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Case study

A case-control study of periodontal diseases related to *Porphyromonas gingivalis* in cats

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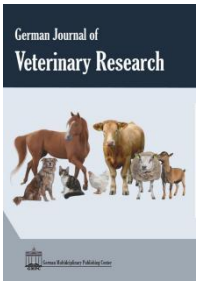

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Abstract

Porphyromonas (*P.*) *gingivalis* is a bacterium known to cause chronic periodontal disease in cats. However, no epidemiological studies have been conducted to assess the prevalence of periodontal diseases related to *P. gingivalis* in Indonesia. This study aimed to identify the factors influencing the development of periodontal diseases in cats associated with *P. gingivalis* infection. A total of 170 cats from the Yogyakarta special region in Indonesia were involved, divided into case and control groups. The case group included 85 cats with periodontal disease, while the control group included 85 cats without periodontal disease, all examined by veterinarians from 11 animal clinics and hospitals in the region. Dental plaque samples were collected using sterile cotton swabs, and *P. gingivalis* was identified using polymerase chain reaction (PCR). Based on PCR, 81/85 and 57/85 samples from the case and control groups, respectively, were tested positive. Several risk factors for periodontal disease were identified, including age >3 years (odds ratio [OR] 8.25; p=0.001; CI, 3.66–18.56), wet and dry feed combination (OR, 11.63; p= 0.001; CI, 5.63–23.99), dry food (OR, 0.13; p= 0.001; CI, 5.63–23.99) and presence of *P. gingivalis* in the teeth (OR, 9.95; p= 0.001; CI, 3.31–29.92). In contrast, feeding exclusively dry food was associated with a reduced risk of periodontal disease (OR 0.13; p=0.001; 95% CI, 5.63–23.99). These findings suggest that age >3 years, a combination of wet and dry feeding, and the presence of *P. gingivalis* are significant risk factors for periodontal disease in cats, while dry food feeding may reduce the risk of disease.

Keywords: Cat, Periodontal diseases, Risk factors, *Porphyromonas gingivalis*

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Introduction

Periodontal diseases are common infectious conditions in cats and are strongly associated with pathogenic bacteria. These periodonto-pathogenic microorganisms induce inflammatory responses in periodontal tissues, leading to damage and degradation of the affected areas (Özavci et al., 2019; Niemiec et al., 2020). The etiological factors of periodontal diseases related to plaque formation and dental microbes cause halitosis (Perrone, 2021). Age, species, breed, genetics, diet, health status, dental care, and condition of the oral microbiota influence the incidence of periodontal diseases Özavci et al., 2019; Niemiec et al., 2020; Kim and Amar, 1994). Previous studies have reported that this disease affects nearly 90% of dogs (Queck et al., 2018; Stella et al., 2018). Periodontal disease has been recognized as one of the most common diseases in cats, affecting approximately 70% of domestic cats over two years of age (Mata, 2015).

Some pathogenic bacteria are responsible for periodontal diseases, for example,

Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus, Fusobacterium nucleatum, Selenomonas, and *Campylobacter* (Özavci et al., 2019; Niemiec et al., 2020). *P. gingivalis* is frequently identified in cats, dogs, and humans suffering from periodontal diseases (Özavci et al., 2019; Gaetti-Jardim et al., 2010; Venkataraman et al., 2015). This Gram-negative bacterium thrives in anaerobic conditions and requires heme and vitamin K (Xu et al., 2020). This bacteria also plays a crucial role in the pathogenesis of periodontal diseases and the development of periodontitis in companion animals (Lenzo et al. 2016).

Information regarding the incidence of periodontal diseases in cats in Indonesia is minimal. Based on data from the Agriculture and Food Service of Gunungkidul Regency, the number of cats in the Yogyakarta special region, Indonesia, has reached 38,000 heads. Veterinary practitioners reported symptoms of periodontal diseases in many cat patients; however, no study of these cases has been conducted. Although the prevalence of this disease is relatively high, Niemec et al. (2020) noted that periodontal diseases were underdiagnosed because of the lack of knowledge regarding this disease. As a result, a case-control study was initiated to explore the factors associated with periodontal diseases related to *P. gingivalis* in cats within the Yogyakarta special region of Indonesia.

Material and methods

Ethical approval

This study was approved by the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia, under Certificate No. 051/EC-FKH/Ex./2023, and was carried out in compliance with the ARRIVE guidelines.

Sample collection

This case-control study included cat patients from clinics and animal hospitals in the Yogyakarta special region, Indonesia, covering five districts: Bantul, Gunungkidul, Yogyakarta, Kulon Progo, and Sleman. Dental plaque samples were collected from these cats. Specimen testing was performed at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia. The study was conducted between May and September 2023.

Before collecting dental plaque samples, each non-anesthetized cat underwent an intraoral examination by a veterinarian to assess periodontal status. All patients were examined following the American Animal Hospital Association (AAHA) dental care guidelines for dogs and cats (Bellows et al., 2019).

A total of 85 cats diagnosed with gingivitis and periodontitis symptoms were categorized as the periodontal disease group (case group), while 85 healthy cats without any periodontal disease formed the control group. A sterile cotton swab was used to collect samples from the canine and premolar teeth, gingiva mucosa, and dental plaque of all cats in both the case and control groups. These plaque samples were sent to the laboratory for the detection of *P. gingivalis*. The collected specimens were placed in tubes containing 5 mL of sterile phosphate-buffered saline (PBS), stored in a cooling box at +4°C, and transported to the Veterinary Public Health Laboratory. Specimen collection was adapted from (Kačírová et al., 2022) with some modifications. Information on risk factors causing periodontal diseases was collected from the cat owners using a questionnaire. Data were collected, including owner identity, patient characteristics, and animal care management.

Molecular detection of *P. gingivalis*

Swab specimens in phosphate buffer saline (PBS) were centrifuged at 10,000 ×g for 5 min, and the supernatant was discarded. The remaining sediment collected was dissolved in 200 µL of sterile PBS. DNA extraction was performed on each sample using a DNA extraction kit, Zymo Research® (Irvine, CA, USA), according to the manufacturer's instructions.

Primers for amplifying the 16S rRNA gene of *P. gingivalis* were designed using the Primer3 online tool. The forward primer was 5'-TCCTA AGGATTGTAAACTTCTTTTA-3', and the reverse primer was 5'-AACTGTTA GCAACTACCGATGT-3', generating a DNA product of 712 bp. The primer set was based on the nucleotide sequence of the 16S rRNA gene of *P. gingivalis* (Access no. OP456623.1). For PCR amplification, a total of 5 µL of DNA sample and 45 µL of PCR master mix were used. The amplification protocol included 35 cycles as follows: Initial denaturation at 95°C for 5 minutes, followed by denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 60 seconds. A final post-

extension step was performed at 72°C for 5 minutes. The PCR products were then electrophoresed on a 1.5% agarose gel stained with a DNA dye and visualized using a UV transilluminator. The PCR protocol was based on the methods described by (Ozavci et al., 2019) with some modifications.

Statistical analysis

Data from the owner questionnaire, patient characteristics, animal care management, and PCR results were collected and analyzed descriptively. The association between periodontal disease and risk factors was determined using the chi-squared test (X^2) and odds ratio (OR).

Results

Based on the clinical examination, 85 cats in the case group were diagnosed with gingivitis and periodontitis symptoms (Figure 1). In contrast, cats in the control group showed no clinical signs of periodontal disease. Table 1 presents the demographic characteristics of the population and association risk factors of periodontal diseases.

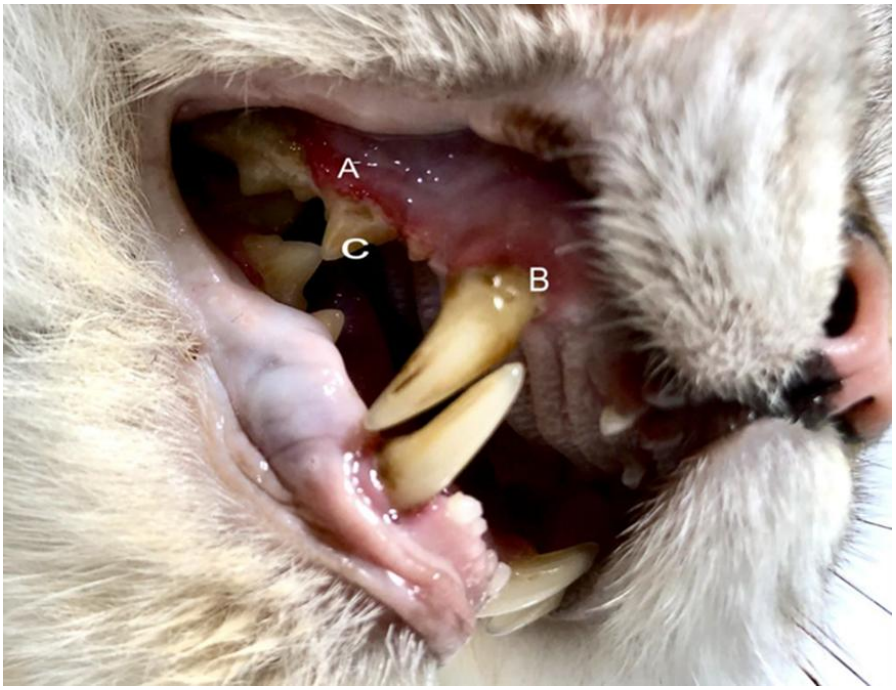

Figure 1: The periodontal disease of the cat in the case group showed gingivitis (A), periodontitis (B), and dental plaque (C).

Table 1: Demographic characteristics of the population and association risk factors of periodontal diseases.

Risk factor		Group		Total	Chi-square	*p*-value	Odds ratio	95% CI	
		Case n=85	Control n=85					Lower	Upper
Sex	Male	60	48	108	3.65	0.056	1.85	0.98	3.49
	Female	25	37	62					
Breed	BSH	2	1	3	1.67	0.560	2.02	0.18	22.75
	Domestic	22	33	55		0.071	0.55	0.29	1.06
	Himalaya	3	2	5		0.65	1.52	0.25	9.32
	Mix domestic	49	42	91		0.282	1.39	0.76	2.55
	Munchkin	1	0	1		0.316	∞	0.662	1.554
	Persia	8	7	15		0.787	1.16	0.40	3.35
Age	<1 year	9	37		38.19	0.001**	0.15	0.07	0.35
	1–3 years	34	39			0.439	0.79	0.43	1.45
	>3 years	42	9			0.001**	8.25	3.66	18.56
Types of feed	Wet	0	5	5	50.31	0.023*	0	0.62	1.43
	Homemade	0	1	1		0.500	0	0.64	1.51
	Dry	23	63	86		0.001**	0.13	0.07	0.26
	Combination (dry and wet)	62	16	78		0.001**	11.63	5.63	23.99

*significant $p<0.05$; **significant $p<0.001$; Case: periodontal diseases; Control: nonperiodontal disease, BSH: British shorthair.

These results can be interpreted to show that cats over 3 years old are 8.25 times more likely to develop periodontal diseases than cats under 3 years old (Table 1). The owner's feeding behavior, such as providing food combinations (sometimes dry food and other times wet food), increases the incidence of periodontal diseases by 11.63 times, which is significantly higher than other feeding types. On the other hand, dry food significantly reduces the probability of periodontal disease by 0.13 times.

Moreover, some variables were not significantly associated with the development of periodontal diseases. These variables included sex (p=0.056), breed (p=0.56), and tooth brushing (p=0.156). This suggests that males and females have an equal likelihood of developing periodontal diseases. Similarly, purebred cats such as BSH, domestic, Munchkin, Persian, and mixed-breed (mixed domestic) cats have the same risk of developing periodontal diseases. Furthermore, the history of oral hygiene, such as whether the cats received tooth brushing or not, was not identified as a causal factor for periodontal diseases.

Periodontal diseases related to *P. gingivalis*

The results of the PCR analysis from P. gingivalis are demonstrated in Figure 2. In summary, the result of PCR identification from 170 plaque swab specimens is shown in Table 2, amount 138 (81.2%) were positive for *P. gingivalis* — 81 from the case group and 57 from the control group. As shown in Table 2, *P. gingivalis* plays a vital role in developing periodontal disease (OR=9.95; p=0.0001; CI=3.31–29.92). Risk factors for periodontal diseases in cats were identified in this study. For cats older than 3 years, a combination of dry and wet food, along with the presence of *P. gingivalis*, increases the likelihood of periodontal diseases in cats. Meanwhile, dry food plays a protective role against periodontal diseases.

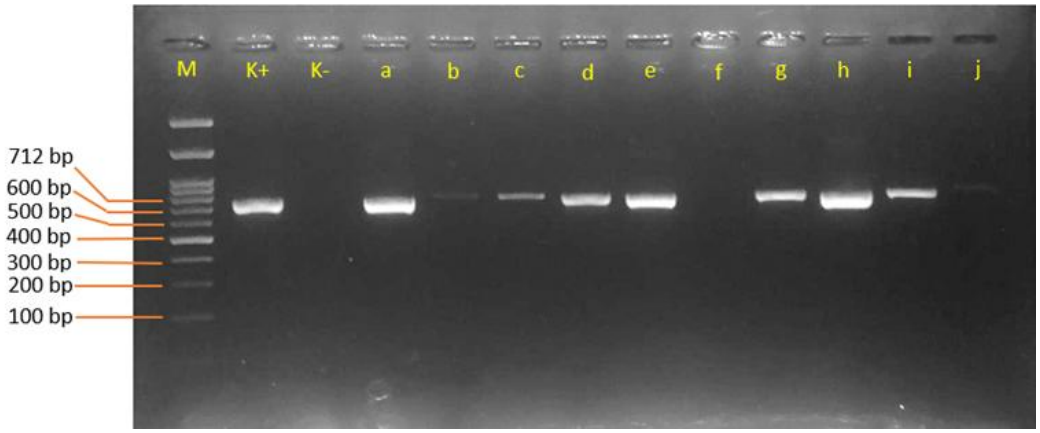

Figure 2: Result of PCR for *P. gingivalis*, left column: M: marker, K+: positive control, K-: negative control, positive sample detected at: a, b, c, d, e, g, h, and i.

Table 2: Role of *P. gingivalis* in periodontal disease in cats around Yogyakarta special region, Indonesia.

P. gingivalis	**Group**		**Total**	**Chi square**	***p*-value**	**Odds ratio (95% CI)**	**95% CI**	
	Case (n=85)	Control (n=85)					Lower	Upper
Positive	81	57	138	20.36	0.001**	9.95	3.31	29.92
Negative	4	28	32					

Case: periodontal diseases; control: nonperiodontal diseases.

Discussion

This study showed that sex does not have a significant role in the development of periodontal disease. These findings confirm previous research indicating that the sex of cats does not significantly influence the incidence of periodontal diseases (Girard et al., 2009). However, other studies have reported contrasting results. Zaccone et al. (2022) found that male cats are 1.78 times more at risk of developing periodontal diseases than female cats. O'Neill et al. (2023) revealed that male cats have a higher prevalence of periodontal diseases but did not investigate its effect as a risk factor.

Porphyromonas gingivalis is a significant periodontal pathogen that can invade host cells, activate immune response pathways, and release harmful exotoxins, leading to chronic inflammation and tissue destruction in periodontal disease. Both age and sex can affect the oral microbial community in cats, influencing the abundance of specific bacterial groups and contributing to variations in microbial diversity and composition across different age groups (Yang et al., 2024).

The breed of cats does not play a significant role in causing periodontal diseases. All breeds are equally susceptible to periodontal diseases. Previous research has shown different results, with some studies suggesting that purebred cats are more likely to suffer from gingivitis than mixed-breed cats (Girard et al., 2009; Lommer et al., 2001). The age of the cat has been shown to be an important factor in the development of periodontal diseases.

Previous studies stated that increasing food requirements are related to a cat's age (Mestrinho et al., 2018). This can potentially increase plaque formation on the teeth and the incidence of periodontal diseases (Palmeira et al., 2022). These findings are confirmed by this study, which shows that cats older than 3 years have an 8.25 times greater risk of developing periodontal diseases compared to those aged under 3 years (Ingham et al., 2022) because periodontal disease is a progressive condition that tends to worsen if the initial cause is not treated, resulting in conditions like gingivitis or periodontitis (Ingham et al., 2022; Palmeira et al., 2022; Wilson et al., 2002). The age of the cats was the strongest predictor and correlated strongly with the presence of periodontal diseases (O'Neill et al., 2023; Palmeira et al., 2022). Overall, cat age appears to be a significant risk factor for the increasing presence and severity of periodontal diseases in cats. Veterinarians should encourage heightened vigilance for periodontal diseases in cats older than 3 years and recommend routine screening focusing on dental health (O'Neill et al., 2023; Ray et al., 2021).

Indeed, 86 (50.58%) owners fed their cats dry food, and 78 cat owners provided a combination of dry and wet food. This finding is higher than Toribio et al. (2009) findings in Australia, where 38.1% of owners provided a combination of commercial dry and canned food. This study also showed that a combination of dry and wet food produces more periodontal disease than other types of feeding. Dental calculus and plaque were more frequently found in cats fed wet food rather than dry food (Gawor et al., 2006). However, dry food significantly reduces the probability of periodontal disease (p=0.001; OR: 0.13). Calculus and dental plaque occur less frequently in cats fed dry food compared to those fed wet food (Mata, 2015). The rough texture of dry food helps to remove plaque from a cat's teeth. The crunchiness of dry food requires more chewing than wet food, which can help clean the teeth and gums mechanically. This mechanical action might reduce plaque buildup caused by periodontal disease. This result does not align with Clarke et al. (1998), which found that feeding a combination of dry and wet food to domestic cats compared with wild cats had no role in developing periodontal diseases.

This study revealed that *P. gingivalis* increases the risk of periodontal diseases in cats by 9.95 times. According to Krumbeck et al. (2021), three of the five dominant bacteria in clinically healthy cats are genus *Porphyromonas* [*P. gulae* (10%), *Porphyromonas* spp. (2.8%), and *P. circumdentaria* (2.6%)]. *P. gingivalis* has been reported as a dominant anaerobic bacterium, and it can change the composition of the oral microbiota (Ozavci et al., 2023). The presence of *P. gingivalis*, combined with other bacteria, can contribute to forming periodontal pockets and chronic periodontitis (Ozavci et al., 2023; Nomura et al., 2020). The virulence factor of *P. gingivalis* primarily induces inflammatory mediators in periodontal tissue, which is considered one of the most influential pathogenic bacteria in the incidence of periodontal diseases and tooth loss (Xu et al., 2020; Zhang et al., 2021; Mysak et al., 2014).

Dental plaques provide a suitable environment for growing Gram-negative bacteria such as *P. gingivalis* (Ozavci et al., 2023). The combination of plaque, pellicle, and pathogenic bacteria damages the gingival tissue, leading to the progression from gingivitis to periodontitis (Perrone, 2021; Ozavci et al., 2023). The presence of *P. gingivalis* in the oral cavity of cats can cause rheumatoid arthritis, bacterial vaginosis, and osteomyelitis in humans through cat bites. It can be a risk factor for zoonotic diseases (Ozavci et al., 2019). Due to imbalanced data from the case and control groups, this study could not analyze some risk factors, such as living conditions

(indoor vs outdoor) or tooth brushing activity. There may be confounding factors among the risk factors (age and sex), which require further analysis, which has not been conducted in this study.

Conclusions

The risk factors for periodontal diseases in cats in the Yogyakarta special region were age >3 years, a combination of wet and dry feeding, and the presence of *P. gingivalis*, but dry food reduces the risk. Cat owners must pay more attention to care management, particularly dental health.

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Conflicts of Interest. The authors declare no conflict of interest.

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Authors contribution. H.B.P: sampling, testing samples, and data analysis; W.S.N: Conceptualization, study design, data analysis, manuscript final editing; I.T: sampling, manuscript editing. All authors have read and agreed to publish the manuscript.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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