

Prevalence of *Capnocytophaga canimorsus* in dogs in relation to the severity of periodontal disease

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Abstract

Capnocytophaga canimorsus is a bacterium found in the oral cavity of dogs, with a well-documented zoonotic potential. The aim of this study was to assess the prevalence of *C. canimorsus* in dogs at different stages of periodontal disease. This was designed as a prospective clinical study. A total of 84 dogs were enrolled and classified into three groups based on clinical examination and the most severely affected tooth according to the stage of periodontitis: dogs with healthy periodontal status, dogs with mild periodontal disease (PD₁ and PD₂), and dogs with moderate-to-advanced periodontal disease (PD₃ and PD₄). The presence of *C. canimorsus* was detected using rtPCR from oral swab samples. Chi-squared test ($P < 0.05$) was used to assess differences in the frequency of *C. canimorsus*-positive cases between groups. The overall prevalence of *C. canimorsus* among the examined dogs was 71%. A significantly higher occurrence of *C. canimorsus* was observed in group of dogs with PD₁ and PD₂ compared to group with a healthy periodontium ($P = 0.0452$) and those with PD₃ and PD₄ ($P = 0.0318$). No significant differences were found between dogs with healthy periodontal status and those with PD₃ and PD₄. These findings suggest that *C. canimorsus* may play an important role in the early stages of periodontal disease pathogenesis, or alternatively, its presence may reflect natural shifts in microbial composition across different stages of periodontitis. Further studies are warranted to clarify its exact role in disease progression.

Canine oral health, microbiota, veterinary dentistry

Periodontitis is the most commonly diagnosed oral disease in dogs. In the vast majority of cases, it progresses subclinically for an extended period, often delaying the initiation of therapy. Progressive inflammation can result not only in local but also systemic consequences (Niemić 2008).

The development of periodontitis is multifactorial, with the composition of the oral microbiota playing an important role. Primary pathogens include *Porphyromonas gulae* and *Tannerella forsythia* (Di Bello et al. 2014; Niemić et al. 2021; Yasuda et al. 2024).

Capnocytophaga canimorsus is a bacterium capable of causing severe zoonotic infections in humans, typically following bites or other forms of exposure to canine saliva (Gaastra and Lipman 2010). The presence of *Capnocytophaga* species in the canine oral cavity has been repeatedly documented (Oba et al. 2021; Nogueira et al. 2021). Although *C. cynodegmi* has been linked to different stages of periodontal disease, a similar association has not yet been demonstrated for *C. canimorsus* (Nogueira et al. 2021).

The aim of this study was to assess the prevalence of *C. canimorsus* in dogs at different stages of periodontitis and to clarify its potential role in the disease's aetiopathogenesis.

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Based on previous studies demonstrating shifts in the oral microbiome during the progression of periodontal disease (Reiter 2018; Santibáñez et al. 2021), we hypothesize that the prevalence of *C. canimorsus* may differ according to the severity of periodontitis.

Materials and Methods

Study design

This prospective clinical study was performed in accordance with the current law on animal protection and the consent of Ethics Committee of the University of Veterinary Sciences Brno. All animal owners provided informed consent to have their animals participate in the study.

Study animals and inclusion criteria

Dogs enrolled in the study were patients of the Small Animal Clinic, Faculty of Veterinary Medicine, University of Veterinary Sciences Brno. Clinically healthy status was confirmed based on history, general clinical examination, and haematological and biochemical analysis of venous blood. All dogs fasted for 12 h with free access to water. Sample collection was performed under general anaesthesia following a full oral examination, in accordance with American Veterinary Dental College (AVDC) guidelines. Following the clinical examination and dental cleaning, all dogs underwent a complete occlusal radiographic examination. Dogs for which full dental treatment was not performed and, consequently, complete occlusal radiographs were unavailable were included only if they met one of the following criteria: (i) animals younger than two years with at most mild signs of gingivitis or early periodontitis (Wallis et al. 2019; Yasuda et al. 2024), or (ii) older animals with clinical evidence of at least one tooth affected by moderate or severe periodontitis (Santibáñez et al. 2021).

Procedure

Sterile swabs were used to collect samples by sweeping along the buccal gingival margin from molar 209 to molar 109 and from molar 309 to molar 409. Samples included material from gingival tissue, tooth surfaces (with dental plaque when present), and saliva. Full-mouth radiographs were obtained for all dogs, and the stage of periodontitis was determined based on combined clinical and radiographic assessment.

All procedures were performed in accordance with standard veterinary practice and did not impose any additional stress or discomfort on the animals.

Samples

For each sample, two sterile swabs were collected and immediately placed into Amies transport medium (Amies transport medium, Copan Italia S.p.A., Brescia, Italy; REF 114C; Amies transport medium, Syntesys Disposable Labware, Helsinki, Finland; REF 313521). Samples were stored at 4 °C for a maximum of 14 days prior to transport to the laboratory for microbiological analysis to detect *C. canimorsus*.

Patient classification

Patients were assigned to groups based on the stage of periodontal disease, following the criteria described by Kirby and Miller (2018), which align with the classification system of AVDC. Classification was determined according to the most severely affected tooth within the entire dentition. Based on this assessment, dogs were categorized into groups corresponding to the respective stages of periodontal disease (Table 1; Kirby and Miller 2018).

Table 1. Classification of patients according to the severity of periodontal disease (according to Kirby and Miller 2018).

Healthy periodontal status	PD ₀	Clinically normal	- Attachment loss: none - Radiographic changes: none
Mild periodontal disease	PD ₁	Gingivitis only without attachment loss	- Attachment loss: none - Radiographic changes: none
	PD ₂	Early periodontitis	- Less than 25% of attachment loss or stage 1 furcation involvement in a multi-rooted tooth - Early radiographic signs of bone loss
Moderate-to-advanced periodontal disease	PD ₃	Moderate periodontitis	25–50% attachment loss or stage 2 furcation involvement in a multi-rooted tooth - Moderate radiographic signs of bone loss
	PD ₄	Advanced periodontitis	- More than 50% attachment loss or stage 3 furcation involvement in a multi-rooted tooth - Advanced radiographic signs of bone loss

DNA extractions

Samples were collected in the form of smears taken from the dogs' gingiva and teeth and were stored in Amies medium. These swabs were used for DNA extraction using Genomic DNA Mini Kit (Tissue) (Geneaid Biotech Ltd., New Taipei City, Taiwan) following manufacturer's instructions. Extracted DNA samples were stored at -20°C for further use.

rtPCR

The reaction mixture for rtPCR was prepared in 20 μl volume per sample and contained 12 μl LightCycler[®] 480 SYBR Green I Master (Roche, Basel, Switzerland), 4 μl ddH₂O, 1 μl of forward primer, 1 μl of forward reverse primer (Suzuki et al. 2010), and 2 μl of template DNA. Samples known to be culture positive for *C. canimorsus* were used as positive control, while above mentioned reaction mixture containing 2 μl of ddH₂O instead of DNA was used as negative control. The amplification included 40 cycles of: denaturation at 95°C for 20 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. LightCycler[®] 480 (Roche, Basel, Switzerland) was used for amplification.

Statistical analysis

Statistical analysis was performed using statistical software UNISTAT version 6.5 (London, UK). As part of the analysis, the frequency of PCR positive and negative samples between individual groups were compared. Main analysis was performed using Pearson's chi square test, sub-analyses were performed using chi square test with Yates correction. The significance level was set at 0.05.

Results

The study included a total of 84 dogs representing 30 breeds, 33 males (22 intact, 11 castrated) and 51 females (36 intact, 15 spayed), aged 4.9 ± 4.3 years and weighing 19.3 ± 16.1 kg. Of these, 16 dogs (19%) had healthy periodontal status, 41 dogs (49%) had PD₁ or PD₂, and 27 dogs (32%) had PD₃ or PD₄.

Capnocytophaga canimorsus was detected in 60 dogs (71%). Among positive dogs, 9 (15%) had a healthy periodontal status, 35 (58%) had mild periodontal disease, and 16 (27%) had moderate-to-advanced periodontal disease. Among negative dogs, 7 (29%) had a healthy periodontal status, 6 (25%) had mild periodontal disease, and 11 (46%) had moderate-to-advanced periodontal disease.

A significant difference in detection rates among the three periodontal status groups was observed ($P = 0.0216$). Dogs with mild periodontal disease had a significantly higher prevalence of *C. canimorsus* (85%) compared to dogs with a healthy periodontal status (56%; $P = 0.0452$) and those with moderate-to-advanced periodontal disease (59%; $P = 0.0318$). No significant difference ($P = 0.8467$) in the prevalence of *C. canimorsus* was observed between dogs with healthy periodontal status (59%) and dogs with moderate-to-advanced periodontal disease (56%).

Discussion

Capnocytophaga canimorsus is a slow-growing, Gram-negative bacterium that is a commensal of the oral cavity in dogs and cats and has confirmed zoonotic potential (Gaastra and Lipman 2010). Transmission to humans may occur through bites, scratches, or exposure to saliva, potentially resulting in severe systemic infections such as sepsis, meningitis, disseminated intravascular coagulation, or gangrene (Lion et al. 1996; Gaastra and Lipman 2010). Since the first reported human infection in 1976, approximately 200 additional cases have been documented (Macrea et al. 2008; Gaastra and Lipman 2010). Although the incidence of severe *C. canimorsus* infections is generally low (Pers et al. 1996; van Dam and Jansz 2011), mortality remains high, with fatal outcomes observed in approximately 26% of cases (Butler 2015). Clinical manifestations include sepsis with septic shock, meningitis, endocarditis, osteomyelitis, and pulmonary or intra-abdominal infections. Individuals with predisposing conditions such as asplenia, chronic alcoholism, or immunosuppression are typically affected, although up to 40%

of cases occur in previously healthy subjects (Lion et al. 1996; Gaastra and Lipman 2010). Hess et al. (2018) reported that approximately 91.8% of human infections are caused by capsular serovars A–C. Although these virulent serovars represent only 7.6% of isolates from dogs, heightened vigilance regarding their presence is warranted, particularly in dogs owned by individuals with predisposing risk factors.

Given the clinical severity of *C. canimorsus* infections in humans and the potential risk of transmission from companion animals, the present study aimed to assess its prevalence in dogs with varying stages of periodontitis. To date, this prevalence has been scarcely investigated, and unlike *C. cynodegmi* (Nogueira et al. 2021), an association with periodontitis has not been established. This study represents the first in a planned series designed to provide a comprehensive overview of the epidemiology and clinical relevance of this pathogen in dogs.

Inclusion criteria for dogs without full-mouth radiographs were based on previously published data. According to the longitudinal study by Wallis et al. (2019) which evaluated periodontitis progression in young Yorkshire Terriers, most teeth did not exceed PD₂ by 1.5 years of age, with only a single tooth reaching PD₃. These findings are corroborated by Yasuda et al. (2024), who reported that in small-breed puppies under one year, significant periodontal lesions (PD₁) were absent. From one year of age, initial signs of early periodontitis (PD₂) appeared, while from two years onward, moderate forms of the disease (PD₃) became more frequent. Advanced periodontitis (PD₄) was predominantly observed in dogs aged three years or older (Yasuda et al. 2024). Considering that small breeds are generally more predisposed to periodontitis than medium or large breeds (Harvey et al. 1994; Wallis et al. 2021), the risk of misclassifying young dogs of medium or large breeds as free of moderate-to-severe periodontitis is likely even lower. The second criterion is based on prior studies demonstrating that the oral microbiome of dogs undergoes changes even in the early stages of periodontal disease, with progressive shifts corresponding to lesion severity. Santibáñez et al. (2021) reported significant alterations in microbial populations in dogs with periodontitis, including increased abundance of anaerobic genera such as *Porphyromonas*, *Fusobacterium*, and *Bacteroides*, and decreased representation of *Proteobacteria* and *Actinobacteria* (Santibáñez et al. 2021). Based on these findings, we hypothesize that the presence of even a single tooth affected by advanced periodontitis can substantially influence the overall oral microbiota, justifying the inclusion of such individuals in the study.

Previous studies have also investigated the prevalence of *Capnocytophaga* species. Nogueira et al. (2021) reported a significantly higher prevalence of *C. cynodegmi* in dogs with advanced periodontitis (PD₄) compared to those with gingivitis only (PD₁). In contrast, no significant association was observed for *C. canimorsus* (Nogueira et al. 2021). Notably, their study did not include dogs with clinically healthy periodontium and classified patients solely based on the extent of periodontal damage, which may have influenced their statistical analysis and contributed to the differing outcomes relative to our findings.

Oba et al. (2021) analysed the oral microbiome in dogs, including saliva, supragingival, and subgingival plaque samples. The dogs included in their study did not present with severe periodontal disease. *Capnocytophaga* was found to be most abundant in supragingival plaque, and its prevalence was associated with a higher Oral Health Score, indicative of poorer oral health (Oba et al. 2021). In contrast, the present study focused specifically on *C. canimorsus* rather than the entire genus, and included dogs across different clinical stages of periodontitis. This design allows for a detailed assessment of the distribution of a single pathogen at various disease stages, providing novel insights.

The prevalence of *C. canimorsus* in dogs may be interpreted from two complementary perspectives. On one hand, this bacterium could represent a commensal of the canine oral

cavity, whose relative abundance varies according to the severity of periodontal disease. On the other hand, its presence might contribute causally to the progression of periodontitis, indicating a potential role in the disease's pathogenesis.

During the progression of gingivitis and periodontitis, the oral microbiome undergoes gradual shifts. In healthy dogs or those with early gingivitis, Gram-positive aerobic bacteria predominate, whereas maturation of dental plaque favours Gram-negative anaerobes (Reiter and Harvey 2010; Reiter 2018). Oxygen depletion in deeper plaque layers creates an anaerobic environment, which is likely mirrored in deep periodontal pockets, where bacterial metabolism shifts toward fermentation and anaerobic glycolysis (Santibáñez et al. 2021). *Capnocytophaga canimorsus* is a facultative anaerobe (Gaastra and Lipman 2010), and early stage periodontitis may provide a suitable niche for its proliferation. Its occasional detection prior to clinical signs may reflect early subclinical changes in the oral cavity. In contrast, lower prevalence in advanced periodontitis may result from dominance of other obligate anaerobes.

The possibility that *C. canimorsus* acts as a causal factor in the development of periodontal disease cannot be dismissed. Its higher prevalence in dogs with gingivitis or early periodontitis suggests that this microorganism may play a significant role during the initial phase of disease, contributing to its pathogenesis. A key question remains whether *C. canimorsus* functions as an independent periodontal pathogen or acts synergistically with other members of the oral microbiota. Based on the findings of Nogueira et al. (2021), it is also conceivable that the relative abundance of individual *Capnocytophaga* species shifts during disease progression, with each species potentially playing a distinct role at different stages of periodontitis.

This study has several limitations that should be considered when interpreting the results. The relatively small sample size ($n = 84$) limits the generalizability of the findings to the broader canine population. Additional potential bias may arise from the fact that the study population was drawn primarily from a single geographic region. Finally, the exclusive focus on *C. canimorsus* precludes a comprehensive assessment of the dynamics of the entire oral microbiome and its role in the pathogenesis of periodontal disease.

Capnocytophaga canimorsus is a significant component of the canine oral microbiome and exhibits a high prevalence in dogs with gingivitis or early periodontitis. The precise role of this bacterium in the pathogenesis of periodontal disease in dogs, particularly whether its presence exerts a significant causal influence on disease progression, warrants further investigation.

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