Praca oryginalna *Original paper*

Identification and prevalence of *Capnocytophaga canimorsus* and *Capnocytophaga cynodegmi* from the dog population in Wrocław, Poland

ZUZANNA CZEKAJ[1], MAŁGORZATA D. KLIMOWICZ-BODYS[2], MAGDALENA KARWAŃSKA[3], MAGDALENA SIEDLECKA[3], KRZYSZTOF RYPUŁA[2]

[1]Student Science Club "AnthraX", Department of Epizootiology and Clinic of Birds and Exotic Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, pl. Grunwaldzki 45, 50-366 Wroclaw, Poland

[2]Division of Infectious Diseases of Animals and Veterinary Administration, Department of Epizootiology and Clinic of Birds and Exotic Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, pl. Grunwaldzki 45, 50-366 Wroclaw, Poland

[3]Department of Epizootiology and Clinic of Birds and Exotic Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, pl. Grunwaldzki 45, 50-366 Wroclaw, Poland

Received 01.03.2024 *Accepted 30.04.2024*

Czekaj Z., Klimowicz-Bodys M. D., Karwańska M., Siedlecka M., Rypuła K.

Identification and prevalence of *Capnocytophaga canimorsus* and *Capnocytophaga cynodegmi* from the dog population in Wrocław, Poland

Summary

The bacterium *Capnocytophaga* spp. is a part of the oral microbiome of dogs, which has zoonotic potential. This study aimed to assess factors that influence the prevalence of *Capnocytophaga cynodegmi* and *Capnocytophaga canimorsus* in Wrocaw's dog population. Molecular detection (PCR) involved 105 swabs, examining parameters such as oral cavity condition, underlying health issues, recent antibiotics, age, medications, material collection, and dog chews. The study highlighted significant associations between the presence of *C. cynodegmi* and oral cavity condition, material collection method, and use of dog's chews. Correlations obtained in this study between the oral health of Wroclaw's dog population and the presence of *C. cynodegmi* suggest its significance in the oral microbiome of dogs. Limitations, including the small sample size, highlight the need for more research to comprehensively understand additional factors that affect the prevalence of *Capnocytophaga* spp. in dogs.

Keywords: *Capnocytophaga canimorsus*, *Capnocytophaga cynodegmi*, *Capnocytophaga* spp., dog

Capnocytophaga spp. are Gram-negative bacteria that belong to the family *Flavobacteriaceae*, order *Flavobacteriales* (https://lpsn.dsmz.de/genus/capnocytophaga, 15). *Capnocytophaga* spp. characterise a high degree of polymorphism, with a variation in the size and appearance depending on the strain and culture conditions. Microscopic observation revealed that microorganisms are short or elongate flexible fusiform to rod-shaped cells with round or tapered ends. The diameter of the microorganism is from 0.42 to 0.6 µm, while its length is from 2.5 to 5.7 µm (9). Bacteria of the genus *Capnocytophaga* are fastidious, slow growing, aerotolerant anaerobes with a requirement for CO_2. Typically they form thin, spreading gray colonies on solid media (22). Multiple suitable culture media are used for cultivation of the bacteria, including chocolate agar and Columbia agar with 5% sheep's blood (5, 22). Yet not all blood based media support typical growth type of *Capnocytophaga* (22). The genus *Capnocytophaga includes* 12 species validly published under the ICNP (International Code of Nomenclature of Prokaryotes), including *Capnocytophaga canimorsus* and *C. cynodegmi*, which are part of dogs' and cats' oral microbiomes but also zoonotic pathogens (7, 20, 54).

C. canimorsus is the most frequently identified microorganism of this genus with reported pathogenicity and the ability to be transmitted from dogs' oral cavity to humans. Probably the first case of *C. canimorsus* infection in humans occurred in 1961 in California. The patient was a 17-year-old man who had undergone splenectomy 3 years earlier and had been bitten by a dog a few days before his illness. Most patients are middle-aged or elderly people, more often men than women, suffering from chronic diseases, alcoholism or drug addiction. These are often people who have undergone splenectomy (7). In the period from 1961 to 2014, nearly 500 cases of *C. canimorsus* infections in humans were reported. The most common cause of human infection was contact with a dog, with 60% of cases being associated with dog bites and 24% of cases were associated with contact such as licking or scratching. Cat exposures were sporadic, occurring in 3% of cases. Therefore, it is believed that dog contact is the main source of *C. canimorsus* infection. Other risk factors are: median age (55 years), sex (two-third patients are male), splenectomy or spleen disorders (asplenia, functional hyposplenism), alcoholism, diabetes mellitus, cirrhosis, corticosteroid use and other immunocompromising conditions (leukaemia, myeloma, macroglobulinemia and other lymphoproliferative diseases, HIV) (7, 37). The most clinical presentation were sepsis or sepsis and septic shock. In these cases, patients demonstrated: purpura or petechiae, disseminated intravascular coagulation (DIC), gangrene (including dry gangrene), abdominal pain, distention, diarrhoea, haemolytic uremic syndrome (HUS) and/or myocardial infarction. The second common clinical presentation was meningitis. Less commonly, endocarditis or myotic aneurysms have been reported (7, 13). Table 1 presents selected confirmed cases of *C. canimorsus* infections in humans described in the literature over the last 5 years.

Due to the great popularity of keeping dogs at home and the very close contacts with dogs of people with different health status, it seems important to analyse whether bacteria of the *Capnocytophaga* genus, and in particular *C. canimorsus* and *C. cynodegmi*, may be a potential threat to pet owners. The prevalence of these microorganisms in the oral cavity of dogs ranges from 8% to 74% (9). So far, the frequency of these bacteria in dogs in Poland has not been assessed. This study aimed to evaluate the prevalence and the possible factors influencing the prevalence of *C. canimorsus* and *C. cynodegmi* in the dog population in Wroclaw, Poland. All samples were analysed for the influence of various factors, including: the condition of the dog's oral cavity, concomitant diseases, antibiotics taken up to 6 months before the examination, age, long-term medications taken, material taken directly from the teeth and chews, and the presence of the abovementioned bacteria.

Material and methods

Study population and sample procedures. A total of 105 swabs were taken from dogs for molecular detection of the *Capnocytophaga* genus. All the samples were analysed for various parameters, including the oral cavity condition of the dog, underlying conditions, antibiotics taken up to 6 months before the examination, age, long-term medications, material collected directly from the teeth, and oral chews used for the dogs.

Tab. 1. Selected cases of *Capnocytophaga canimorsus* infections in humans in 2019-2024

Age, sex	Animal contact	Medical history	Symptoms	Diagnosis	Ref.
70, M	no data	– splenectomy immune thrombocytopenia	– general malaise, – chills, – vomiting, – diarrhoea	purpura fulminans (catastrophic acute severe coagulopathy associated with disseminated intravascular coagulation (DIC) and mild-sized vessel obstruction)	34
47, M	no data	– ex-smoker	– dyspnoea at rest, – reduced exercise tolerance, – low-grade fever, – night sweats, – weight loss	infective endocarditis of the native aortic valve	40
37, M	dog bite	– smoking habit	– fever, – headache, – myalgia, – epigastralgia, – nausea, – diarrhoea	Bobo-Newton acute myocarditis	6
72, M	dogs contact	– alcoholic liver cirrhosis, – episodes of ascitic decompensation, – an episode of gastrointestinal bleeding secondary to oesophageal varices, – chronic portal thrombosis, – hepatocarcinoma, – umbilical hernia	– fever, – abdominal pain	bacterial peritonitis	25
70, W	face licking by dog	– osteoarthritis in both hands	– fever, – rashes on abdomen and mouth	tricuspid valve endocarditis	23
81, M	face licking by dog with poor dentition	– 10 years after trabeculectomy, – radial keratotomy, – penetrating keratoplasty	– blebitis, – perforated bleb	bacterial blebitis	53

Tab. 1. continuation

Age, sex	Animal contact	Medical history	Symptoms	Diagnosis	Ref.
76, M	dog bite	– hypertension, – type two diabetes, – smoking habit, – alcoholism	– fatigue, – anorexia, – dyspnoea	double native valve (aortic and mitral) endocarditis	14
68, M	dog bite and scratch	– prostatic hyperplasia, – distal gastrectomy (gastric cancer), – smoking habit	– persistent lower abdominal pain, – back pain, – melena	septic shock	26
58, W	dog bite	– left total hip arthroplasty	– insidious onset of left hip pain	periprosthetic joint infection	17
80, W	dog licking excoriations brought on by eczema	– coronary artery disease – type two diabetes, – flare of nummular eczema on her bilateral lower and upper extremities	– fever, – chills, – dull, – ill-defined lower back, – bilateral buttock, – pelvis pain	aortitis	1
61, M	dog bite	– hypertension, – chronic obstructive pulmonary disease, – occasional smoking	– progressive abdominal pain radiating to the lower back, – fatigue, – nausea, – muscle strains, – cold chill	mycotic aortic aneurysm	4
38, W	dog scratch	– hyperthyroidism, – microscopic haematuria	– worsening headache radiating down the back of head and neck, – fever, – congestion, – cough, – myalgias, – fatigue, – vomiting, – confusion, – distorted hearing	meningitis	10
41, W	facial dog bite	– chronic alcoholism with long-term abstinence, – obesity	– visible petechial bleeding, – fever, – rash with marbled skin on whole back, extremities and face, – necrotic toes	sepsis	16
31, M	dog bite	– type one diabetes, – depression, – smoking habit	– rhinorrhoea, – fever, – dyspnoea, – fatigue, – postnasal drip, – nausea, – mild, sharp, left-sided chest pain made worse by deep inspiration	bloodstream infection associated with an urticarial exanthem	12
63, M	no dog or cat contact	– coronary heart disease, – atrial fibrillation, – chronic pulmonary disease, – smoking habit, – splenectomy for immune thrombocytopenia, – chronic myelomonocytic leukaemia	– fever, – cough, – asthenia	septic shock	28
51, M	minor injury made by dog	– excellent healthy sportsman	– fever, – non-itchy exanthema all over the body, – sparing the oral mucosa	exanthema associated with bacteraemia	45
43, M	dog bite	– alcoholism, – heroin and crack cocaine using	– painful rash across torso, back and limbs, – vomiting, – difficulty walking, – long-standing cough	purpura fulminans	18
70, W	dog bite	– hypothyroidism, – celiac disease, – centrilobular emphysema, – osteoporosis, – osteoarthritis, – anxiety, – dyslipidaemia, – smoking habit	– severe headache, – confusion, – expressive aphasia, – rigours, – photophobia, – neck pain, – general malaise, – nausea, – vomiting	meningitis	52

Tab. 1. continuation

Age, sex	Animal contact	Medical history	Symptoms	Diagnosis	Ref.
74, M	dog bite	– type two diabetes, – arterial hypertension, – dyslipidaemia, – liver steatosis, – cholecystectomy, – abdominal hernia surgery, – partial gastric resection for ulcer disease	– headache, – stiff neck, – nausea, – fever	purulent meningitis	35
50, M	dog scratch and bite	– no data	– fever, – shiver, – arthromyalgia, – vomiting, – odynophagia, – cough, – weight loss	sepsis	47
75, M	dog contact	– no data	– confusion, – abdominal pain, – faecal incontinence, – fever	meningitis and bacteraemia	33
66, W	dog contact	– no data	– headache, – fever	meningitis	11
33, M	face licking by dog	– splenectomy	– tachypnoea, – tachycardia, – severe abdominal pain, – fever	purpura fulminans and sepsis	51
61, W	dog bite	– splenectomy, – Lupus erythematodes (without immunosuppressive therapy), – arterial hypertension, – mitral valve regurgitation, – angiodysplasia of the colon with recurrent haemorrhages, – polyarthrosis, – fibromyalgia	– cephalgia, – nausea, – diarrhoea	Waterhouse-Friederichsen Syndrome (sepsis associated with bilateral adrenal haemorrhage)	39
49, W	sleeping with cat	– type two diabetes, – continuous ambulatory peritoneal dialysis, – chronic subcutaneous tunnel infection with abscess around the catheter	– fever, – abdominal, – cloudy dialysis effluent	peritonitis	43
58, M	dog bite	– no data	– fever, – mild consciousness disorder, – ischemic necrosis on feet and hands (dry gangrene)	sepsis and hemophagocytic lymphohistiocytosis (HLH)	44
57, M	dog contact	– intravenous drug ex-user, – chronic hepatitis C, – HIV positive, – bilateral hip prosthesis, – mixed sensorimotor polyneuropathy	– pain in the left knee, – difficulty walking, – fever	septic arthritis	48
42, W	dog bite	– asthma	– diffuse headaches, – myalgias, – nonspecific abdominal pain	bacteraemia complicated by haemolytic uremic syndrome	27
52, M	dog bite	– allergy to bee stings, – smoking cigarettes and marihuana habit	– pain throughout the body, – vomiting, – diarrhoea, – coldness, – shortness of breath, – rash, – hematomas over the whole body	sepsis with peripheral necrosis (of the hands, feet, genitals, face, and later the forearms and lower legs)	36
86, W	dog scratch and licking	– aortic valve repair (porcine), – bilateral total hip joint replacements	– progressively worsening left iliac fossa pain, – acute deterioration, – lethargy	ruptured mycotic common iliac aneurysm	3

The material was collected by a veterinarian with Σ-TRANSWAB® (MWE, Corsham, Wiltshire, United Kingdom) and frozen the same day at –20°C until DNA extraction. The animals were included in the examination group after receiving permission from the owners to take samples from the dogs' mouths. In addition, each owner was asked to complete a survey about the examined dog and its dental care. Among the collected data, essential elements included information on the oral cavity condition of the dogs, such as the occurrence of pathological changes, the process of tooth replacement, and the absence of teeth. Additionally, detailed information on routine oral hygiene, including regular brushing of the teeth, was included in the analysis. Swabs were taken from each animal, from the teeth, tongue, lips, or entire oral cavity, without the possibility of defining the exact collection site, depending on the dog's cooperation and aggressiveness.

Isolation and identification of *Capnocytophaga* spp. DNA was isolated directly from the collected swabs using a Genomic Mini commercial kit (A&A Biotechnology, Gdansk, Poland). If additional tests were necessary, the swabs were frozen. PCR was performed using Thermo Scientific DreamTaq Green DNA Polymerase and dNTP Mix (Thermo Scientific, Waltham, MA, United States) following the reaction protocol created by the same company. The species identification of microorganisms was carried out using species-specific primers. Based on the study by Tabatabaei et al. (42), the *Capnocytophaga* spp., *C. canimorsus*, and *C. cynodegmi* gene primers (Genomed S.A., Warsaw, Poland), shown in Table 2. The solution for the reaction mixture was prepared in a volume of 25 μL for each reaction. It comprised 19.4 μL of dH_2O, 2.5 μL of PCR buffer, 0.5 μL of dNTPs (200 mM), 0.2 μL of each oligonucleotide primer (100 nmol), 0.2 μL of Taq DNA polymerase (5 U/μL), and 2 μL of template DNA. Following an initial denaturation step at 95°C for 5 minutes, the amplification process involved denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds, repeated for 37 cycles in a 96-well T100 PCR thermal cycler (BIO-RAD, Berkeley, California, USA), with a final extension at 72°C for 7 minutes. The PCR products were subsequently subjected to electrophoresis on a 2% agarose gel (BASICA LE Agarose; Prona, Burgos, Spain) coloured with Midori Green DNA Stain (Nippon Genetics Europe GmbH, Düren, Germany).

Statistical analysis. The characteristics of the dogs and the responses to the questionnaire were compared to the prevalence of *Capnocytophaga* species. The data were analysed using Pearson's chi-square test and Fisher's exact tests. $P \leq 0.05$ was considered to indicate a statistically significant association. Statistical analysis was performed using PQStat (v. 1.8.6).

Tab. 2. Primers used in the study

Bacterium	Primer	Sequence
Capnocytophaga genus	CAL2	5'GTAGAGTGCTTCGGCACTTG3'
	AS2	5'GTGATGCCACCAAACAATACTA3'
C. canimorsus	CAL2	5'GTAGAGTGCTTCGGCACTTG3'
	CaR	5'GCCGATGCTTATTCATACA3'
C. cynodegmi	CAL2	5'GTAGAGTGCTTCGGCACTTG3'
	CyR	5'GCGGATGCTTATTCGTATG3'

Results and discussion

All samples were taken from 2022 to 2023 and were analysed at the Department of Epizootiology and Clinic of Birds and Exotic Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Poland. In some cases, more than one dog was swabbed from one household. The data of the animals investigated are presented in Table 3.

In 55.2% of the total collected swabs, at least one species of *Capnocytophaga* was isolated. A total of 45.7% of the samples examined were positive for *C. cynodegmi*, and 39% were positive for *C. canimorsus*. Additionally, according to the owner's survey and examination of the dog, two subgroups were distinguished: dogs with lesions in the oral cavity and those without changes. The prevalence of *Capnocytophaga* bacteria in this group is shown in Figure 1.

Tab. 3. Characteristics of the investigated dogs

		Dogs with changes in the oral cavity	Dogs without changes in the oral cavity
Number of samples	n (%)	73 (69.52)	32 (30.48)
Location of swabs (%)	Teeth	65.75	46.88
	Oral cavity excluding teeth	34.25	52.12
Age of dog (%)	<1 year	5.48	9.38
	1-5 years	36.99	65.62
	6-10 years	36.99	21.88
	>10 years	20.55	3.12
Tooth brushing (%)	Yes	26.03	34.38
	No	73.97	65.62
Giving chews to dog (%)	Yes	68.49	62.50
	No	31.51	37.50

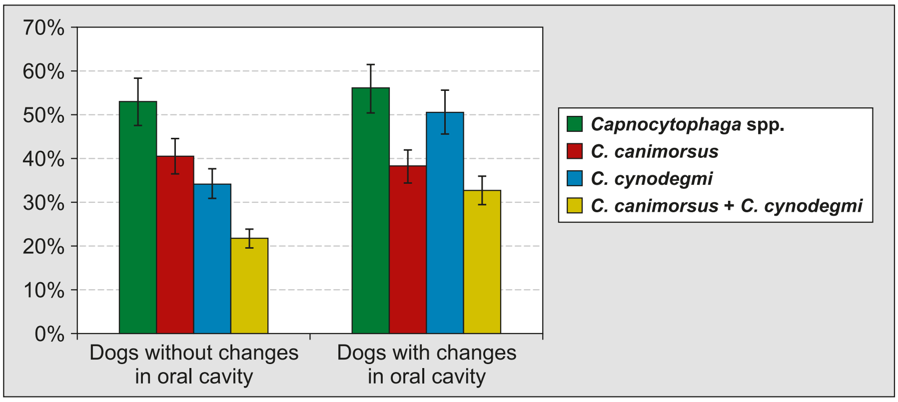

Fig. 1. Prevalence of *Capnocytophaga* species in healthy and sick dogs

Among the dogs with changes in the oral cavity, the following clinical signs were observed: tartars (n = 62), ongoing replacement of primary teeth (n = 3), absence of teeth (n = 3), lumps (n = 3), and other lesions (n = 2).

During statistical analysis of the data in the positive *Capnocytophaga* spp. group, there was a significant correlation between the presence of *C. cynodegmi* and lesions in the oral cavity in dogs (p = 0.019), collecting material directly from the teeth (p = 0.015), and chewing for dogs (p = 0.015). No significant results of the presence of *C. cynodegmi* were obtained for underlying conditions, taking antibiotic up to 6 months before the examination, age, or the using of long-term medications. A strong correlation (p = 0.000001) between the presence of *C. canimorsus* and *C. cynodegmi* was also observed.

As a result of the comparison of the presence of *C. canimorsus* and *Capnocytophaga* spp., no significant differences were obtained for underlying conditions, antibiotic use up to 6 months before examination, age, long-term medication use, material collection directly from the teeth, chewing for dogs, or lesions in the oral cavity in dogs.

This study included households with more than one dog, the results of which are presented in Table 4. Although interesting, the obtained results did not reach statistical significance. Nevertheless, in the 16 analysed cases of houses with several dogs, only in four of them the presence of *Capnocytophaga* gene was found in the oral cavity of all animals. Of these four cases, in three houses, all dogs tested positive for *C. canimorsus* while in one of them, the presence of *C. cynodegmi* was found.

This research was preceded by preliminary studies in order to develop the methodology and strategy of the main study. Swabs from the preliminary study were inoculated onto blood agar and chocolate agar and cultured at 37°C for 72 hours in a modified atmosphere with 5% CO_2, according to the literature (19, 32). Interestingly, none of the cultured colonies exhibited morphological characteristics similar to those reported in the literature for *Capnocytophaga* colonies (24). Due to the difficulties in obtaining positive culture results, a freeze-dried reference strain of *C. canimorsus* was selected as a positive sample for bacteriological testing. Unfortunately, no culture, either in solid or liquid media (Brain-Heart Infusion Broth), was successful, confirming the difficulties in cultivating bacteria of this type, which is consistent with the literature. However, even under ideal growing conditions, growth may take up to a week and failure to identify may result if the culture plates are discarded earlier (13). According to the literature data, blood cultures were positive in 54% of 37 patients analysed, with a median growth time of four days (13). Prolonged incubation period, resulting with overgrowth of presented in clinical specimen contaminants, together with the possibility of atypical appearance of the obtained colonies of *Capnocytophaga* make it difficult to isolate the bacteria. Additionally, several authors reported culture-negative infections caused by the bacteria in question, that required genetic approach for the detection of the pathogen (21, 50). The percentage of positive cultures and the time required to obtain a positive result indicate that the PCR method is of great importance in the identification of *Capnocytophaga* spp. and establishing the diagnosis in cases of infections with these microorganisms.

Tab. 4. Presence of *Capnocytophaga* spp., *C. cynodegmi*, and *C. canimorsus* in dogs in the same household

Household	Number of dogs	Positive dogs n (%)		
		Capnocytophaga spp.	*C. cynodegmi*	*C. canimorsus*
1	2	1 (50)	0 (0)	1 (50)
2	2	1 (50)	1(50)	1 (50)
3	2	2 (100)	1 (50)	2 (100)
4	2	2 (100)	2 (100)	1 (50)
5	4	2 (50)	2 (50)	0 (0)
6	2	1 (50)	1(50)	1 (50)
7	2	0 (0)	0 (0)	0 (0)
8	3	1 (33.33)	1 (33.33)	1 (33.33)
9	2	0 (0)	0 (0)	0 (0)
10	3	2 (66.66)	2 (66.66)	2 (66.66)
11	2	1(50)	1 (50)	0 (0)
12	2	1(50)	1 (50)	1 (50)
13	2	1(50)	1 (50)	1 (50)
14	3	3 (100)	2 (66.66)	3 (100)
15	2	0 (0)	0 (0)	0 (0)
16	2	2 (100)	0 (0)	2 (100)

Various studies conducted in veterinary medicine have shown that bacteria of the genus *Capnocytophaga* spp. are common in the oral microflora of dogs and cats. However, the incidence of *C. canimorsus* and *C. cynodegmi* vary significantly depending on the identification method used. Based on PCR analysis, the presence of *C. canimorsus* was found in 70-74% of dogs, while *C. cynodegmi* in 86-96% of dogs (8, 41, 46). On the other hand, *Capnocytophaga* species were found in 49.2% of dogs, but 21.7% of them hosted *C. canimorsus* while 11.7% of them carried *C. cynodegmi* (9). In the present study, the prevalence was similar to another report in which 19% of the samples were positive for *C. canimorsus* and 66.94% for *C. cynodegmi* (29).

The presence of the *Capnocytophaga* genus is linked to a decline in oral health, which was also confirmed by the results obtained for *C. cynodegmi* (31). In other study studies, the prevalence of *C. cynodegmi* was greater in dogs with a high incidence of periodontal disease (21). However, in the above mentioned research, there was no significant correlation between *C. canimorsus* and periodontal disease, as shown in the results of the present study. The promising results obtained in this study suggest the potential confirmation of the connection between *C. cynodegmi* and reduced oral health in dogs. The findings of this analysis offer valuable insights that could be crucial for future research on preventing oral health problems in dogs and understanding the impact of various factors on the prevalence of *C. cynodegmi*. However, some authors reported that the concentration of *Capnocytophaga* spp. COT-339 decreased over time in the group of canine patients with progressive periodontitis, and the prevalence of *C. cynodegmi* COT-254 decreased in both the progression group and the group without progression (49). However, the mentioned references did not discuss specific changes or characteristics of *Capnocytophaga* in the oral microbiota of dogs during the transition from mild gingivitis to the early stages of periodontitis. The role of *C. cynodegmi* as a biomarker of dental health in dogs has not yet been entirely clear because some studies indicate that it is a core microbiota of healthy dogs with high prevalence, but it was also found in many progressive stages of periodontal disease, with the exception of the initial stages (23, 29, 31, 49).

The oral environment influences the presence of bacteria; the prevalence of *Capnocytophaga* was greater in subgingival and supragingival plaques than in saliva (30). In examined dogs, more positive results for *C. cynodegmi* were also observed in swabs collected from the teeth surrounding the area.

The study conducted by Ruparell et al. showed that daily oral care chews influenced the composition of the plaque microbiota in dogs (38). Some dog foods reduced dental plaque build-up and gingival inflammation, and chews helped reduce the accumulation of dental substrates (plaques and tartars) through mechanical abrasion. Some chewable products may also contain active agents that further help reduce dental calculus (hardened plaque) formation and prevent bad breath (2). In this study, a correlation between giving chews to dogs and the presence of *C. cynodegmi* was observed, as well as lesions in the oral cavity. The owners of the dogs participating in the present study were given oral chews to improve their dogs' oral health, which is why oral chews and the presence of *C. cynodegmi* could be connected in this investigation. In a study by Oba et al (32) a control group of dogs showed a lower prevalence of *Capnocytophaga* spp. Than in dogs fed chews, which indicated dental chews may positively affect the abundance of this group in the oral cavity. To the authors' knowledge, no studies were conducted to examine the impact of oral chew types on the prevalence of *C. cynodegmi* specifically. However, the findings of this study suggested that the type and frequency of chew use may significantly impact dogs' oral health and the prevalence of *C. cynodegmi*. On the other hand, consuming dry food positively impacted the presence of this bacterium (32). It can be concluded that the toughness of food and chewing items can affect the prevalence of *C. cynodegmi*, which can also be observed in present study due to correlations with giving chews to dogs.

C. canimorsus and *C. cynodegmi* have different strains, which can have different transferring potentials. Genetic analysis revealed that *C. canimorsus* can be classified into two main groups, with group I potentially being transmitted to humans and group II indigenous to the oral cavities of dogs and cats (46). The results of the previously mentioned study could prove that transmission of various strains of *Capnocytophag*a spp. are different. The results obtained in this research may suggest that direct contact between animals, or even those sharing bowls or toys, is insufficient to transfer bacteria from one dog to another. This process is crucial for the possibility of transferring bacteria from dogs to humans. However, this requires and more detailed research to understand this issue in its entirety.

The current study had some limitations. The first was a small group of positive *C. canimorsus* samples. Although, based on various studies, we could expect correlations between the presence of *C. canimorsus* and the survey results, the group of positive samples was too small to yield statistically significant results. Another limitation of the study was the small amount of DNA in swabs, especially in samples taken from saliva, not directly from teeth. During PCR, a few bands were weak compared to the positive control, which indicated that some of the positive samples could be omitted due to the weak band.

In conclusion, the study underscores the connection between the oral cavity condition of dogs in the Wroclaw population, the material collected directly from their teeth, and the administration of oral chews. These factors were found to be linked to the presence of *C. cynodegmi*. *Capnocytophaga* seems to be an important part of the oral microbiome of dogs in both the healthy and sick phases. Despite these promising results, further research is essential to explore additional parameters influencing the prevalence of *Capnocytophaga* spp. comprehensively.

References

1. *Ashley P. A., Moreno D. A., Yamashita S. K.*: Capnocytophaga canimorsus aortitis in an immunocompetent host. Hawaii. J. Health. Soc. Welf. 2020, 79, 324-325.
2. *Barbosa E., Pires P. G. S., Hauptli L., Moraes P.*: Strategies to improve the home care of periodontal disease in dogs: A Systematic Review. Res. Vet. Sci. 2023, 154, 8-14, doi: 10.1016/j.rvsc.2022.10.025.
3. *Barry I., Sieunarine K., Bond R.*: Ruptured mycotic common iliac aneurysm due to Capnocytophaga canimorsus, acquired from dog saliva: A case report. Int. J. Surg. Case Rep. 2021, 78, 12-15, doi: 10.1016/j.ijscr.2020.11.149.
4. *Berndsen R. H. A., Hulshof P. B. J. E., van Meer M. P. A., Saleem B. R., Scholtes V. P. W., The R. M., Jongkind V., Yeung K. K.*: Capnocytophaga canimorsus mycotic aortic aneurysm after a dog bite. EJVES Vasc. Forum. 2022, 55, 64-67, doi: 10.1016/j.ejvsvf.2022.04.005.
5. *Boer M. G. J. de, Lambregts P. C. L. A., Dam A. P. van, Wout J. W. van't*: Meningitis caused by Capnocytophaga canimorsus: When to expect the unexpected. Clin. Neurol. Neurosurg. 2007, 109, 393-398, doi: 10.1016/j.clineuro.2007.02.010.
6. *Brenton C. Le, El Braidy B., Durup F., Garot J.*: Bobo-Newton acute myocarditis caused by Capnocytophaga canimorsus: a case report. Eur. Heart. J. Case Rep. 2023, 7, 1-4, doi: 10.1093/ehjcr/ytad209.
7. *Butler T.*: Capnocytophaga canimorsus: an emerging cause of sepsis, meningitis, and post-splenectomy infection after dog bites. Eur. J. Clin. Microbiol. Infect. Dis. 2015, 34, 1271-1280, doi: 10.1007/s10096-015-2360-7.
8. *Dam A. P. Van, Weert A. Van, Harmanus C., Hovius K. E., Claas E. C. J., Reubsaet F. A. G.*: Molecular characterization of Capnocytophaga canimorsus and other canine Capnocytophaga spp. and assessment by PCR of their frequencies in dogs. J. Clin. Microbiol. 2009, 47, 3218-3225, doi: 10.1128/JCM.01246-09.
9. *Dilegge S. K., Edgcomb V. P., Leadbetter E. R.*: Presence of the oral bacterium Capnocytophaga canimorsus in the tooth plaque of canines. Vet. Microbiol. 2011, 139, 437-455, doi: 10.1016/j.vetmic.2010.12.010.
10. *Fennel A. G., Wilson K. S., Caja K. R., Parikh P. M.*: Capnocytophaga canimorsus meningitis in a 38-year-old immunocompetent woman from household

pet exposure. Am. J. Emerg. Med. 2022, 56, 396.e1-396.e3, doi: 10.1016/j.ajem.2022.03.043.
11. *Galles A., Monlun E., Villeneuve L., Poirot-Mazères S.*: Méningite à Capnocytophaga canimorsus. Med. Mal. Infect. 2020, 50, 754-756, doi: 10.1016/j.medmal.2020.09.001.
12. *Goetzinger J. C., LaGrow A. L., Shibib D. R., Thind S. K.*: Capnocytophaga canimorsus bloodstream infection associated with an urticarial exanthem. Case Rep. Infect. Dis. 2021, 9932170, doi: 10.1155/2021/9932170.
13. *Hansen M., Crum-Cianflone N. F.*: Capnocytophaga canimorsus meningitis: diagnosis using Polymerase Chain Reaction Testing and systematic review of the literature. Infect. Dis. 2019, 8, 119-136, doi: 10.1007/s40121-019-0233-6.
14. *Harrigan A., Murnaghan K., Robbins M.*: Double valve infective endocarditis due to Capnocytophaga canimorsus. IDCases. 2022, e01448, doi: 10.1016/j.idcr.2022.e01448.
15. *Holt C. S.*: Capnocytophaga, [in:] Whitman B. W. (ed): Bergey's Manual of Systematics of Archaea and Bacteria. Publisher John Wiley & Sons, Inc. 2015, doi: 10.1002/9781118960608.gbm00299.
16. *Hundertmark M., Willians T., Vogel A., Motitz M., Bramlage P., Pagonas N., Ritter O., Sasko B.*: Capnocytophaga canimorsus as cause of fatal sepsis. Case Rep. Infect. Dis. 2019, 3537507, doi: 10.1155/2019/3537507.
17. *Kelly B. C., Constantinescu D. S., Foster W.*: Capnocytophaga canimorsus periprosthetic joint infection in an immunocompetent patient: A Case Report. Geriatr. Orthop. Surg. Rehabil. 2019, 10, 1-2, doi: 10.1177/2151459318825199.
18. *Killington K., Lee N., Asher R., Farrant O., Stnoe N.*: Purpura fulminans secondary to Capnocytophaga canimorsus bacteraemia following a dog bite: A case report and review of literature. Access Microbiol. 2023, 5, 000505.v3, doi: 10.1099/acmi.0.000505.v3.
19. *Kotrashetti V., Idate U., Bhat K., Kulkarni R., Kugaji M., Kumbar V.*: Comparison of culture and Polymerase Chain Reaction – restriction fragment length polymorphism for identification of various Capnocytophaga species from subgingival plaque samples of healthy and periodontally diseased individuals. J. Oral Maxillofac. Pathol. 2022, 26, 287, doi: 10.4103/jomfp.jomfp_172_21.
20. *Lai C. H., Lin Y. S., Wang C. M., Chang P. C., Shia W. Y.*: A novel 16S RRNA PCR-Restriction Fragment Length Polymorphism Assay to accurately distinguish zoonotic Capnocytophaga Canimorsus and C. Cynodegmi. Microbiol. Spectr. 2023, 11, e0291622, doi: 10.1128/spectrum.02916-22.
21. *Lam J. H., Horvath R., Amodeo M.*: Culture-negative Capnocytophaga canimorsus meningitis diagnosed by 16s ribosomal RNA polymerase chain reaction in an immunocompetent veterinarian and a review of the literature. Int. Med. J. 2023, 53, 1054-1057, doi: 10.1111/imj.16110.
22. *Leadbetter E. R.*: The genus Capnocytophaga, [in:] Dworkin M., Falkow S., Rosenberg E., Schleifer K.-H., Stackebrandt E. (eds) The Prokaryotes. A Handbook on the Biology of Bacteria. Vol. 7: Proteobacteria: Delta and Epsilon Subclasses. Deeply Rooting Bacteria. Springer New York, NY, 2006, doi: 10.1007/0-387-30747-8.
23. *Linden S., Gilje P., Tham J., Lindstedt S., Rasmussen M.*: Capnocytophaga canimorsus tricuspid valve endocarditis. IDCases. 2021, 24, e01083, doi: 10.1016/j.idcr.2021.e01083.
24. *Lisjak A., Correa Lopes B., Pilla R., Nemec A., Suchodolski J., Tozon N.*: A comparison of the oral microbiota in healthy dogs and dogs with oral tumors. Animals 2023, 13, 3594, doi: 10.3390/ani.13233594.
25. *Martines D. L., Perez B. H., Seoane M. S. S., Gonzales P. G.*: Bacterial peritonitis secondary to Capnocytophaga canimorsus. Rev. Esp. Enferm. Dig. 2024, 116, 228-229, doi: 10.17235/reed.2023.9571/2023.
26. *Nakayama R., Miyamoto S., Tawara T., Aoyagi A., Oguro T., Kobayashi N., Suzuki M., Takeyama Y.*: Capnocytophaga canimorsus infection led to progressively fatal septic shock in an immunocompetent patient. Acute. Med. Surg. 2022, 9, e738, doi: 10.1002/ams2.738.
27. *Naoussi F., De Neef A., Gilbert A., Résimont G., Weekers L., Misset B.*: Complication méconnue des morsures canines: syndrome hémolytique et urémique suite à une bactériémie à Capnocytophaga Canimorsus. Rev. Med. Liege. 2022, 77, 551-556.
28. *Nisi F., Dipasquale A., Constantini E., Giustiniano E., Ripani U., Cecconi M.*: Surviving Capnocytophaga Canimorsus septic shock: intertwining a challenging diagnosis with prompt treatment. Diagnostics 2022, 12, 260, doi: 10.3390/diagnostics12020260.
29. *Nogueira B. S., Martini A. de C., Menezes I. de G., Souza R. L. de, Maruyama F. H., Rodrigues J. Y., Nakazato L., Dutra V.*: Detection of Capnocytophaga canimorsus and Capnocytophaga cynodegmi in dogs with periodontal disease of Brazil. Res. Soc. Dev. 2021, 10, e245101321146, doi: 10.33448/rsd-v10i13.21146.
30. *Oba P. M., Carroll M. Q., Alexander C., Somrak A. J., Keating S. C. J., Sage A. M., Swanson K. S.*: Dental chews positively shift the oral microbiota of adult dogs. J. Anim. Sci. 2021, doi: 10.1093/jas/skab100.
31. *Oba P. M., Carroll M. Q., Alexander C., Valentine H., Somrak A. J., Keating S. C. J., Sage A. M., Swanson K. S.*: Microbiota populations in supragingival plaque, subgingival plaque, and saliva habitats of adult dogs. Anim. Microbiome 2021, 3, doi: 10.1186/s42523-021-00100-9.
32. *Oba P. M., Sieja K. M., Keating S. C. J., Hristova T., Somrak A. J., Swanson K. S.*: Oral microbiota populations of adult dogs consuming wet or dry foods. J. Anim. Sci. 2022, doi: 10.1093/jas/skac200.
33. *O'Riordan F., Ronayne A., Jackson A.*: Capnocytophaga canimorsus meningitis and bacteraemia without a dog bite in an immunocompetent individual. BMJ Case Rep. 2021, 12, e242432, doi: 10.1136/bcr-2021-242432.
34. *Parisi X., Pohan G.*: Purpura fulminans due to Capnocytophaga canimorsus. Br. J. Haematol. 2023, 202, 455, doi: 10.1111/bjh.18837.
35. *Prasil P., Ryskova L., Plisek S., Bostik P.*: A rare case of purulent meningitis caused by Capnocytophaga canimorsus in the Czech Republic – case report and review of the literature. BMC Infect, Dis. 2020, 20, 100, doi: 10.1186/s12879-020-4760-2.
36. *Peeters M., Pelzer J., Yazar O., Salemans P., Wong C., Decraemer G., Bouwman L.*: Fatal sepsis with peripheral necrosis due to Capnocytophagus canimorsus infection after dog bite: a case report. Ann. Med. Surg. 2023, 85, 982-985, doi: 10.1097/MS9.0000000000000280.
37. *Peterson Ch. J., Abel W. F., Reedy V. P. K.*: Furry, fomite, and facultative anaerobe: a unique case of Capnocytophaga canimorsus. Cerreus 2023, 15, e47747, doi: 10.7759/cereus.47747.
38. *Ruparell A., Warren M., Staunton R., Deusch O., Dobenecker B., Wallis C., O'Flynn C., McGenity P., Holcombe L. J.*: Effect of feeding a daily oral care Chew on the composition of plaque microbiota in dogs. Res. Vet. Sci. 2020, 132, 133-141, doi: 10.1016/j.rvsc.2020.05.001.
39. *Schuler F., Padberg J.-S., Hullermann C., Kümpers P., Lepper J., Schulte M., Uekötter A., Schaumburg F., Kahl B. C.*: Lethal Waterhouse-Friderichsen syndrome caused by Capnocytophaga canimorsus in an asplenic patient. BMC Infect.Dis. 2022, 22, 696, doi: 10.1186/s12879-022-07590-1.
40. *Sri A., Droscher E., De Palma R.*: Capnocytophaga canimorsus and infective endocarditis-making a dog's dinner of the aortic valve: a case report. Eur. Heart. J. Case Rep. 2021,5, 1-6, doi: 10.1093/ehjcr/ytab278.
41. *Suzuki M., Kimura M., Imaoka K., Yamada A.*: Prevalence of Capnocytophaga canimorsus and Capnocytophaga cynodegmi in dogs and cats determined by using a newly established species-specific PCR. Vet. Microbiol. 2010, 144, 172-176, doi: 10.1016/j.vetmic.2010.01.001.
42. *Tabatabaei M., Rouhani H. R.*: Polymerase Chain Reaction detection of Capnocytophaga canimorsus and Capnocytophaga cynodegmi as the emerging zoonosis. Avicenna J. Clin. Microbiol. Infect. 2019, 6, 21-25, doi: 10.34172/ajcmi.2019.05.
43. *Tanabe K., Okamoto S., Asano S. H., Wada J.*: Capnocytophaga canimorsus peritonitis diagnosed by mass spectrometry in a diabetic patient undergoing peritoneal dialysis: a case report. BMC Nephrol. 2019, 20, 219, doi: 10.1186/s12882-019-1415-x.
44. *Terashima S., Hayakawa K., Saito F., Wada D., Iwamura H., Kuro, A., Ozaki Y., Nakamori Y., Ishii K., Kuwagata Y.*: Hemophagocytic syndrome with severe sepsis caused by Capnocytophaga canimorsus. Am. J. Emerg. Med. 2020, 38, 1540.e5-1540.e8, doi: 10.1016/j.ajem.2020.03.017.
45. *Uçkay I., Stirnemann J.*: Exanthema associated with Capnocytophaga canimorsus bacteremia. Int. J. Infect. Dis. 2019, 82, 104-105, doi: 10.1016/j.ijid.2019.03.006.
46. *Umeda K., Hatakeyama R., Abe T., Takakura K., Wada T., Ogasawara J., Sanada S., Hase A.*: Distribution of Capnocytophaga Canimorsus in dogs and cats with genetic characterization of isolates. Vet. Microbiol. 2014, 171, 153-159, doi: 10.1016/j.vetmic.2014.03.023.
47. *Vecilla D. F., Muro F. E. C., Renzi F., de Tuesta del Arco1 J. L. D.*: Sepsis por Capnocytophaga canimorsus en un paciente inmunocompetente. Rev. Esp. Quimioter. 2022, 35, 304-306, doi: 10.37201/req/006.2022.
48. *Vecilla D. F., Vivanco C. A., López I. A., Artetxe J. M. B.-E., Renzi F., de Tuesta Del Arco J. L. D.*: A case of septic arthritis caused by Capnocytophaga canimorsus in an HIV patient. Access Microbiol. 2022, 4, acmi000368, doi: 10.1099/acmi.0.000368.
49. *Wallis C., Marshall M., Colyer A., O'Flynn C., Deusch O., Harris S.*: A longitudinal assessment of changes in bacterial community composition associated with the development of periodontal disease in dogs. Vet. Microbiol. 2015, 181, 271-282, doi: 10.1016/j.vetmic.2015.09.003.
50. *Wareham D. W., Michael J. S., Warwick S., Whitlock P., Wood A., Das S. S.*: The dangers of dog bites. J. Clin. Pathol. 2006, 60, 328-329, doi: 10.1136/jcp.2006.037671.
51. *Wendt R., Schauff Ch., Lübbert Ch.*: An asplenic with life-threatening Capnocytophaga canimorsus sepsis. IDCases. 2020, 21, e00828, doi: 10.1016/j.idcr.2020.e00828.
52. *Wildman E. C., Brockman N., Meatherall B.*: A case report of Capnocytophaga canimorsus meningitis with failure of ceftriaxone therapy. J. Assoc. Med. Microbiol. Infect. Dis. Can. 2024, 9, 46-51, doi: 10.3138/jammi-2023-0003.
53. *Yang M. C., Ling J., Mosaed S.*: Capnocytophaga canimorsus blebitis: case report and review of literature. BMC Ophthalmol. 2021, 21, 59, doi: 10.1186/s12886-021-01823-8.
54. *Zangenah S., Abbasi N., Andersson A. F., Bergman P.*: Whole genome sequencing identifies a novel species of the genus Capnocytophaga isolated from dog and cat bite wounds in humans. Sci. Rep. 2016, 6, doi: 10.1038/srep22919.

Corresponding author: Zuzanna Czekaj, Student Science Club of Animal Infectious Diseases "AnthraX", Department of Epizootiology and Clinic of Birds and Exotic Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, pl. Grunwaldzki 45, 50-366 Wroclaw, Poland; e-mail: 115804@student.upwr.edu.pl; malgorzata.klimowicz-bodys@upwr.edu.pl