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Porphyromonas gulae infection in canines, pet owners and veterinarians in China: an epidemiological study and risk factor analysis

Yang Bai[1†], Peijia Song[1†], Zhangqi Shen[1], Hao Shi[1], Zimo Jiang[1], Jiahao Lin[1,2*] and Yipeng Jin[1*]

Abstract

Porphyromonas gulae is a clinically prevalent, anaerobic, oral bacteria in canines, that may be a causative agent of canine periodontal disease, and a potential threat to human oral health. Research on *P. gulae* pathogenicity in canines, their owners, and veterinarians is lacking in China. This study aimed to determine the isolation and detection rates of *P. gulae* in gingival crevicular fluid (GCF) samples from 101 canines in Beijing, using anaerobic culture techniques and 16S rRNA gene sequencing. The main risk factors for the transmission of *P. gulae* from canines to humans were also analyzed through analyzing the statistical data on risk factor variables from 103 canine owners and 60 veterinarians in Beijing who tested positive for *P. gulae* detection in GCF samples. The isolation and detection rates of *P. gulae* in canines were 31.5% (29/92) and 92.1% (93/101), respectively, compared with detection rates of 24.3% (25/103) in canine owners, 43.3% (26/60) in veterinarians, and 52.0% (13/25) in dentists. The degree of contact with canines ($P=0.001$, $P<0.01$) and smoking ($P=0.021$, $P<0.05$) were significant risk factors for *P. gulae* detection in owners. Moreover, the degree of contact during ultrasonic scaling ($P=0.065$, $0.05<P<0.1$) was the most important risk factor for the positive detection of *P. gulae* in veterinarians. These findings suggest that *P. gulae* may colonize the human oral cavity through intimate contact with canines or participation in dental ultrasonic scaling operations.

Keywords *Porphyromonas gulae*, Canine periodontal disease, Bacterial spread, Public health, Risk factor analysis

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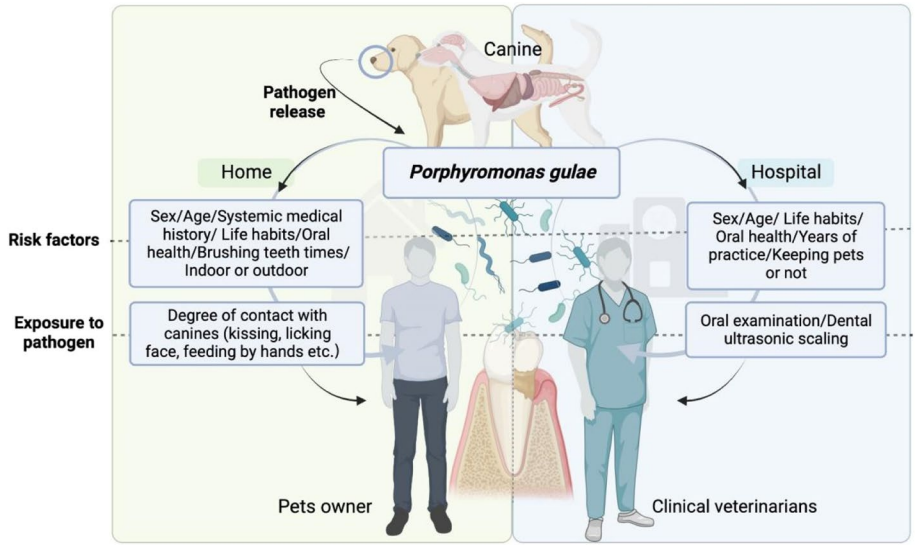
Graphical Abstract



Introduction

Periodontitis disease (PD) is a prevalent oral disease among dogs and cats worldwide [1, 2]. It initially manifests as gingivitis in clinical practice and then progresses to periodontitis gradually. The bones and ligaments that supporting the teeth are destroyed, leading to serious oral complications, such as clinical attachment loss and gingival atrophy. Canine PD is caused by dental plaque mainly composed of various of bacteria that gradually erode the periodontal tissue, causing inflammation [3–5]. The oral microbial environment of canines is complex, and the distribution of bacteria varies significantly between species, with varying quantities from site to site [6]. In North America, 50–70% of adult canines have PD [7]. In Japan, approximately 78% of canines over 5 years of age have gingivitis [8]. In the Czech Republic, the prevalence of PD in canines is approximately 60% [2]. However, to date, no statistical study has been conducted on the prevalence and incidence of PD in canines in China.

PD is also a common disease in humans, impacting the oral health. According to a statistical survey, approximately 80% of the adult population in China suffers from PD to varying degrees, during much of the researchers' attention [9]. The current incidence of gingivitis among Chinese adolescents is approximately 48.8%, which is higher than in developed countries [10]. In addition to swollen, painful gums, tooth loss, and bleeding, human PD can cause halitosis, which may have a significant negative impact on the social lives of patients. The World Health Organization regards oral health as a key indicator of overall human health, welfare, and quality of life. Therefore, the widespread problem of PD warrants greater attention. Although, there is a certain degree of correlation between canine PD and human PD, the research on canine PD is less extensive than that on human stomatology, and the etiology of canine PD remains unclear. Studies have shown that the number of endogenous bacteria in the subgingival region of canines changes as PD develops, which is not thought to be caused by the invasion of exogenous pathogens [6].

The *Porphyromonas gingivalis* has been recognized as the most important pathogen in human PD, requiring the same culture conditions and showing similar ability to infect epithelial cells as *Porphyromonas gulae*, an animal periodontal pathogen, can be isolated from the gingival sulcus of canines, cats, kangaroos, monkeys, sheep, wolves, and other animals [11, 12]. *P. gulae* may be the key pathogen of PD in canines and felines, with the fimbria, lipopolysaccharide, proteases, and several other virulence factors affecting the periodontium [13]. This bacterium is reportedly more likely to be detected in canines with PD than in those without PD. Previously, *P. gulae* was proposed as the animal biotype of *P. gingivalis,* given their similar virulence characteristics and immunological features [14]. Therefore, it is reasonable to suspect that *P. gulae* may also have a negative impact on human oral health.

An increased abundance of *Synergistales bacterium COT-178*, *Moraxella* sp. and *Porphyromonas* spp.

significantly correlated with the progression of PD in canines [15–17]. In 2011, Senhorinho et al. [18] were the first to report that *P. gulae* could be detected in 92% and 56% of canines with and without periodontitis, respectively. In 2013, Pérez-Salcedo et al. reported a *P. gulae* detection rate of 86% in felines with PD ($n=50$), while the positive detection rate among canines was 67–96% [12].

In 2021, Yamasaki et al. [19] reported the first discovery of an animal-derived bacterium in dental plaque samples taken from 66 canines and 81 individuals from 64 families in Japan. They found that the carrier rate of *P. gulae* in canines was 71.2%, and *P. gulae* was identified in 13 owners and their canines. Inaba et al. [20] subsequently found that *P. gulae* could colonize both healthy and diseased human gingival tissues, induce inflammatory reactions in cells, and reduce cell motility. This bacterium was highly cytotoxic, showing adherence to and invasion of human oral epithelial cells, suggesting that it may cause or aggravate human PD. The possibility exists that *P. gulae* may be transmitted from canines to humans, potentially allowing the spread of drug-resistant bacteria of the same genus, thereby presenting a public health risk. At present, the companion animal-related industry in China has grown rapidly, with the number of companion animals reaching 120 million in 2010. As the relationship between humans and companion animals has become increasingly close, daily interactions with canines may include hugging, getting licked and kissing, which could greatly raise the risk of infection. Previous studies have shown that companion animals could transmit periodontal pathogens to humans through close contact. For example, cats can transmit *Tannerella forsythia* (a member of the red complex and an important pathogen of PD) to their owners [21]. Therefore, it is suspected that *P. gulae* may also be transmitted from a canine's mouth to its owner in a similar way. However, few studies have been reported on the spread of *P. gulae* among animals and humans.

In Chinese animal hospitals, dentists handle ultrasonic scaling procedures every day, and many oral bacteria may exist in the aerosols produced during ultrasonic scaling, which might lead to an increased likelihood of bacterial infection among veterinarians. Studies have demonstrated that numerous droplets and aerosols are created when high-speed ultrasound equipment is used for dental cleaning [22]. These aerosols may combine with blood and salivary germs in the mouth to form infectious aerosols, which could infect medical staff and other susceptible individuals. Therefore, further investigation is required to determine whether *P. gulae* may be transmitted to veterinarians via infectious aerosols, potentially leading to colonization of their oral cavity.

This study aims to investigate the carriage rate of *P. gulae* among canine owners and veterinarians in China and investigate the risk factors for colonization. To track the spread of *P. gulae*, aerosol samples obtained during ultrasonic scaling procedures were also examined. Research on veterinarians contracting bacteria during ultrasonic scaling has never previously been conducted (Fig. 1).

In summary, exploring the transmission of *P. gulae* between canines and humans is of public health importance. Only a few studies have investigated the bacterial composition of canine PD and the abundance of *P. gulae* using high-throughput sequencing techniques, and even fewer studies have been conducted on the risk factors for colonization with this bacterium. The environmental risk factors for colonization and the possible transmission pathways for *P. gulae*, taking owners and veterinarians into consideration, were areas in which research was lacking and were therefore the focus of this study.

Results

Morphological description of *P. gulae* strains

P. gulae exhibits the following characteristics: black-pigmented colonies, obligate anaerobic growth, Gram stain-negative, non-motile, non-spore-forming, and rod-shaped in appearance under a light microscope. This bacterium grows slowly (7–10 days) on modified brain-heart infusion (BHI) agar containing hemin (Solarbio, 5 μg/mL) and VK1 (Solarbio, 1 μg/mL). Smooth, uniformly-shaped colonies were observed on BHI agar medium containing 5% fetal bovine serum (FBS) after 5–10 days of culture. In liquid medium, *P. gulae* was more difficult to enrich. Refer to Fig. 2 for the growth of *P. gulae* in BHI agar medium with hemin and VK1.

The separation rate of *P. gulae*

Gingival crevicular fluid (GCF) samples were collected from 72 canines with PD attending the Teaching Animal Hospital of China Agricultural University from February 2021 to February 2022. Anaerobic culturing was performed on all samples. A total of 22 species and 92 strains of anaerobic bacteria were isolated and purified, including *P. gulae, Prevotella intermedia, Porphyromonas crevioricanis, Porphyromonas macacae* and other canine oral *Porphyromonas* spp. The isolation and purification results were consistent with the high-throughput sequencing results. Based on these findings, *P. gulae* appeared to be the predominant pathogen of canine PD, with a detection rate of 31.5% (29/92). The strain information obtained by culture was confirmed by the results of first generation sequencing of cloned 16S rRNA gene.

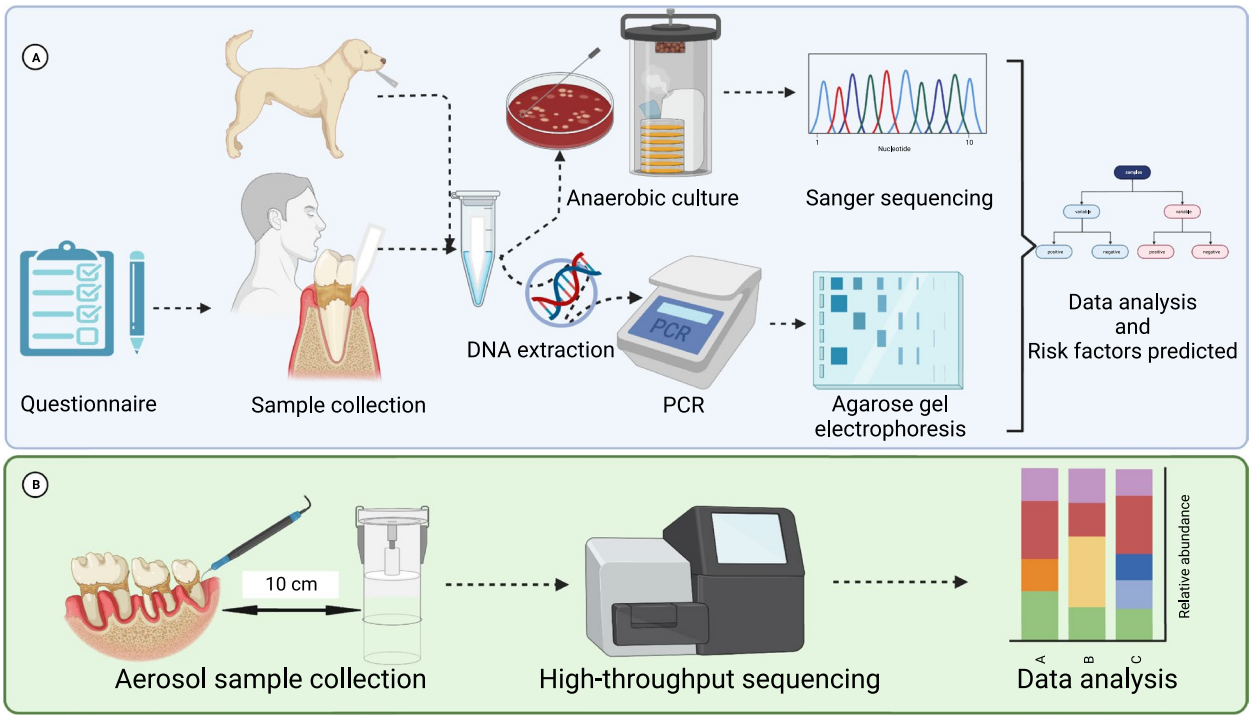


Fig. 1 Experimental procedure. Part **A**: Statistical analysis of the detection and isolation rates of *P. gulae* in canines, canine owners and veterinarians in China, and the questionnaire survey of risk factors for owners and veterinarians to integrate the main risk factors. Part **B**: Analysis of the composition and abundance of bacterial species that may be transmitted in the aerosol by high-throughput sequencing methods

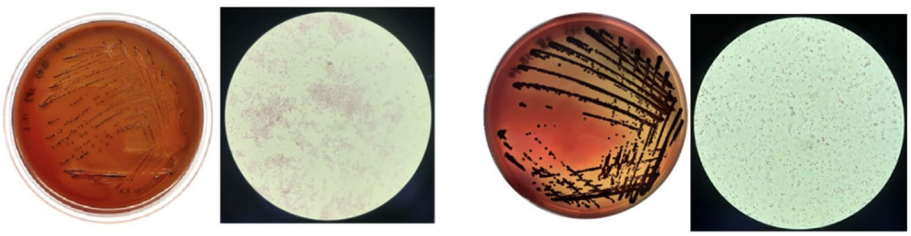


Fig. 2 SB42 and SB40 of *P. gulae*. The modified BHI plates were incubated anaerobically, then cells were Gram stained and examined by microscopy (100× magnification)

The positive detection rate and risk factors of *P. gulae* in canine owners

Of the 103 canine owners tested for *P. gulae*, 25 were positive (24.3%), including 19 males and 6 females. The age range was from 20 to 61 years, with an average age of 46.3 (±12.7) years. Sex and age can affect the immune system and the progression of PD. We classified the degree of contact between canine owners and canines into eight levels, designated A–H (based on the research of Yamasaki et al. [19]). The highest level of contact (level A) included raising at home, kissing and other close-contact behaviors. The lowest level of contact (level H) indicated no direct contact. Among those individuals testing positive for *P. gulae*, 19 (76.0%) were classified as level A (raising at home, kissing, and other direct intimate contact behaviors), 6 (24.0%) were classified as level B (raising at home, licking hands, feeding by hands, and other indirect intimate contact behaviors), and no individuals were classified as level C to H. Systemic diseases are considered to increase the incidence and severity of PD directly or indirectly. In our study, 8 out of 25 individuals with a history of systemic disease tested positive for *P. gulae*. Smoking and drinking alcohol are directly related to PD as these behaviors alter the physical and chemical environment of the periodontium. Among the individuals that tested positive, 12 (48.0%) consumed alcohol and 16 (64.0%) were smokers. Oral health status indirectly affects the composition and abundance of microbes

Table 1 Statistical analysis between the *P. gulae* detection test results of canine owners and the sample variables

Variables		Positive samples	Negative samples	Total
Age(a)	A. 20 ≤ a < 40	6(5.8%)	25(24.3%)	31(30.1%)
	B. 40 ≤ a < 60	17(16.5%)	33(32.0%)	50(48.5%)
	C. 60 ≤ a	2(1.9%)	20(19.4%)	22(21.4%)
	Total	25(24.3%)	78(75.7%)	103
Sex	Male	19(18.4%)	30(29.1%)	49(47.6%)
	Female	6(5.8%)	48(46.6%)	54(52.4%)
	Total	25(24.3%)	78(75.7%)	103
Degree of Contact with pet canines	A. Raising at home,kissing, licking face and other direct intimate contact behaviors	19(18.4%)	23(22.3%)	42(40.8%)
	B. Raising at home,licking hands, feeding by hands and other indirect intimate contact behaviors	6(5.8%)	14(13.6%)	20(19.4%)
	C. Raising at home, only physical contact behaviors such as hugging	0	25(24.3%)	25(24.3%)
	D. Raising in the yard, kissing, licking face and other direct intimate contact behaviors	0	4(3.9%)	4(3.9%)
	E. Raising in the yard, licking hands, feeding by hands and other indirect intimate contact behaviors	0	2(1.9%)	2(1.9%)
	F. Raising in the yard, only physical contact behaviors such as hugging	0	9(8.7%)	9(8.7%)
	G. Raising at home, no contact	0	1(1.0%)	1(1.0%)
	H. Raising in the yard, no contact	0	0	0
	Total	25(24.3%)	78(75.7%)	103
General Systemic Disease	Positive	8(7.8%)	15(14.6%)	23(22.3%)
	Negative	17(16.5%)	63(61.2%)	80(77.7%)
	Total	25(24.3%)	78(75.7%)	103
Smoking	Positive	16(15.5%)	14(13.6%)	30(29.1%)
	Negative	9(8.7%)	64(62.1%)	73(70.9%)
	Total	25(24.3%)	78(75.7%)	103
Drinking alcohol	Positive	12(11.7%)	20(19.4%)	32(31.1%)
	Negative	13(12.6%)	58(56.3%)	71(68.9%)
	Total	25(24.3%)	78(75.7%)	103
Oral health	A. Healthy	12(11.7%)	46(44.7%)	58(56.3%)
	B. Unhealthy	13(12.6%)	32(31.1%)	45(43.7%)
	Total	25(24.3%)	78(75.7%)	103
Brushing teeth	A. Never	0	0	0
	B. Once/day	6(5.8%)	11(10.7%)	17(16.5%)
	C. Twice/day	19(18.4%)	67(65.0%)	86(83.5%)
	D. Three times/day	0	0	0
	Total	25(24.3%)	78(75.7%)	103

Note: Systemic history included heart disease, diabetes, hypertension

in the oral cavity. Among those testing positive for *P. gulae*, 12 had good oral health (48.0%), 9 had gingivitis (36.0%), 3 had periodontitis (12.0%) and 1 had dental caries (4.0%). Oral cleaning can destroy and remove microorganisms in the oral cavity. Typically, an individual may develop gingivitis after not brushing their teeth for 10 days. Among those who tested positive for *P. gulae*, 19 individuals brushed their teeth twice a day and 6 brushed their teeth once a day. Detailed statistical data are shown in Table 1.

Next, we cross-checked the canine survey information, including *P. gulae* test results, years of feeding, and periodontal status evaluation, with the *P. gulae* test results of the owners. Of the 101 canine samples tested for *P. gulae*, 93 (92.1%) were positive. Generally, if the owner tested negative for *P. gulae*, so did their dog. Four canine samples without corresponding owner

Table 2 Statistical analysis between the *P. gulae* detection test results of canine owners and canines

	Variables		Positive owners	Negative owners	Total
canines	Feeding years	A. $0 < r \leq 5$	20(20.6%)	41(42.3%)	61(62.9%)
		B. $5 < r < 10$	6(6.2%)	22(22.7%)	28(28.9%)
		C. $10 \leq r$	2(2.1%)	6(6.2%)	8(8.2%)
		Total	28(28.9%)	69(71.1%)	97
	Periodontal condition	A. Healthy	8(8.2%)	17(17.5%)	25(25.8%)
		B. Gingivitis	8(8.2%)	30(30.9%)	38(39.2%)
		C. Periodontitis	12(12.4%)	22(22.7%)	34(35.1%)
		Total	28(28.9%)	69(71.1%)	97

Note: The four canine samples without corresponding owner samples were not included in the statistics, so the total number of canine samples was 97

samples were not included in the statistics, resulting in a total number of 97 canine samples shown in Table 2.

The owners of 28 canines tested positive for *P. gulae*, including 8 canines without PD, 8 canines with gingivitis, and 12 canines with periodontitis. Among all samples, 61 canines had been raised for 5 years or fewer (62.9%), and of the corresponding owners, 20 tested positive (80.0% of positive owners); 28 canines had been raised for 5 to 10 years (28.9%), and of the corresponding owners, 6 tested positive (24.0% of positive owners); and, finally, 8 canines had been raised for 10 years or longer (8.2%), and of the corresponding owners, 2 tested positive (8.0% of positive owners). Among the canines raised for 5 years or fewer, the positive rate of their corresponding owners was 32.8%. The positive rate of owners who raised canines for 5 to 10 years was 21.4%. And the positive rate of owners who raised canines for 10 years or longer was 25.0%. The results showed that the positive rate did not increase with an increase in feeding years. The detailed statistical data are shown in Table 2.

The positive detection rate of *P. gulae* was taken as the basic variable, and the relationship among the test variables was analyzed using a logistic regression model. The classification regression model calculation of *P. gulae* detection in the canine owners revealed a significant difference ($P = 0.001$, $P < 0.01$) between the degree of contact with canines and the infection of canine owners with *P. gulae*. Smoking was another risk factor affecting the positive detection of *P. gulae* in canine owners ($P = 0.021$, $P < 0.05$). The other variables tested had no significant effect on the detection of *P. gulae.* The detailed statistical data are shown in Table 3.

All variables had a variance inflation factor (VIF) lower than 5, indicating that there was no collinear relationship between independent variables.

To determine the contribution of each variable to the *P. gulae* test result, we established a random forest model (Fig. 3). The model showed that the most important factor affecting the positive detection of *P. gulae* in canine owners is the degree of contact with canines (importance value 0.30), followed by smoking (importance value 0.15). This finding was consistent with the results obtained using the logistic regression model above.

Table 3 Correlation analysis between the sample variables and the *P. gulae* detection test results of the canine owners

Variables	Estimate	Std.Error	Z Value	*P*	VIF
Age	−0.472	0.589	−0.802	0.423	1.597
Sex	0.776	0.880	0.882	0.378	1.617
Contact degree	−1.415	0.421	−3.357	0.001 ***	1.226
Systemic Disease	1.230	0.846	1.453	0.146	1.448
Smoking	2.329	1.013	2.300	0.021 *	2.002
Drinking alcohol	−0.034	0.831	− 0.041	0.967	1.475
Owners' Oral health	0.570	0.691	0.825	0.410	1.300
Brushing teeth	−0.542	1.090	−0.497	0.619	1.134
Feeding years	−0.073	0.532	−0.138	0.890	1.218
Canines' Oral health	0.265	0.434	0.610	0.542	1.262

Note: *** ($P < 0.001$); **($P < 0.01$); *($P < 0.05$) indicates significant difference between groups

We also generated a decision tree model to predict *P. gulae* colonization of canine owners based on the input variables. The model judged the results at different branches based on the input variables at the top root node, and finally output the probability that the sample was positive. A sample decision tree model is shown in the attachment.

The positive detection rate and risk factors of *P. gulae* in veterinarians

After analyzing the risk factors in canine owners, we further surveyed veterinarians working in operating rooms related to dental surgery in several animal hospitals in Beijing. A total of 60 veterinarians were tested for *P. gulae*, whose age ranged from 23 to 58 years (average age of 30.0 ±5.7 years). Of these veterinarians, 25 (41.7%)

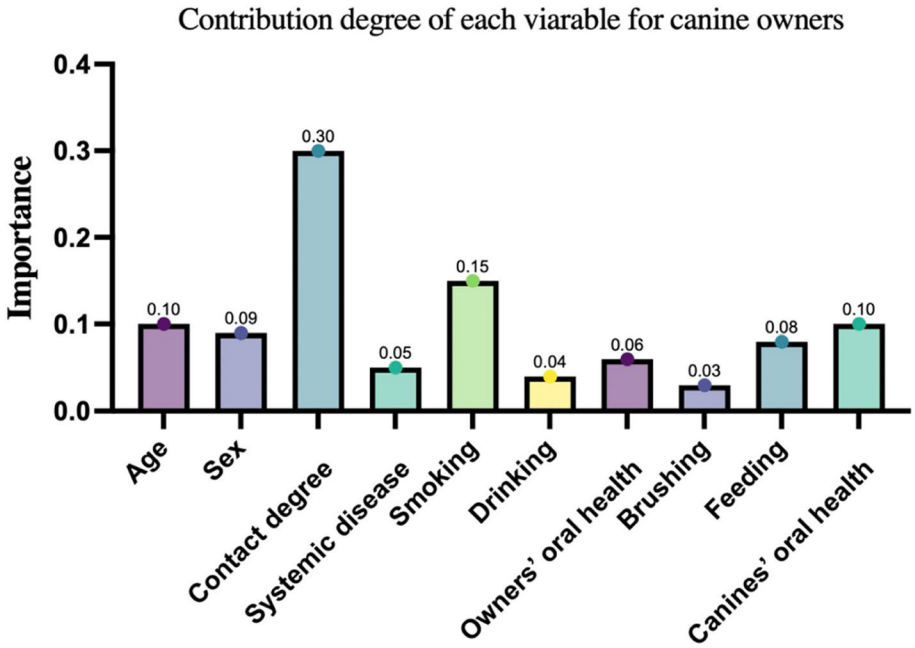


Fig. 3 Random forest model calculation chart for the *P. gulae* detection test results of canine owners and the sample variables

directly participated in ultrasonic scaling, 24 (40.0%) were anesthesiologists, and 11 other operators and assistants (18.3%) worked in the operating room without participating in ultrasonic scaling. Among the veterinarian workers, 37 (61.7%) had pets at home, including 12 with dogs, 15 with cats, 9 with both dogs and cats, and 1 with a hamster. Of these 37 individuals, 32 (53.3%) had close contact with their pets, such as hugging and kissing, letting pets lick their hands or face, and feeding pets with their hands.

Of the 60 veterinarians tested for *P. gulae*, 26 (43.3%) tested positive, including 13 males and 13 females. The age range was 23 to 58 years, with an average age of 30.1 ± 7.4 years. Among the 26 individuals who tested positive, 13 were directly involved in the operation of ultrasonic scaling (50.0%), 10 were anesthesiologists (38.5%) and 3 were not involved in ultrasonic scaling operations (11.5%). Of the 25 individuals who directly participated in ultrasonic scaling, 13 (52.0%) tested positive. Among those who tested positive, 15 underwent no more than three rounds of dental cleaning per week (57.7%) and 11 underwent more than three rounds of dental cleaning per week (42.3%). In this profession, among those who tested positive, 15 had worked fewer than 5 years (57.7%), 8 had worked between 5 and 10 years (30.8%) and 3 had worked more than 10 years (11.5%). Among the individuals who tested positive, 17 had pets (65.4%) and among these, 16 had close contact with their pets (61.5%) and 5 were smokers (19.2%). Of the individuals who tested positive, 8 had good oral health (30.8%), 16 had gingivitis or periodontitis with different degrees of dental calculus (61.5%) and 2 had dental caries (7.7%). Detailed statistical data are shown in Table 4.

The logistic regression model was used to analyze the relationship among test variables, with the positive detection rate of *P. gulae* as the basic variable, and the relationship among the test variables was analyzed using a logistic regression model. The classification regression model calculation of *P. gulae* detection by veterinarians, as shown in Table 5, revealed a significant difference ($P=0.065$, $0.05<P<0.1$) between the degree of contact with ultrasonic scaling and the infection of veterinarians with *P. gulae*. The other variables tested had no significant effect on the detection of *P. gulae*.

All variables had a VIF detection value lower than 5, indicating that there was no collinear relationship between independent variables. We established a random forest model to determine the contribution of each variable to the *P. gulae* test result (Fig. 4). According to this model, the most important factors affecting the positive detection of *P. gulae* in veterinarians were oral health, the degree of contact with ultrasonic scaling and working years, with importance values of 0.17, 0.16, and 0.16, respectively. Therefore, veterinarians with poor oral health, close contact with ultrasonic scaling and a high number of working years would have a higher probability of *P. gulae* colonization.

Table 4 Statistical analysis between the *P. gulae* detection test results of veterinarians and the sample variables

Variables		Positive samples	Negative samples	Total
Age(a)	A. $20 \leq a < 30$	17(28.3%)	15(25.0%)	32(53.3%)
	B. $30 \leq a < 40$	6(10.0%)	18(30.0%)	24(40.0%)
	C. $40 \leq a$	3(5.0%)	1(1.7%)	4(6.7%)
	Total	26(43.3%)	34(56.7%)	60
Sex	Male	13(21.7%)	16(26.7%)	29(48.3%)
	Female	13(21.7%)	18(30.0%)	31(51.7%)
	Total	26(43.3%)	34(56.7%)	60
Contact degree with ultrasonic scaling	A. Operator	13(21.7%)	12(20.0%)	25(41.7%)
	B. Anesthesiologist	10(16.7%)	14(23.3%)	24(40.0%)
	C. General practioners	3(5.0%)	8(13.3%)	11(18.3%)
	Total	26(43.3%)	34(56.7%)	60
Contact frequency with ultrasonic scaling	A. ≤ 3 operations / week	15(25.0%)	23(38.3%)	38(63.3%)
	B. >3 operations / week	11(18.3%)	11(18.3%)	22(36.7%)
	Total	26(43.3%)	34(56.7%)	60
Working years(w)	A. $0 < w < 5$	15(25.0%)	17(28.3%)	32(53.3%)
	B. $5 \leq w < 10$	8(13.3%)	12(20.0%)	20(33.3%)
	C. $10 \leq w$	3(5.0%)	5(8.3%)	8(13.3%)
	Total	26(43.3%)	34(56.7%)	60
Keeping pet	Positive	17(28.3%)	20(33.3%)	37(61.7%)
	Negative	9(15.0%)	14(23.3%)	23(38.3%)
	Total	26(43.3%)	34(56.7%)	60
Close contact with pets	Positive	16(26.7%)	16(26.7%)	32(53.3%)
	Negative	10(16.7%)	18(30.0%)	28(46.7%)
	Total	26(43.3%)	34(56.7%)	60
Smoking	Positive	5(8.3%)	3(5.0%)	8(13.3%)
	Negative	21(35.0%)	31(51.7%)	52(86.7%)
	Total	26(43.3%)	34(56.7%)	60
Oral health	Healthy	8(13.3%)	14(23.3%)	22(36.7%)
	Unhealthy	18(30.0%)	20(33.3%)	38(63.3%)
	Total	26(43.3%)	34(56.7%)	60

Table 5 Correlation analysis between the sample variables and the *P. gulae* detection test results of veterinarians

Variables	Estimate	Std.Error	Z Value	*P*	VIF
Sex	−0.362	0.620	−0.585	0.559	1.256
Age	−0.734	0.749	−0.980	0.327	3.057
Contact degree with ultrasonic scaling	−0.680	0.368	−1.846	0.065*	1.391
Contact frequency with ultrasonic scaling	0.098	0.637	0.153	0.878	1.353
Working years	0.469	0.694	0.675	0.500	3.239
Keeping pet	−0.346	0.813	−0.426	0.670	1.912
Close contact with pets	0.798	0.823	0.969	0.332	2.195
Smoking	1.29	0.906	1.422	0.155	1.235
Oral health	0.606	0.472	1.283	0.200	1.181

Note: *($P < 0.05$) indicates significant difference between groups

Taking into account the results of both models, it is evident that the primary risk factor for *P. gulae* carriage among veterinarians is close contact with ultrasonic scaling ($P = 0.065$, $0.05 < P < 0.1$), with an importance value of 0.16. Veterinarians who directly participate in ultrasonic scaling have the highest risk of

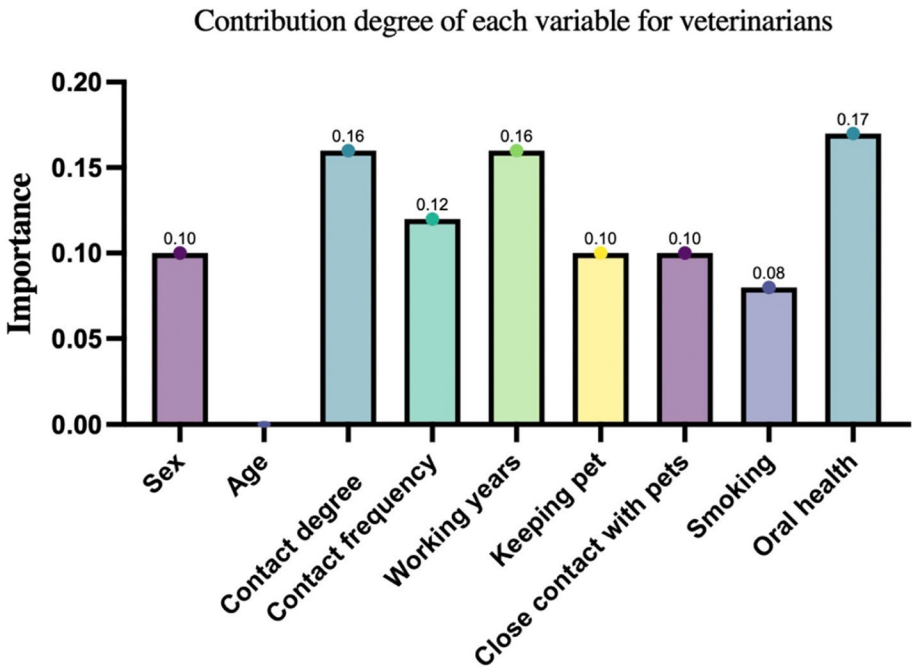


Fig. 4 Random forest model calculation chart for the *P. gulae* detection test results of veterinarians and the sample variables

being colonized with *P. gulae*, showing a positive detection rate of 52%. Other factors that also impacted positive *P. gulae* detection were the number of working years (importance value 0.16) and oral health (importance value 0.17).

Finally, we generated a decision tree model to predict *P. gulae* colonization of veterinarians based on input variables. One of the decision tree models is shown in the attachment.

High-throughput detection of aerosol samples

A Venn diagram showing the interaction among the operational taxonomic units (OTUs) of the four air samples (Environment sample1–Environment sample4) is shown in Fig. 5(a). Different colors represent distinct samples. The numbers in the overlapping regions indicate the number of species shared by multiple samples, while the numbers in the non-overlapping regions indicate the number of species that are unique to a sample. The four air samples contained 74 OTUs, with Environment sample1, Environment sample2, Environment sample3 and Environment sample4 containing 31, 293, 65, and 342 unique OTUs, respectively. Among them, the Environment sample2 had the most OTUs, up to 384, while Environment sample3 sample had the least number of OTUs, with only 153.

Figure 5(b) shows a pie chart of microbial community at the genus level classification, showing the distribution of unique or common species in different samples. Different colors represent different species, and the size of each slice represents the number of species within the total number of species, presented as a percentage. The genus *Porphyromonas* was detected in all OTUs, accounting for 6.12% of the total species.

Figure 5(c) shows the relative abundance of bacteria at the genus level. The abscissa is the sample name, and the ordinate is the proportion of species in the sample. Columns of different colors represent different species, and the length of each column represents the proportion of the species. The species represented by color are arranged from largest to smallest proportion. *Streptococcus* was the dominant strain with the highest relative abundance, followed by *Pseudomonas*, *Porphyromonas*, *Herbaspirillum*, and *Ralstonia*. The relative abundance of *Porphyromonas* ranked third. Each sample contained different species, with Environment sample1 and Environment sample3 showing a large number of *Porphyromonas* bacteria.

The results of the community heatmap are shown in Fig. 5(d). The abscissa is the sample name and the ordinate is the species name. The color gradient represents the change in abundance of different species in the sample, with red indicating a high value and blue indicating a low value. All four samples showed a relative increase in the abundance of *Porphyromonas*.

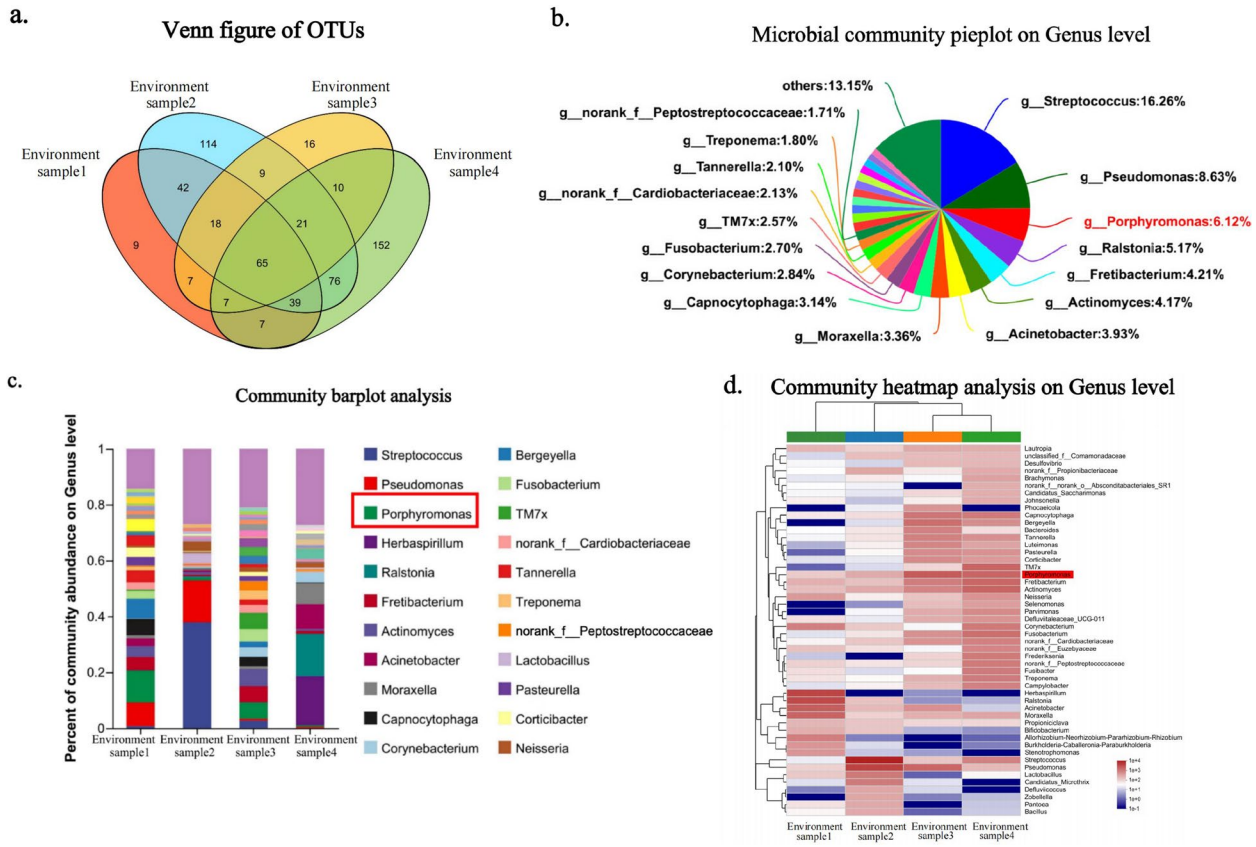


Fig. 5 16S rRNA test results for the aerosol samples. (**a**) Venn diagram of the OTUs; (**b**) Microbial community pie plot at the genus level; (**c**) Community bar plot analysis at the genus level; (**d**) Community heatmap analysis at the genus level

Discussion

P. gulae is a major periodontal pathogen in canines, that can be transmitted to their owners. However, the isolation and culture of *P. gulae*, an anaerobic bacterium, are technically challenging. Anaerobic bacteria have distinct nutritional requirements and life cycles compared to aerobic bacteria. Anaerobic bacteria require specialized culture conditions, display long growth cycles, and are highly sensitive to oxygen, with trace amounts of oxygen in the atmosphere potentially causing irreversible damage. These factors have made the primary culture of anaerobic bacteria from canine PD difficult. No previous studies had successfully isolated *P. gulae* in China; however, successful culturing was needed for in-depth studies of canine PD.

Our findings revealed that the positive detection rate of *P. gulae* in the oral cavity of operating room veterinarians was higher than that of canine owners, while the positive detection rate for dentists was higher than that for general operating room veterinarians. This indicates that the risk of clinical veterinarians being colonized by *P. gulae* is higher than that of canine owners who live with their pet, while the risk of dentists being colonized was the highest due to long-term contact and exposure to a high-risk environment.

Close contact with canines is the most important risk factor for *P. gulae* colonization in canine owners. We calculated that the most intimate contact with canines, i.e., kissing or being licked on the face, would increase the risk of this bacterium colonizing the owner's mouth by nearly 140 times.

Smoking is a well-established risk factor for periodontal deterioration and periodontitis, which can increase the risk of PD by four times, and significantly impact the efficacy of PD treatment. Studies have shown that smoke aerosols from cigarettes can weaken the phagocytosis ability of neutrophils, thereby reducing the bacterial clearance and promoting bacterial colonization in the gums. In addition, smoking can also reduces blood flow and the gingival crevicular fluid volume of the gingiva, making it harder for immune cells to reach the gingival crevice.

The study, it was hypothesized that the positive rate of *P. gulae* colonization in canine owners would increase with the number of years they have own their dogs. However, as shown in Table 2, there was no positive

correlation between the two variables, indicating that the primary factor contributing to *P. gulae* colonization in owners was the degree of contact with their dogs. Even if owners had raised their canines for a long time, they were not be easily colonized by *P. galue* if there was no intimate contact behavior.

The analysis of veterinarians revealed that the risk of *P. gulae* colonization was primarily related to the degree of contact with ultrasonic dental cleaning. Among the veterinarians included in our study, 13 out of 25 who directly participated in ultrasonic scaling tested positive for *P. gulae* (52.0%), 10 out of 24 who were anesthetists tested positive (41.7%) and 3 out of 11 other veterinarians who did not participate in ultrasonic scaling tested positive (27.3%). Thus, dentists were the most high-risk group for *P. gulae* colonization, and should take the necessary precautions in their daily work.

Research has demonstrated that high levels of aerosol pollution are detectable for 30–60 minutes after the initiation of ultrasonic scaling, and the number of bacteria in these aerosols starts to decrease 10 minutes after ultrasonic scaling has ceased [23]. Serious aerosol pollution was detected within 150 cm around the mouth, with levels being particularly high within 50 cm around the mouth. With increased distance, the degree of pollution gradually decreased, but the difference was not significant.

We collected a total of 20 aerosol samples from the environment in an ultrasonic dental cleaning operating room in the animal hospital at China Agricultural University in Beijing. Different breeds, ages and feeding habits of the canines sampled may result in differences in the type and abundance of oral microorganisms. However, the results showed that *P. gulae* could be detected in the aerosol generated during ultrasonic dental cleaning, and this bacterium was detected in all OTUs, accounting for 6.12% of the total detected bacteria. These finding suggests that aerosols can be an important transmission route by which *P. gulae* may spread to veterinarians.

This is the first study in China on *P. gulae* and its transmission via ultrasonic dental cleaning from canines to veterinarians. Further studies are needed to minimize its impact of this bacterium on human and animal health.

Conclusion

P. gulae may colonize the human oral cavity through intimate contact with canines or by participating in dental ultrasonic scaling operations. Canine owners and veterinarians can use the information provided in this article to reduce their risk of becoming infected with animal-derived *P. gulae* by avoiding major risk factors. With the potential link between canine PD and human oral health, veterinarians could have a positive impact on the life quality of canines and potentially on the health of canine owners and dentists by controlling suspected pathogenic bacteria in canine PD.

Materials and methods

Sampling standards

Diagnostic grading criteria were referenced from the Saunders Solution in Veterinary Practices Small Animal Dentistry for PD. The inclusion criteria for the sample were as follows: canines meeting the diagnostic criteria for PD without serious complications; aged 3 to 10 years; no PD treatment within the past 6 months; no antibiotics or NSAIDs used in the last 3 months; and owners agreed to the sampling procedure, understood the purpose of the trial, and were willing to participate in this clinical trial. The exclusion criteria were as follows: canines with PD younger than 3 years of age or older than 10 years of age; canines with other serious diseases or tumors; and canines whose owners were not willing to cooperate with this clinical study.

Design of analysis variables

We designed different risk factor variables for different populations (canine owners or clinical veterinarians) and collected data through questionnaires. The variables of the canine owners included sex, age, degree of contact with canines, systemic medical history, smoking, drinking, oral health and the daily frequency of teeth brushing. The variables of the veterinarians were adjusted according to the working environment and included sex, age, degree of contact and frequency of ultrasonic dental cleaning, years of work, domestic pets, intimate behaviors with pets, smoking and oral health. The positive detection rate of *P. gulae* was taken as a basic variable, and the association with the above variables was inferred through analysis.

Clinical sampling

Sterilized triangular fiber chromatography filter paper (cut into a right triangle with a height of 10 mm and a base length of 5 mm, was sterilized by ultraviolet irradiation for 30 minutes). The filter paper was inserted into the gingival crevices on the buccal surface of the right mandibular canine teeth of the subject on both the left and right sides. After 10 seconds, the filter paper was removed and placed into a centrifuge tube (121 °C for 30 minutes) containing 1.5 mL of sterile physiological saline. The examinees were surveyed by questionnaire and information for all of the variables was recorded. The samples were stored at 4 °C after collection, and PCR

detection was carried out within 1 week. For long-term storage, samples were stored at −20 °C.

Bacterial isolation

The collected gingival crevicular fluid samples were shaken and then spread, using disposable loops, onto four areas of 10% BHI blood plates containing hemin chloride (5 μg/mL) and VK1 (1 μg/mL). Plates were then incubated at 37 °C and 80% humidity, with a triple gas mixture (80% N_2, 10% H_2, 10% CO_2) in an anaerobic environment for 7–15 days. Single colonies were selected and inoculated again for purification. The resulting single colonies were then inoculated with BHI liquid medium supplemented with 5% FBS, yeast extract (1 mg/mL), hemin chloride (5 μg/mL) and VK1(1 μg/mL) to enrich the bacteria. The cultured bacteria were stored at −80 °C. The American Type Culture Collection (ATCC) strain 51700 of *P. gulae* was used as a quality control bacterium.

16S rRNA gene sequencing

PCR was performed using KOD One™ PCR master mix Toyobo (Osaka, Japan) with bacterial 16S rRNA gene universal primers 27F and 1492R. After agarose gel electrophoresis, the band size and uniqueness of the PCR products were determined, and the PCR products were sent to GENEWIZ (Jiangsu, China) for one-generation Sanger sequencing. The resulting sequences were checked using the Ape app and Blast homology comparisons were performed with known sequences in the NCBI GenBank database (www.ncbi.nlm.nih.gov/genbank/). Homology with known sequences showing >99% similarity was considered as confirmation of the same bacterium, and the results were accurate to the species level.

Statistical analyses

The collected variable data were processed, and analyzed using SPSS. A single sample *t*-test was conducted to determine the confidence interval for the positive detection rate of *P. gulae*.

Logistic regression was used as the multivariate analysis method.

The binary logistic regression model was expressed mathematically as follows:

$$\log\left(p/(1-p)\right) = \alpha + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_n x_n$$

In the above model, p on the left side of the equation was the probability that *P. gulae* was positively detected, α was the slope of the equation, which corresponded to the ln value of the ratio (odds) of the number of positive samples, and x was the independent variable included in the test that may be related to whether the sample was positive, β was the coefficient of each independent variable. The model calculates the β value for set β. The corresponding ratio of the variable could be obtained by the exponential function transformation of the value with the natural constant e as the base, so as to infer the correlation between the variable and the dependent variable, and to infer the risk factors.

$P < 0.05$ indicated that the difference was statistically significant.

Next, the model was tested to ensure that it met the necessary assumptions. The coefficient of variance inflation factor (VIF) was used to test multicollinearity. It was generally considered that if VIF was greater than 5, there was multicollinearity, and if VIF was greater than 10, there was high-level multicollinearity.

Then, we classified the data through the decision tree model to achieve a prediction. Decision tree models are widely used in classification prediction because they can display interaction effects among variables. The advantage of the stochastic forest model is that it can evaluate the relative importance of input variables while training the model. The importance of variables could reflect the relative contribution of characteristic variables in the model, and the importance of each variable to the model could be evaluated through out-of-pocket errors. A regression tree is a type of decision tree and a component unit of a random forest. Its establishment process is based on the tree structure and mainly consists of root nodes, internal nodes and leaf nodes. The root node is at the top. The establishment process of the tree is the process of node differentiation. Each node division results in one more node. The root node is divided into internal nodes. When the conditions for the end of the division are met, the output of each leaf node can be determined. As the complexity of the model increases, the size of the regression tree also increases. When we input the relevant information for each case in our study, the decision tree model was able to predict whether a case would be colonized with *P. gulae.*

Logistic regression could express the dependency relationship between dependent variables and their respective variables, while the decision tree model represented the interaction between variables. The decision tree model and logistic regression model complement each other, thereby more fully explaining the relationship between variables.

The software used was python 3.11.1, and the analysis and calculation results were tabulated.

Aerosol sampling and analysis

When a canine with severe dental calculus or PD was undergoing ultrasonic scaling, a Petri dish with a sterilized quartz fiber filter membrane (diameter: 90 mm) was opened and placed 10 cm away from the canine's

mouth to collect the aerosol directly. The collection time was from the beginning of ultrasonic scaling to 10 minutes after surgery. After sample collection, the filter membrane was placed in a sterile self-sealing bag using sterilized forceps, which was sealed and then stored prior to 16S rRNA gene detection.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44280-023-00007-x.

Additional file 1. Decision tree model operation diagram of canine owners. Decision tree model operation diagram of veterinarians.

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Authors' contributions
Y.B. and P.S. designed the experiments; Y.B., P.S. and Z.J. executed the experiments in collection of clinical samples; P.S. executed the experiments in isolation of strains; Y.B. and P.S. executed in sequencing and analyzing the data; Y.B. and P.S. wrote the manuscript; Z.S. and H.S. participated in critical review of the manuscript; J.L. and Y.J. participated in the conception and experimental design and critical review of the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Availability of data and materials
Data will be shared upon request by the readers.

Declarations

Ethics approval and consent to participate
All animal studies were reviewed and approved by China Agricultural University Laboratory Animal Welfare and Animal Experimental Ethical Committee (Approval ID: AW31103202–2-4).

Consent for publication
All patients signed informed consent to publish their personal details in this article.

Competing interests
There are no competing interests to declare.

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