raise the suspicion of the disease, therefore the final diagnosis requires confirmation in histopathological examination and isolation and identification of the etiological factor [37-44]. In prophylaxis in humans, it is recommended to thoroughly wash wounds resulting from bites and to follow the rules of hygiene when dealing with sick animals.

The seriousness of the problem requires researching what factors can cause and accelerate inflammation during its development stage and which can prevent and help fight it. The list of factors given above requires further research. Ways to work out possible factors may come from researchers or use deep learning tools such as the neural network approach, in particular self-organizing maps [45]. Determining all indicators and their possible features emerging during the co-creation of the system and studying the impact of such a set of possibilities is far beyond the scope of this article, therefore we presented two factors, diet and zoonotic diseases based on known research methods.

Aim of the Work

- Examining the order of microbial colonization in the oral cavity of humans and animals depending on the availability of certain types of diets containing nutrients.
- Examining how the type of nutrients affects the specific aerobic and anaerobic bacterial flora according to scientific nomenclature described by Socransky [37,38].
- Testing whether the biofilms of humans and domestic animals living with them (on the example of dogs) are identical or different.

Materials and Methods

Diet applications

The physiological flora of the oral cavity in humans has been described detail in Rowińska [7,8] and Kucia [39]. The research was conducted on volunteers (n = 40, divided into 4 groups) who consumed traditional meals containing proteins, simple carbohydrates, fats, vegetables (control diet, diet F) or experimental diets (diet B, W and T). The patients of experimental groups consumed different products, but ended each meal with a sugar-free protein product such as kefir, yoghurt, cheese etc. (diet B); vegetables such as radish, watercress, kale, broccoli, kohlrabi, etc. (diet W) or foods containing omega-3 fatty acids, e.g. fish, especially salmon, mackerel, sardines, seafood, rapeseed and linseed oil (diet T). Dogs weighing less than 10 kg are fed a complete and balanced diet adapted to the age of the dogs that includes: 40% protein, 10% carbohydrates, 25% vegetables and healthy fats, calcium and fatty acids. Briefly, three days before the visit, they were advised:

- Standard oral hygiene for you—do not change your hygiene habits (lub do not change your hygiene habits - standard oral hygiene).
- Eat meals consisting mainly of products from the recommended control diet (typical meal - diet F) and at least finish each meal with a product from the recommended diet B, W or T).
- On the third day of the diet, meeting at the MSCKZiU dental office in Toruń.
- Collection of the bacterial inoculum with saliva on plates with an appropriate culture medium (noninvasive collection—without breaking the tissue continuity)—procedure A.
- Performing hygienic and periodontal measurements (indicators)—procedure B.
- Removal of tartar—procedure C.
- After next following 3 days, another bacterial inoculum with saliva on plates with an appropriate culture medium and sequencing (noninvasive collection—without damaging the tissue continuity)—procedure D.
- Description and symbol of containers:
    - Material collected supragingivally before the procedure.
    - Material collected before the procedure from the gingival fissure.
    - Material collected supragingivally 3 days after the procedure.
    - Material collected 3 days after collected material (surgery) from the gingival fissure.
- Pan coding:
    - 1st item-ordinal number of the subject from the collective research document 2 diet item F or B or W or T.

Analysis of bacterial biofilms

The analyzed material was analyzed using the methodology described in the publication of Rowińska [8,9] and Kucia., *et al*. 2020 [39]. Statistical analyses were conducted using Statistica software (version 12, StatSoft, Tulsa, OK, USA). The Tukey test was used to determine

differences between groups. A p value ≤0.05 was considered statistically significant. The obtained sequences of bacterial pathogens were analyzed with the use of specialized heuristic software of the Basic Local Alignment Search Tool 2.0 algorithm (Blast 2.0) and compared to the sequences already existing in the databases for calculating sequence similarity. Only sequences with a 99.99-100% probability of similarity match were taken into account.

Research techniques

Modern dentistry as a branch of medicine dealing with the functioning, pathologies and treatment of teeth, periodontium, tongue, mucosa and other tissues of the oral cavity and surrounding it, as well as the temporomandibular joint of humans, has developed many indicators over the years, including the state of oral hygiene. Oral hygiene determines its health condition, and deviations from generally accepted norms are a sign of existing or emerging pathological conditions. The characteristics of the main indicators used in human dentistry were described in detail in the publications Rowińska., *et al*. 2021 [7,8]. The pads from both studied groups of people: working with having pets are presented in table 1 and 2, respectively.

Dental indicators will be were used to assess the presence and location of bacterial plaque and tartar in the oral cavity and to determine the needs of specialist treatment—periodontics. The presence of residual bacterial plaque and tartar is a determinant of the presence of inflammation of soft tissues, very often chronic inflammation. The results will be used for further research analysis and for inference (Table 1 and 2).

The subject of our considerations

Our research determined the influence of a traditional diet on the formation of oxidative stress and inflammation caused by the formation of bacterial biofilm in the oral cavity of humans and animals. It then investigated whether biophilic bacteria could be transmitted from animals to humans through prolonged exposure and whether they could cause zoonoses through dental, biomedical and laboratory tests.

We will determine whether the biofilm is identical in the case of the owners of pets, livestock and the animals themselves. For this, we have examined:

- The effect of food on the induction of inflammation of the soft tissues in the oral cavity in the presence of biofilm in humans after the use of equal types of diet, which have been described in detail in the works of Rowińska [7,8].
- The influence of food on the over- and sub-section biofilm, is it the same case of domestic animals such as dogs.
- The influence of nutrients in terms of the possibility of slowing down the development of inflammation in the human oral cavity in the development of zoonoses.
- Various food products (diets) in causing the greatest oxidative stress in the oral cavity of mammals caused by zoonoses.

Ethical statement

According to the Polish law standards, the presented preliminary and basic research in the publication, Do not require the consent of the local bioethics commission. Basic research was carried out non-invasively with animals. The authors also declare that they have no competing interests.

The research presented in the study "The effect of diet on the induction of pathogens in the oral cavity of humans and animals", submitted to the *Plos One* Journal, was conducted non-invasively and painlessly in humans and animals. In accordance with Polish law, tests

with the use of bacterial inoculum from the oral cavity of humans and animals do not require the consent of national, local or institutional bioethics committees. In our research, we do not apply the principles of the Helsinki Declaration of 1975, as amended in 2013. All applicable international, national and institutional guidelines for the use of oral bacterial strains have been applied and have been carried out in accordance with the recommendations of ISO 11133: 2014. The authors also declare that they have no competing interests.

Results

Analysis of bacterial biofilms in search of periopathogens of bacterial complexes in humans, domestic animals

In our work, we analyzed bacterial biofilms in people working professionally with dogs as pets (Table 1) in search of periopathogens of all bacterial complexes and their molecular analysis to see if they are similar and different. To confirm or exclude possible transmission of periopathogens from of animals to humans in the development of zoonoses after sequential analysis of bacterial biofilms in the analyzed nutritional groups The analysis of biological material in the form of an inoculum collected non-invasively from periodontal tissues in all types of groups human and domestic animals was carried out using microbiological methods with the use of sowing on plates with a specific culture medium in order to analyze the complete microbiota on the analyzed dishes, as described in Rowinska [7,8] and in the paper Kucia [39].

L.P.	cod	Margenta	Pl.I	OHI	API	PBI	CPITN	Diet
1	20.F.(A, B, C, D)	3, 5 sufficient	2, 13 moderate inflammation gums	2 good/ sufficient	51, 85% average	11, 11 condition requiring improvement of hygiene	2 (does not require perio treatment.)	F
2	15.F.(A, B, C, D)	4bad	2, 38 moderate inflammation	1, 25 good	84, 21% bad	5, 26% clinical healthy	2 (does not require perio treatment.)	F
3	25.F.(A, B, C, D)	2, 5 sufficient	1, 81 mild inflammation	2 good/ sufficient	80% bad	50% moderate inflammation	3 the patient is eligible for periodontal treatment	F
4	24.F.(A, B, C, D)	2, 67 sufficient	1, 54 moderate inflammation	1, 67 good	45, 83% average	8, 33 % clinical healthy	2 (does not require perio treatment)	F
5	23.F.(A, B, C, D)	2, 17 sufficient	1, 63 moderate inflammation	2, 83 sufficient	43, 48 average	21, 74 moderate inflammation	3 the patient is eligible for periodontal treatment	F
6	13.T.(A, B, C, D)	2, 5 sufficient	1, 83 mild inflammation	1, 08 good	65, 38% quite good	3, 85% clinical healthy	1 (does not require perio treatment)	T
7	07.T.(A, B, C, D)	1, 83 good	0, 63 healthy gums	0, 5 very good	18, 18% condition requiring improvement of hygiene	0% clinical healthy	0 (does not require perio treatment)	T

8	11.T, (A, B, C, D)	1, 67 good	1, 17 mild inflammation	1, 08 good hygiene	35, 71% fairly good	10, 71% condition requiring improvement of hygiene	4 patient requires periodontal treatment	T
9	35.T.(A, B, C, D)	2, 83 sufficient	2, 13 moderate inflammation	2, 83 sufficient	100% bad	43, 48% moderate inflammation	2 (does not require perio treatment)	T
10	40.T.(A, B, C, D)	2, 8 sufficient	1, 88 good	3, 2 insufficient	85, 71% bad	52, 38% severe unlimited gingivitis	3 the patient is eligible for periodontal treatment	T
11	18.W.(A, B, C, D)	2 good	1, 63 mild inflamation	0, 83 very good	91, 3% bad	0, 43% clinical healthy	2 (does not require perio treatment)	W
12	31.W.(A, B, C, D)	3 sufficient	2, 06 moderate inflammation	2, 25 sufficient	85, 71% bad	0% no therapy needed	3 the patient is eligible for periodontal treatment	W
13	32.W.(A, B, C, D)	2, 6 sufficient	2, 2 moderate inflammation	2, 75 sufficient	100% bad	5% no therapy needed	3 the patient is eligible for periodontal treatment	W
14	38.W.(A, B, C, D)	2 sufficient	1, 88 good	2, 33 sufficient	63, 16 % average	26, 32 moderate infllamation	3 the patient is eligible for periodontal treatment	W
15	33.W.(A, B, C, D)	1, 4 good	1, 6 mild inflammation	2 sufficient	94, 44% bad	11, 11 moderate inflammation	2 (does not require perio treatment)	W
16	08.B.(A, B, C, D)	1, 5 good	1, 79 mild inflammation	0, 9 good	83, 33% bad	0% clinical healthy	3 the patient is eligible for periodontal treatment	B
17	33.W.(A, B, C, D)	1, 4 good	1, 6 mild inflammation	2 sufficient	94, 44% bad	11, 11 moderate inflammation	2 (does not require perio treatment)	B
18	10.B.(A, B, C, D)	2, 6 sufficient	1, 55 good	1, 25 good	59, 09% average	0% clinical healthy	3 the patient is eligible for periodontal treatment	B
19	26.B.(A, B, C, D)	2 good/sufficient	1, 8 mild inflamation	2 good/ sufficient	20, 85% moderate inflammation	8, 33% clinical healthy	2 (does not require perio treatment)	B
20	28.B.(A, B, C, D)	2, 6 sufficient	1, 67 mild inflammation	2, 5 sufficient	42, 85% average	0% no therapy needed	2 (does not require perio treatment)	B

Table 1: *A group of test subjects who had contact with domestic animals for several hours.*

Methods of isolating bacterial DNA from the analyzed biofilms were also used and the obtained sequences by the Sanger method were analyzed using the Blast software as described in [7,8,39,44], (Figure 1, panel A, C, D). Additionally, in both groups in humans (Table 1), dental indicators of the oral cavity microbiota were used after eating a specific diet marked as F, B, W or T [7,8]. The exact composition of the diet and examples of its use along with the detailed characteristics of dental indicators were presented in the works of Rowińska., *et al*. 2021 [7,8]. The growth of pathogenic bacterial strains was observed in each of the analyzed groups (Figure 1 Panel A, B, C, D). Domestic animals remained on traditional types of diets commonly used for their species.

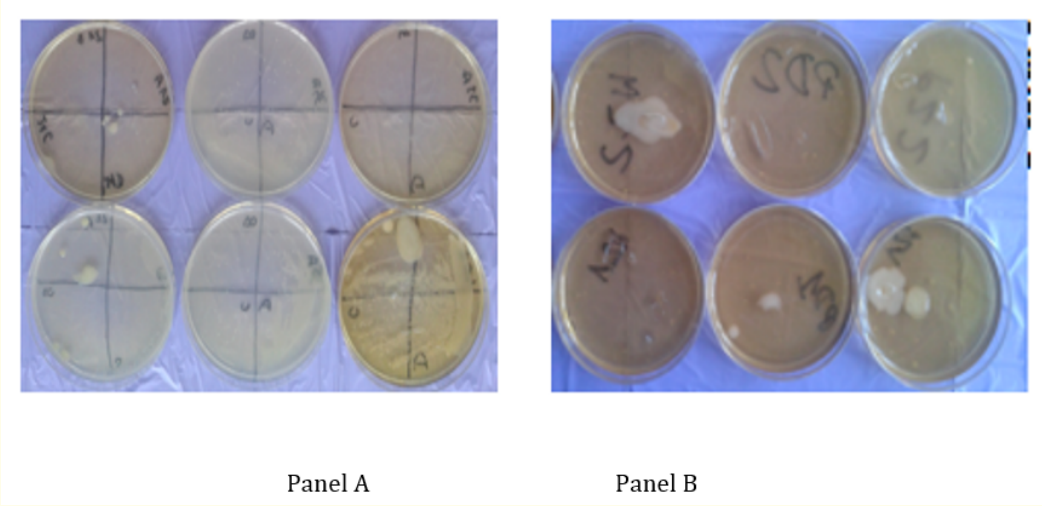

Figure 1: *Examples of pathogenic bacteria growth on growth media after collecting the bacterial inoculum from periodontal tissues in each of the analyzed groups. Panel A - inoculum taken from people with dogs who are also on various types of diets, as described in the works of Rowińska [7,8]. Panel B - bacterial inoculum from dogs. Only the grown bacterial colonies were used for sequencing (See details in supplementary materials as S1 figure).*

Sequence analysis of bacterial biofilms grown on plates revealed the presence of all bacterial complexes in humans in both types of groups. both for people working professionally for dog owners. The bacteria of the orange, yellow and red complexes predominated in the analyzed bacterial biofilms (Table 2). The obtained results clearly show that the influence of a particular type of diet can stimulate the development of beneficial, such as, for example, *L. salivarius* or negative microorganisms, such as, for example, *P.gingivalis*. The presence of *L. salivarius* in many dietary supplements and yoghurts is a rich supplement to the existing beneficial bacterial microflora and has a competitive effect against pathogenic bacteria present in the oral cavity. The results obtained from sequencing using the Sanger method [43] with the appropriate sets of primers for the identification of bacterial biofilms from the periodontal surfaces show the identification of 99.99–100% of the analyzed species or strains of pathogenic bacteria of the next six complexes in all analyzed research groups. The first is group of subjects with people who had a contact with dogs for several hours every day (Table 2). The second group is a group of tested only domestic animals (Table 3).

lp	Type	lp	Type	lp	Type	lp	Type
21a	*Streptococcus pygenes*	21b	*Rothia dentocariosa)*	21c	*Capylobacter concisus*	21d	*Streptococcus gordonii*
22a	*Porhyromonas gingivalis*	22b	*Tanarella forsythia*	22c	*Prevotella nigrescens*	22d	*A. meyeri*
23a	*Rothia dentocariosa*	23b	*Arachnia propionica (Actinomyces propionicus*	23c	*Peptostreptococcus micros*	23d	*Streptococcus mutans*
24a	*Streptococcus sanguinis*	24b	*Capnocytophaga sputigena*	24c	*Capnocytophaga ochracea*	24d	*Streptococcus sanguinis*
25a	*Capnocytophaga ochracea*	25a	*Peptostreptococcus micros*	25c	*Bifidobacterium dentium*	25d	*Bifidobacterium dentium*
26a	*Escherichia. coli R2*	26b	*Escherichia. coli R2*	26c	*Eubacterium nodatum*	26d	*Trichomonas tenax*
27a	*Lactobacillus acidophilus*	27b	*Lactobacillus buchneri*	27c	*Lactobacillus fermentum*	27d	*Lactobacillus salivarius*
28a	*Lactobacillus casei*	28b	*Lactobacillus plantarum*	28c	*Bifidobacterium dentium*	28d	*Lactobacillus casei*
29a	*Pyrococcus sp. OT3*	29b	*Streptococcus oralis*	29c	*Streptococcus gordoni*	29d	*Peptostreptococcus micros*
30a	*Streptococcus constellatus*	30b	*E.coli R4*	30c	*Escherichia. coli R3*	30d	*E. coli R3*
31a	*Thermospiro melanesiensis BI429*	31b	*Thermanaerovibrio acidaminovorans DSM 6589*	31c	*Propionibacterium acnes*	31d	*Lactobacillus plantarum*
32a	*Actinomyces. israeli,*	32b	*Actinomyces odontolyticus*	32c	*Actinomyces. israeli,*	32d	*Actinomyces. naeslundi*
33a	*Actinomyces odontolyticus*	33b	*A. gerencseriae*	33c	*Eubakterium timidum*	33d	*Lactobacillus casei*
34a	*Propionibacterium acnes*	34b	*Actinomyces odontolyticus*	34c	*Bifidobacterium dentium*	34d	*Lactobacillus buchneri*
35a	*Lactobacillus buchneri*	35b	*Lactobacillus buchneri*	35c	*Bifidobacterium dentium*	35d	*Bifidobacterium dentium*
36a	*Lactobacillus plantarum*	36b	*Lactobacillus fermentum*	36c	*Lactobacillus fermentum*	36d	*Lactobacillus salivarius*
37a	*Bifidobacterium dentium*	37b	*Peptostreptococcus micros*	37c	*Arachnia propionica*	37d	*Desulfonatronospira thiodismutans Aso3-1*
38a	*Fusobacterium polymo*	38b	*Fusobacterium nucleatum*	38c	*A. viscosus rphum*	38d	*A. odontolyticus,*
39a	*Lactobacillus casei*	39b	*Actinomyces meyeri*	39c	*Actinomyces meyeri*	39d	*Eubacterium nodarum*
40a	*Bifidobacterium dentium*	40b	*Bifidobacterium dentium*	40c	*Lactobacillus delbrueckii*	40d	*Lactobacillus delbrueckii*

Table 2: *Sanger sequencing of bacterial inoculum from specific dishes taken as A, B, C and D (contact people with dogs).*

lp	Dog (Panel B)
1	*E. coli R2*
2	*Pyrococcus sp. OT3*
3	*Rothia dentocariosa*
4	*Capnocytophaga sputigena*
5	*A. hordeovulneris*
6	*Capnocytophaga ochracea*
7	*A. canis*
8	*Peptostreptococcus micros*
9	*Arachnia propionica (Actinomyces propionicus)*
10	*E.coli R4*
11	*Propionibacterium acnes*
12	*Tanarella forsythia*
13	*A. bowdenii pies*
14	*A. viscosus*
15	*Actinomyces odontolyticus*
16	*Peptostreptococcus micros*
17	*Treponema denticola*
18	*Fusobacterium nucleatum*
19	*Actinomyces meyeri*
20	*Fusobacterium polymorphum*

Table 3: *Sanger sequencing of bacterial inoculum from specific dishes.*

The types of diets used in both analyzed types of groups of people poses dogs and dogs affect the inflammation of soft tissues. The best results were seen in people on the W diet, which is in line with our previous research, and the vegetables used are a large injection of vitamins, including antioxidants, which reduce the level of oxidative stress in the oral cavity. Similar results were obtained in people following both types of diets B and T. However, in the last two subgroups two people were found eligible for intensive periodontal treatment. B and T diets also reduce inflammation, but to a lesser extent. The elimination of carbohydrates from food is important for soft tissues, even in the presence of tartar. The best results were obtained by patients who were on the W diet, where the average result was less than 0.1%. Periodontal disease is one of the most common diseases of the oral cavity in humans and, along with caries and its complications, is the main cause of tooth loss. Due to the frequency and prevalence of periodontal diseases, it is classified as a social disease. Experimental and clinical studies allow us to clearly classify periodontitis as an indicator of the risk of many serious diseases, including the cardiovascular system. Moreover, it is a risk factor that can be constantly modified and attempted to control. The high incidence of cardiovascular diseases and the widespread occurrence of periodontal diseases in Polish society indicate the need to investigate the likely relationship in order to introduce appropriate preventive measures. Therefore, it is important that oral evaluation is included in the routine patient examination, especially in patients at high cardiovascular risk. In these people, prophylaxis of periodontal diseases should be carried out, mainly consisting in proper oral hygiene. An important element of prophylaxis are periodic (every 6 months) visits to the dentist. A family doctor or internist can also evaluate the periodontium by examining the oral cavity. Consideration should be given to swelling, redness of the gums, soreness and bleeding under pressure from dental instruments, sometimes associated with loosening of the teeth; these are

Oral microbiota collected from volunteers working poses domestic pets e.g. dogs

The results obtained from sequencing of all four analyzed groups, collected from each of 20 human and animal specimens and plated by the reduction plating method, showed that in each of the analyzed cases there were bacterial biofilms belonging to one of the six bacterial complexes commonly analyzed in dental microbiology. However, the qualitative composition in individual groups varied considerably. In people with pets, the composition of the oral microflora was shifted towards the yellow, blue and orange complexes. On the other hand, the share of bioflim forming the red complex was unitary. It was different in the case of people working with livestock. Based on the results of sequencing using the Sanger method, we observed the predominance of orange, yellow and red complex species in equal quantity. In both cases, bacterial species characteristic of each animal species predominated, but with a clear tendency to the presence of biofilms also present in the human oral cavity where it was possible to observe the presence of bacteria from each of the 6 bacterial complexes, the largest share of which are bacteria found in the complexes: orange > red > yellow > green > blue > purple, in which the equilibrium of the reaction has been shifted towards a specific pH. Based on the analyzed results, we can conclude that in both cases, groups of people have not found any bacterial species that could induce zoonoses in humans. Despite the specific diet for humans used and the proportion of similar nutrients present in the diets of the analyzed groups of animals.

An example of the API indicator that was presented in both Tables 1 and 2 was presented in the form of tables to show the differences in both types of groups of people living with domestic animals (Table 4).

Type of diet	Magnet indicator	Indicator Pl.I	Indicator OHI	Indicator API	indicator PBI	indicator CPITN
B	10,9 : 5 = 2,18 sufficient	8,56 : 5 = 1,71 mild inflammation	7,75 : 5 = 1,55 good	266,12 : 5 = 53,22% average	23,33 : 5 = 4,67% Clinically healthy periodontitis	2 of the respondents qualify for specialist treatment
T	11,63 : 5 = 2,33 sufficient	10,14 : 5 = 2,03 modearte inflammation	8,69 : 5 = 1,74 good	304,98 :5 = 61% average	110,42:5=22,08% moderate gingivitis	2 of the respondents qualify for specialist treatment
W	11 : 5 = 2,2 sufficent	9,37 : 5 = 1,87 mild inflammation	10,16 : 5 = 2,03 sufficient	434,34 : 5 = 86,87% bad	42,8 6 : 5 = 8,57% Clinically healthy periodontitis	3 of the respondents qualify for specialist treatment
F	14,84 : 5 = 2,97 sufficient	9,49 : 5 = 1,9 mild inflammation	9,75 : 5 = 1,95 good	305,37 : 5 = 61,07% average	96,44 : 5 = 19,29% mild/condition requiring improvement in oral hygiene	2 of the respondents qualify for specialist treatment

Table 4: *Examples of 4 types of diets used on the selected API and PBI indicator (See table 1 and 2 in Results). Comparison of group results - average values in groups of people poses domestic animals.*

On the basis of the graphs, we can conclude that a special diet regulates the pH of our oral mucosa, changing the niche of the bacterial biofilm towards beneficial bacteria, and not pathogenic bacteria causing caries and plays a protective role in people living with pets table 5.

No of Patients/ Type of Diet in Indicator	1B	2B	3B	4B	5B	6T	7T	8T	9T	10T	11W	12W	13W	14W	15W	16F	17F	18F	19F	20F
Indicator API	*	*	*	*	*	*	*	*	*	*	*	*	*		*	***	***	***	**	***
Indicator PBI	**		*		*	**			*	*	*	*	*	*	**	*	**	**	**	**

Table 5: *Statistical analysis of all 40 analyzed patients with different type of diet after application of API I PBI indicator p < 0.05 *, <0.01 **, <0.001 ***. (See section the subject of our considerations in materials and methods).*

The types of diets used in both analyzed types of groups of people poses dogs and dogs affect the inflammation of soft tissues. The best results were seen in people on the W diet, which is in line with our previous research, and the vegetables used are a large injection of vitamins, including antioxidants, which reduce the level of oxidative stress in the oral cavity. Similar results were obtained in people following both types of diets B and T. However, in the last two subgroups two people were found eligible for intensive periodontal treatment. B and T diets also reduce inflammation, but to a lesser extent. The elimination of carbohydrates from food is important for soft tissues, even in the presence of tartar. The best results were obtained by patients who were on the W diet, where the average result was less than 0.1%. Periodontal disease is one of the most common diseases of the oral cavity in humans and, along with caries and its complications, is the main cause of tooth loss. Due to the frequency and prevalence of periodontal diseases, it is classified as a social disease. Experimental and clinical studies allow us to clearly classify periodontitis as an indicator of the risk of many serious diseases, including the cardiovascular system. Moreover, it is a risk factor that can be constantly modified and attempted to control. The high incidence of cardiovascular diseases and the widespread occurrence of periodontal diseases in Polish society indicate the need to investigate the likely relationship in order to introduce appropriate preventive measures. Therefore, it is important that oral evaluation is included in the routine patient examination, especially in patients at high cardiovascular risk. In these people, prophylaxis of periodontal diseases should be carried out, mainly consisting in proper oral hygiene. An important element of prophylaxis are periodic (every 6 months) visits to the dentist. A family doctor or internist can also evaluate the periodontium by examining the oral cavity. Consideration should be given to swelling, redness of the gums, soreness and bleeding under pressure from dental instruments, sometimes associated with loosening of the teeth; these are symptoms of gingivitis or acute periodontitis. However, in patients with already diagnosed periodontal disease, risk factors for cardiovascular disease should be assessed and appropriate periodontal treatment instituted. Our research on the basis of the analyzed dental hygiene and peridontological indicators and the results obtained in all analyzed types of groups is an attempt to respond to the processes of formation of a specific microbiome under the influence of diet taking place in the oral cavity..

Hygiene in the analyzed people was worse. Two subsequent measurement indicators indicated the presence of tartar diagnosed with a periodontometer and were at a good level according to the indicator evaluation criteria. On their basis, the obtained results were entered into the test evaluation criteria. Bacterial plaque and tartar initiate a chronic condition of gingivitis that the subject may not be aware of. Hygienic dental indicators help us to better diagnose the state of oral hygiene: magenta indicator stains food deposits on the tooth, soft and hard bacterial plaque; Pl.I determines the thickness of the bacterial plaque; OHI - diagnoses the presence of soft and hard bacterial plaque; API - informs about the presence of bacterial plaque in the interdental spaces; PBI - informs about inflammation in the interdental spaces, using for this purpose one of the symptoms of inflammation, namely bleeding of the gingival papilla; CPITN index - diagnoses the need for periodontal treatment, which at the same time suggests a higher probability of the presence of red and yellow complex bacteria in the oral cavity. Measurements were made on different teeth and at different locations on the dental arches. Using the above indicators, the results in each group were analyzed and the analysis of the results is presented below:

1. The magenta index visually determines the presence of plaque deposits: soft and hard, plaque from the use of colored drinks and food. After averaging the results obtained in each of the analyzed subgroups, the analyzed values were in the range of 2-3, which indicates a sufficient level

of oral hygiene in each of the studied groups.

2. The Pl.I index indicates the thickness of the bacterial plaque. After analyzing the mean of the results in each of the studied subgroups, the obtained values ranged from 1 to 2, which proves that the oral hygiene in each of the studied groups was good.

3. The OHI index determines the presence of tartar. After analyzing the results in each of the analyzed subgroups, the results were obtained from 1 to 2, which also shows good oral hygiene in each of the analyzed groups.

4. The API index determines the presence of plaque present in the interdental spaces, (similar to the PBI index), in places that are difficult to clean and promote its accumulation. The obtained results in the subgroups with diets B, T, W ranged from 53.38% to 69.72%, which indicated an average oral hygiene. In the diet F subgroup, the mean score was 82.42%, indicating very poor oral hygiene.

5. PBI captures the bleeding of the gums, which is one of the symptoms of inflammation of the soft tissues. After analyzing the results, it turned out that with the W diet the index value was 0.09%, and with the B diet the index value was 4.6%, and with the T diet the index value was 5.05%, which suggests no inflammation in the periodontal tissues and the analyzed periodontium is clinically healthy. In the case of the F diet, the index value was 21.3%, which indicates a moderate gingivitis. The differences between the subgroups using the W, B, and T diets differed significantly from the subgroups using the F diet.

6. The CPITN index determines the need for periodontal treatment. In both types of the subgroups where the B and T diets were used, there were two people requiring urgent periodontal treatment, while in the subgroups where the W and F diets were used, one of the people requiring urgent peridontological intervention was found.

The obtained results refer to the research from previous publications by Rowińska [7,8], where simple and cheap tests were developed to estimate the extent to which particular types of diets influence the formation of specific bacterial biofilms within the bacterial complexes described by Socransky [37,38]. Certain types of pathogenic bacteria in the analyzed bacterial biofilms may cause inflammation of periodontal diseases.

Discussion

The analysis of specific periodontal and dental indicators and the above-mentioned test results will be very helpful in the dental environment, especially for doctors, dental hygienists. The described research groups of animals and humans, characterized by sequencing methods, can help to significantly reduce the time of detection of disease entities induced by various bacterial belonging to specific complexes. They will also allow the assessment of the actual inflammation of the periodontal tissues. Based on the indicators and the type of bacterial biofilm, the doctor can directly estimate what type of bacteria he is dealing with and what treatment should be applied. The applied diet does not exclude the use of its various supplements, which can potentially contribute to a significant improvement in the condition of periodontal soft tissues, which was already described in our previous work [42]. Dietary components such as macro- and microelements and vitamins without carbohydrates and fats significantly contribute to the quality and improvement of vitality and the health of the periodontium and the teeth themselves, protecting them against early and rapid loss. From the obtained results (Table 1-5, Figure 1) it can be concluded that a specific diet in humans indicates the selection of a specific organism microflora in the oral cavity and the advantage of beneficial bacteria over pathogens from the six analyzed complexes.

Chronic and severe stomatitis can lead to the destruction of the alveolar bone, leading to tooth loss. This process can be induced by the secreted toxins by pathogenic bacteria. The latest literature reports indicate a significant and real influence of the bacterial biofilm on other organs and systems of man [7,8].

Currently, antibiotic resistance among pathogenic bacteria is becoming more and more common, which leads to their over-resistance.

The results of our research obtained by the method of Sanger sequencing [44] show that in 4 different types of diets all bacteria from the six bacterial complexes (Tables 3 and 4) are present and maintained at a constant level by using specific energy sources for their metabolism derived from metabolism [7,8,36-39]. From bacterial biofilm sequencing data, it is clear that a specific microbiota persists on a given type of diet for the entire duration of the study. These bacteria can induce diseases of various organs, including ischemic heart diseases, and neoplastic diseases related to the gastrointestinal tissues [1-3].

The influence of diet on the soft tissues of the oral cavity in a group of 20 volunteers - Analysis of dental indicators

Dentistry as a field of knowledge about dental diseases has developed indicators defining the state of hygiene and periodontal tissues [7,8]. On the other hand, any anomalies from generally accepted norms are a predictor of the formation of pathological conditions. Twenty volunteers working professionally with posess for example pets (dogs) at home, participated in the study and committed to an appropriate diet for the duration of the study. Research group was additionally divided into four subgroups of five people, each of which was assigned a different specific diet (protein, vegetable, rich in Omega-3 acids and the so-called fast food diet). In each of the subgroups, the volunteers underwent an analysis of dental indicators three days after following a specific diet, while maintaining standard oral hygiene in each of the respondents. The composition of the bacterial plaque was analyzed and qualitative tests were performed. After analyzing all types of indicators, it was found that oral hygiene after averaging was at a similar level in all examined persons. The plaque staining indexes showed similar oral hygiene values. All patients had hard and soft plaque.

The value of the first index was the highest because it was associated with soft and hard plaque and organic sediments formed after consuming colored drinks and food.

The analysis of the values obtained from the various types of diets T, F, S and W shows that all of them can have an antibacterial effect on the bacterial strains responsible for inflammation of the periodontal tissues in the oral cavity. The PBI value in the range of 100–50% is noteworthy, indicating very severe gingivitis. PBI values in the range of 50–20% are described as moderate. People on a type F diet should be included in the 20-10% range which describes a mild period of periodontitis and requires improvement in oral hygiene. It should be noted that the inflammation of the periodontium usually begins with the interdental spaces. Interpreting our data with the obtained values of the results from the analyzed indices, we should not obtain the PBI result below 10%, which determines clinically healthy periodontium. Study participants who used the so-called a targeted diet, they obtained a “clinically healthy periodontium” result.

Conclusion

After the measurements were taken on the subjects, using specific diets and averaging the measurements, the following was observed:

- In the study, all persons had sufficient hygiene - as evidenced by the staining index of dental plaque.
- Pl.I index - in 6 out of 8 cases it indicated mild inflammation (based on the amount of bacterial plaque), and in 2 cases moderate inflammation in each of the 2 groups.
- OHI index - in the first test group he defined hygiene at a sufficient level, in group 2 at a good level, except for the subgroup with diet W (sufficient hygiene).
- API index - in the first group of respondents it defined the hygiene in the interdental spaces at the lowest level, defined in the index criteria as “bad”; and in the second group as “average” in three subgroups, and “bad” in the subgroup with diet W.

- The PBI index - diagnosing a symptom of inflammation (bleeding) in the first group in the subjects with diets B and T was - "mild / condition requiring improvement of oral hygiene", with the diet W it was "0", which is interpreted as - "periodontitis clinically healthy ", and in subjects on the F diet, it is referred to as" moderate gingivitis "; in the second group, in the subgroups with diets W and B, the index indicated "clinically healthy periodontium", in the subgroup with diet F, "mild / condition requiring improvement of oral hygiene", in the subgroup with diet T - "moderate gingivitis".

- CPITN index - in the first group of respondents it run unevenly, but it did not affect the interpretation of the results in any particular way; in the second group it was more even.

- In both groups of respondents, surprisingly good results of PBI measurements are observed in the case of average or even poor hygiene in the interdental spaces, especially in the first group and the subgroup with the W diet, with "poor" hygiene, the index equal to "0"; similarly in the second group, in the subgroup with the same diet, the average measurement was 8.57%, which is defined in the criteria of the indicator as "clinically healthy periodontium", but in this subgroup there were as many as 3 people eligible for periodontal treatment.

- it can be concluded that antioxidants found in the W diet perfectly cope with oxidative stress in the oral cavity, as evidenced by the results of the measurements carried out in both groups of respondents.

Funding Support

This research was funded SUB/2/DN/20/002/2201.

Institutional Review Board Statement

The study did not require a Statement of the Institution's Audit Committee for ethical and bioethical research. The study was conducted in accordance with the guidelines of the Helsinki Declaration.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest

The authors declare no conflict of interest.

Bibliography

1. Chen., *et al*. "Association between periodontal disease, tooth loss and liver diseases risk". *Journal of Clinical Periodontology* 47.9 (2020): 1053-1063.

2. Carrizales-Sepúlveda., *et al*. "Periodontal Disease, Systemic Inflammation and the Risk of Cardiovascular Disease". *Heart, Lung and Circulation* 27.11 (2018): 1327-1334.

3. Sroussi., *et al*. "Common oral complications of head and neck cancer radiation therapy: mucositis, infections, saliva change, fibrosis, sensory dysfunctions, dental caries, periodontal disease, and osteoradionecrosis". *Cancer Medicine* 6.12 (2017): 2918-2931.

4. Güll., *et al*. "Periodontal disease-like bone loss after adjuvant radiotherapy in the head and neck region: A case report and review of the literature". *Journals: QI. Quintessence International* 48.6 (2017): 451-457.

5. Irie., *et al*. "Periodontal therapy for patients before and after radiotherapy: A review of the literature and topics of interest for clinicians". *Medicina Oral, Patologia Oral, Cirugia Bucal* 23.5 (2018): e524-e530.

6. Sohn., *et al*. "Effects of professional oral hygiene care in patients with head-and-neck cancer during radiotherapy: A randomized clinical trial". *Indian Journal of Dental Research* 29.6 (2018): 700-704.

7. Rowińska., *et al*. "Impact of the Diet on the Formation of Oxidative Stress and Inflammation Induced by Bacterial Biofilm in the Oral Cavity". *Materials* 14 (2021): 1372.

8. Rowińska., *et al*. "The Influence of Diet on Oxidative Stress and Inflammation Induced by Bacterial Biofilms in the Human Oral Cavity". *Materials* 14 (2021): 1444.

9. Aguirre., *et al*. "The one health approach to toxoplasmosis: epidemiology, control, and prevention strategies". *Ecohealth* 16.2 (2019): 378-390.

10. Harrison., *et al*. "Brucellosis". *Pediatrics in Review* 39.4 (2018): 222-224.

11. Zhang., *et al*. "Animal brucellosis control or eradication programs worldwide: A systematic review of experiences and lessons learned". *Preventive Veterinary Medicine* 160 (2018): 105-115.

12. Škultéty., *et al*. "Q fever and prevention". *Epidemiologie, Mikrobiologie, Imunologie* 69.2 (2020): 87-94.

13. Bancerz-Kisiel., *et al*. "Yersiniosis - a zoonotic foodborne disease of relevance to public health". *The Annals of Agricultural and Environmental Medicine* 22.3 (2015): 397-402.

14. Swaminathan., *et al*. "The epidemiology of human listeriosis". *Microbes and Infection* 9.10 (2007): 1236-1243.

15. Banyard., *et al*. "Rabies pathogenesis and immunology". *Revue Scientifique et Technique* 37.2 (2018): 323-330.

16. Benavides., *et al*. "Anthrax: safe treatment for children". *Annals of Pharmacotherapy* 36.2 (2002): 334-337.

17. Kamal., *et al*. "Anthrax: an update". *Asian Pacific Journal of Tropical Biomedicine* 1.6 (2011): 496-501.

18. Li., *et al*. "Avian influenza viruses in humans: lessons from past outbreaks". *British Medical Bulletin* 132.1 (2019): 81-95.

19. Gadê-Neto., *et al*. "Microbiota of periodontal pockets and root canals in induced experimental periodontal disease in dogs". *Journal of Investigative and Clinical Dentistry* 10.4 (2019): e12439.

20. Nises., *et al*. "The occurrence of Treponema spp. in gingival plaque from dogs with varying degree of periodontal disease". *PLoS One* 13.8 (2018): e0201888.

21. Pereira Dos Santos., *et al*. "Relation between periodontal disease and systemic diseases in dogs". *Research in Veterinary Science* 125 (2019): 136-140.

22. Wallis., *et al*. "A review of the frequency and impact of periodontal disease in dogs". *Journal of Small Animal Practice* 61.9 (2020): 529-540.

23. Watson., *et al*. "Diet and periodontal disease in dogs and cats". *Australian Veterinary Journal* 71.10 (1994): 313-318.

24. Meyer., *et al*. "Models of invasion of enteric and periodontal pathogens into epithelial cells: a comparative analysis". *Critical Reviews in Oral Biology and Medicine* 8.4 (1997): 389-409.

25. Lawson., *et al*. "Characterization of some Actinomyces-like isolates from human clinical specimens: reclassification of Actinomyces suis (Soltys and Spratling) as Actinobacu-lum suis comb. nov. and description of Actinobaculum schaalii sp. Nov". *International Journal of Systematic and Evolutionary Microbiology* 47 (1997): 899-903.

26. Woldemeskel., *et al*. "Microscopic and ultrastructural lesions of the urether and renal pelvis in sows with regard to Actinobaculum suis infection". *Journal of Veterinary Medicine* 49 (2002): 348-352.

27. Smith., *et al*. "Antimicrobial susceptibility testing of Actinomyces species with 12 antimicrobial agents". *Journal of Antimicrobial Chemotherapy* 56 (2005): 407-409.

28. Hansen., *et al*. "Actinomyces species: A Danish survey on human infections and microbial characteristics". *The Open Microbiology Journal* 3 (2009): 113-113.

29. Brook., *et al*. "Actinomycosis: diagnosis and management". *The Southern Medical Journal* 101 (2008): 1019-1023.

30. Hall., *et al*. "Actinomyces gathering evidence of human colonization and infection". *Anaerobe* 14 (2008): 1-7.

31. Hoyles., *et al*. "Actinomyces canis sp. nov. isolated from dogs". *International Journal of Systematic and Evolutionary Microbiology* 50 (2000): 1547-1551.

32. The Act of 5.XII. on preventing and combating human infections and infectious diseases". *Journal of Laws* 234 (2009): 1570.

33. Gliński., *et al*. "Zoonosis. PWRiL, Warszawa (2008).

34. Sanz., *et al*. "Role of microbial biofilms in the maintenance of oral health and in the development of dental caries and periodontal diseases. Consensus report of group 1 of the Joint EFP/ORCA workshop on the boundaries between caries and periodontal disease". *Journal of Clinical Periodontology* 44.18 (2017): S5-S11.

35. Gomes., *et al*. "Primary cutaneous actinomycosis caused by Actinomycers meyeri as first manifestation of HIV infection". *Dermatology Online Journal* 17 (2011): 5-8.

36. Binda., *et al*. "Actinobacteria: A relevant minority for the maintenance of gut homeostasis". *Digestive and Liver Disease* 50.5 (2018): 421-428.

37. Socransky., *et al*. "Microbial complexes in sub-gingival plaque". *Journal of Clinical Periodontology* 25 (1998): 134-144.

38. Socransky., *et al*. "Periodontal microbial ecology". *Periodontology* 38 (2005): 135-187.

39. Kucia., *et al*. "Effect of LigiLactobacillus salivarius and Other Natural Components against Anaerobic Periodontal Bacteria". *Molecules* 25 (2020): 4519.

40. Hoyles., *et al*. "Actinomyces canis sp. nov. isolated from dogs". *International Journal of Systematic and Evolutionary Microbiology* 50 (2000): 1547-1551.

41. Pascual., *et al*. "Actinomyces bowdenii sp. nov. isolated from canine and feline clinical specimens". *International Journal of Systematic and Evolutionary Microbiology* 49 (1999): 1873-1877.

42. Buchanan., *et al*. "Actinomyces hordeovulneris,a canine pathogen that produces L-phase variants spontaneously with coincident calcium deposition". *American Journal of Veterinary Research* 45 (1984): 2552-2560.

43. Pelle., *et al*. "Actinomycosis in dogs caused by Actinomyces hordeovulneris". *The Journal of Comparative Pathology* 123 (2000): 72-76.

44. Sanger., *et al*. "DNA sequencing with chain-terminating inhibitors". *Proceedings of the National Academy of Sciences of the United States of America* 74 (1977): 5463-5467.

45. Lizut. "AI in determining indicators for FX prediction models in: Economics And Law, University Publishing House UniversiUniwersytet Nicolaus Copernicus, Torun (2020).

Volume 6 Issue 9 September 2021