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Characteristics of *Capnocytophaga canimorsus* and *Capnocytophaga cynodegmi* in the Dog Population

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Simple Summary: *Capnocytophaga* spp. constitute an important part of the oral microbiome of dogs, both healthy and those suffering from oral and periodontal diseases. This microorganism can pose a threat to human health and life if bitten by a cat or dog. Currently, the factors influencing the occurrence of bacteria in the oral cavity of companion animals are not fully known. For our research, we used oral swabs from dogs that were patients of a university veterinary clinic. We then used the PCR technique to identify microorganisms. When analyzing our results, we took into account various factors related to oral health to estimate the potential occurrence of the *Capnocytophaga* microorganism. Our study highlights the relationship between dogs' oral health, material taken directly from their teeth, and the administration of oral chews. These factors were associated with the presence of *Capnocytophaga cynodegemi*. Despite these promising results, further research is needed to investigate additional parameters that influence the incidence of *Capnocytophaga* spp. in a comprehensive way.

Abstract: Background: 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. Methods: 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. Results: The study highlighted significant associations between the presence of *Capnocytophaga cynodegmi* and oral cavity condition, material collection method, and use of dog's chews. Conclusions: Correlations obtained in this study between the oral health of Wroclaw's dog population and the presence of *Capnocytophaga 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; molecular diagnostic

1. Introduction

Capnocytophaga spp. are gram-negative bacteria that belong to the *Flavobacteriaceae* family [1–3]. These slow-growing rods (coccoid and bacillary) include species such as *Capnocytophaga canimorsus* and *Capnocytophaga cynodegmi*, which are part of the oral microbiome but also zoonotic pathogens [2–

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4]. This study aimed to evaluate the possible factors influencing the prevalence of *Capnocytophaga canimorsus* and *Capnocytophaga cynodegmi* in the dog population in Wroclaw, Poland. A total of 105 swabs were taken from dogs for molecular detection of the *Capnocytophaga* genus. All the samples were analyzed 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. Despite the small size of the study group, the study highlighted that in the Wroclaw population of dogs, the conditions of the dog's oral cavities, the collection of material directly from the teeth, and the administration of oral chews to the dog were associated with the presence of *Capnocytophaga cynodegmi.* Due to the limitations of the study, such as the small sample size, additional studies are needed to fully understand the effects of additional parameters on the prevalence of *Capnocytophaga* spp.

2. Materials and Methods

2.1. Study Population and Sample Procedures

In the preliminary study, a veterinarian collected 20 swabs from dogs with FLOQSwabs® 553C (COPAN, Brescia, Italy) and placed them on Columbia blood agar plates based on culture and growth conditions [5]. No growth was observed after the material was incubated at 37°C for 48 and 72 hours with 5% CO_2.

In the main study, 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. A total of 105 swabs were taken from dogs in the city of Wroclaw. 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.

2.2. Isolation and Identification of Capnocytophaga spp.

Swabs from the preliminary study were lightly inoculated onto blood agar and chocolate agar and cultured in a modified atmosphere, according to the literature. Interestingly, none of the cultured colonies exhibited morphological characteristics similar to those reported in the literature for C*apnocytophaga* colonies. Despite this, it was decided to isolate the genetic material by cooking. The DNA suspension was subsequently frozen for further use in PCR. Due to the difficulties in obtaining positive culture results, a freeze-dried reference strain of *Capnocytophaga canimorsus* was selected as a positive sample for bacteriological testing. Unfortunately, no culture, either in solid or liquid media, was successful, confirming the difficulties in cultivating bacteria of this type, which is consistent with the literature. PCR revealed no positive results.

On the basis of the results of the preliminary study, the methods were changed. DNA was isolated directly from the newly collected swabs using a Genomic Mini commercial kit (A&A Biotechnology, Gdansk, Poland) and via classic cultivation methods. 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. Based on the study by Tabatabaei et al., the *Capnocytophaga* spp., *Capnocytophaga canimorsus*, and *Capnocytophaga cynodegmi* gene primers (Genomed S.A., Warsaw, Poland), shown in Table 1, were used for reactions at 0.4 µM concentrations [6].

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Table 1. Primers used in the study.

Bacterium	Primer 1	Primer 2
Capnocytophaga genus; CAL2-AS2	CAL2: 5′GTAGAGTGCTTCGGCACTTG3′	AS2: 5′GTGATGCCACCAAACAATACTA3′
C. canimorsus; CAL2-CaR	CAL2: 5′GTAGAGTGCTTCGGCACTTG3′	CaR: 5′GCCGATGCTTATTCATACA3′
C. cynodegmi; CAL2-CyR	CAL2: 5′GTAGAGTGCTTCGGCACTTG3′	CyR: 5′GCGGATGCTTATTCGTATG3′

The PCR products were subsequently subjected to electrophoresis on a 2% agarose gel (BASICA LE Agarose; Prona, Burgos, Spain) colored with Midori Green DNA Stain (Nippon Genetics Europe GmbH, Düren, Germany). Unfortunately, all the results were negative, except for the positive commercial control. Due to the lack of other positive results, which seemed impossible, a decision was made to modify the PCR mixture by changing the concentration of the reagents involved in the reaction. After the number of primers was increased, positive samples were obtained.

2.3. Statistical Analysis

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

3. Results

A total of 105 samples were taken from 2022 to 2023 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 2.

Table 2. 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

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Giving chews to dog	Yes	68.49	62.50
(%)	No	31.51	37.50

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 changes in the oral cavity and those without changes. The prevalence of *Capnocytophaga* bacteria in this group is shown in Figure 1.

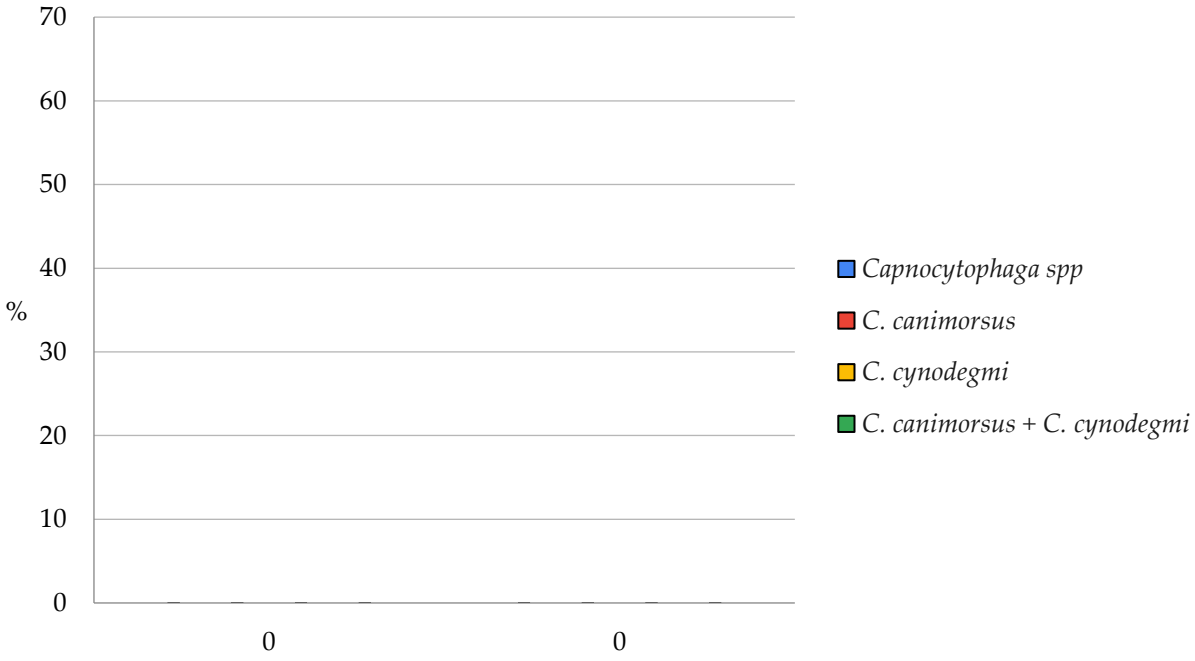

Figure 1. Prevalence of *Capnocytophaga* species in healthy and sick dogs.

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 changes (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 changes in the oral cavity in dogs ($p \leq 0.05$, Fisher's exact test), collecting material directly from the teeth ($p \leq 0.05$, Fisher's exact test), and chewing for dogs ($p \leq 0.05$, Fisher's exact test). No significant differences in the presence of *C. cynodegmi* were detected for underlying conditions, antibiotic use up to 6 months prior to examination, age, or medication use. A correlation ($p \leq 0.05$) was also detected between the presence of *C. canimorsus* and *C. cynodegmi*, using Fisher's exact test.

As a result of the comparison of the presence of *C. canimorsus* and *Capnocytophaga* spp., no significant differences were detected 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 changes in the oral cavity in dogs.

This study included households with more than one dog, the results of which are presented in Table 3. Interestingly, the results obtained did not reach statistical significance. However, among the 16 houses with multiple dogs analyzed, all the dogs tested positive for the *Capnocytophaga* gene in their oral cavities in only four of them. Of these four cases, in three homes, all dogs tested positive for *C. canimorsus*, while in one of them, the presence of *C. cynodegmi* was found.

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Table 3. 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)

4. Discussion

This research conducted a preliminary study to review the study strategy and examine the classic bacterial isolation method. According to a report by Hansen and Crum-Cianflone, blood cultures were positive in 54% of 37 patients analyzed, with a median growth time of four days [7]. One study suggested that PCR could detect the presence of *Capnocytophaga* in cases whether the cultures were negative or not. The percentage of positive cultures and the time required for the cultures to become positive indicate the time required for PCR to establish the diagnosis of *Capnocytophaga* spp.

Various studies conducted in veterinary medicine have shown that the prevalence of *Capnocytophaga canimorsus* is 70–74%, and that of C. *cynodegmi* is 86–96% [8]. In this investigation, the prevalence was similar to that in the study by Nogueira et al., in which 19% of the samples were positive for *C. canimorsus* and 66.94% for *C. cynodegmi* [9]. Presumably, if the material in this study was multiplied, which could increase the concentration of isolated DNA, more positive results could be obtained.

Research by Oba et al. indicated that the presence of the genus *Capnocytophaga* is linked to a decline in oral health, which was also confirmed by the results obtained for *C. cynodegmi* [10]. Nogueira et al. discovered that the prevalence of *C. cynodegmi* is greater in dogs with a high incidence of periodontal disease [9]. However, in the abovementioned research, there was no significant association between *C. canimorsus* and periodontal disease, as shown in the results of this study. The

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promising results obtained in this study suggest a possible 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, Wallis et al. reported that the concentration of *Capnocytophaga* spp. COT-339 decreased over time in patients with progressive periodontitis in dogs, and the concentration of *Capnocytophaga cynodegmi* COT-254 decreased in both the progression group and the group without progression [11]. However, the references mentioned do 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 fully elucidated, because some studies indicate that *C. cynodegmi* is a key microbiota constituent of healthy dogs with a high prevalence, but it is also found in many progressive stages of periodontal disease, with the exception of the initial stages [9,11–13].

Oba et al. discovered that the oral environment influences the presence of bacteria; the prevalence of *Capnocytophaga* was greater in subgingival and supragingival plaques than in saliva [14]. In the dogs examined, more positive results for *C. cynodegmi* were also observed in swabs collected from the teeth surrounding the area.

A study by Ruparell et al. showed that daily oral care chews influence the composition of the plaque microbiota in dogs [15]. Barbosa et al. also reported that some dog foods reduce dental plaque build-up and gingival inflammation and that chews help reduce the accumulation of dental substrates (plaques and tartars) through mechanical abrasion [16]. Some chewable products may also contain active agents that further help reduce dental calculus (hardened plaque) formation and prevent bad breath. In this study, a correlation was observed between the chewing of dogs and the presence of *C. cynodegmi*, as well as changes in the oral cavity in dogs. The owners of the dogs included in the investigation 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 study. In a study by Oba et al., a control group of dogs showed a lower presence of *Capnocytophaga* spp. than dogs fed chews, indicating that dental chews may positively affect the abundance of this group in the oral cavity [14]. To the authors' knowledge, no studies have been conducted to specifically examine the impact of oral chew type on the prevalence of *C. cynodegmi*. However, the findings of this study suggest that the type and frequency of chewing can significantly impact dogs' oral health and the prevalence of *C. cynodegmi*. Oba et al. showed that eating dry food positively impacted the presence of *C. cynodegmi* [10]. 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 this study due to correlations with giving chews to dogs.

Different strains of *Capnocytophaga canimorsus* and *C. cynodegmi* can have different transfer potentials. Genetic analysis by Umeda et al. revealed that *C. canimorsus* can be classified into two main groups, with group I potentially transmitted to humans and group II indigenous to the oral cavities of dogs and cats [17]. The results of the present study demonstrated that the transmission of various strains of *Capnocytophaga* spp. differs. The results obtained in this research may suggest that direct contact between animals, or even those sharing bowls or toys, is insufficient for transferring bacteria from one dog to another. This process is crucial for the possibility of transferring bacteria from dogs to humans. However, further and more detailed research is needed to fully understand this issue.

The current study has several limitations. The first is a small group of positive samples of *C. canimorsus*. 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 those of the positive control, which indicated that some of the positive samples could be omitted due to the weak band.

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5. Conclusions

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 related to the presence of *Capnocytophaga cynodegemi*. *Capnocytophaga* looks like an essential part of the oral microbiome of dogs in both the healthy and sick phases. Despite these promising results, further research is essential for exploring additional parameters that influence the prevalence of *Capnocytophaga* spp. in a comprehensive way.

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Institutional Review Board Statement: The research project was submitted to the Animal Welfare Advisory Team. Due to the non-invasive sample collection procedure, the Animal Welfare Advisory Team qualified the study as research, which did not require any further approval from the Ethics Committee. Each dog owner consented to participate in this study and provided proper documentation.

Informed Consent Statement: Not applicable

Data Availability Statement: All the data generated or analysed during this study are included in this published article.

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