Diversity of Fimbrillin among *Porphyromonas gulae* Clinical Isolates from Japanese Dogs

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ABSTRACT. *Porphyromonas gulae*, a gram-negative black-pigmented anaerobe, is a pathogen for periodontitis in dogs. An approximately 41-kDa fimbrial subunit protein (FimA) encoded by *fimA* is regarded as associated with periodontitis. In the present study, the *fimA* genes of 17 *P. gulae* strains were sequenced, and classified into two major types. The generation of phylogenetic trees based on the deduced amino acid sequence of FimA of *P. gulae* strains along with sequences from several strains of *Porphyromonas gingivalis*, a major cause of human periodontitis, revealed that the two types of FimA (types A and B) of *P. gulae* were similar to type I FimA and types II and III FimA of *P. gingivalis*, respectively. A PCR system for classification was established based on differences in the nucleotide sequences of the *fimA* genes. Analysis of 115 *P. gulae*-positive oral swab specimens from dogs revealed that 42.6%, 22.6%, and 26.1% of them contained type A, type B, and both type A and B *fimA* genes, respectively. Experiments with a mouse abscess model demonstrated that the strains with type B *fimA* caused significantly greater systemic inflammation than those with type A. These results suggest that the FimA proteins of *P. gulae* are diverse with two major types and that strains with type B *fimA* could be more virulent.

KEY WORDS: fimbriae, genotype, periodontitis, *Porphyromonas gulae*, virulence.

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Periodontal diseases are characterized by gingivitis, a reversible condition involving gingival inflammation, and periodontitis, the irreversible destruction of periodontal tissues such as cementum, periodontal ligaments, and supportive bone [15]. In dogs, the prevalence of gingivitis is 95–100% and that of periodontitis, 50–70%, and the severity of periodontal disease tends to worsen with age, as in humans [8, 10].

Numerous periodontitis-related bacterial species have been reported in humans, with *Porphyromonas gingivalis*, a gram-negative black-pigmented anaerobe, considered a major species [15]. The 41-kDa cell surface protein fimbriin (FimA), a subunit of fimbriae, is an important virulence factor of periodontitis, associated with adhesion to and invasion of gingival cells [4]. There are six genotypes of *fimA* encoding FimA (I through V and Ib), among which types II/IV/Ib and I/III/V are considered invasive and non-invasive,

respectively [2, 3, 11–14, 17].

A previous analysis of 11 periodontitis-related bacterial species in oral swab specimens collected from 26 pet dogs in Japan showed that the isolation frequency of *P. gingivalis* was extremely low in dogs [9]. In contrast, *Porphyromonas gulae* was frequently detected in these specimens, accounting for approximately 70% of all *Porphyromonas* isolates. A recent study revealed the presence of a 41-kDa FimA, similar to that in *P. gingivalis*, in *P. gulae* [7]. In the present study, we performed molecular analyses of *fimA* in many clinical strains of *P. gulae* focusing on the association between the gene's diversity and virulence.

MATERIALS AND METHODS

Strains and culture conditions: Table 1 lists the *P. gulae* strains analyzed in the present study, 10 of which were isolated in Tokyo, Japan, previously [9]. The 7 new clinical strains were isolated from 7 dogs in Tokyo, Japan, by a method described previously [9]. Briefly, specimens collected from the gingival margin of the right fourth maxillary premolar using swabs (Seed-Swab^R γ-1 or 2, Eiken Chemical Co., Ltd., Tokyo, Japan) were directly streaked on culture plates (Trypticase Soy Agar, Becton, Dickinson & Co,

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Table 1. *P. gulate* and *P. gingivalis* strains used

Species	Strain	<i>fimA</i> types	Length of <i>fimA</i> gene (bp)	Accession Numbers	References
<i>P. gulate</i>	ATCC 51700 ^{a,b)}	A	1152	AB297918	[7]
	D024	A	1152	AB663087	This study
	D025	A	1152	AB663088	This study
	D028	A	1152	AB663089	This study
	D034 ^{a)}	A	1152	AB663090	This study
	D035 ^{a)}	A	1152	AB663091	This study
	D036 ^{a)}	A	1152	AB663092	This study
	D042	A	1152	AB663093	[9]
	D043	A	1152	AB663094	[9]
	D060	A	1152	AB663095	[9]
	D066	A	1152	AB663096	[9]
	D067	A	1152	AB663097	[9]
	D068	A	1152	AB663098	[9]
	B43	B	972 ^{c)}	CS228034	[5]
	D040 ^{a)}	B	1161	AB663099	[9]
	D044 ^{a)}	B	1161	AB663100	[9]
	D052	B	1161	AB663101	[9]
	D053	B	1161	AB663102	[9]
	D077	B	1161	AB663103	This study
<i>P. gingivalis</i>	381	I	1044	D17794	[3]
	ATCC 33277 ^{b)}	I	1044	D17795	[3]
	BH18/10	I	1044	D17796	[3]
	HW24D1	II	1047	D17797	[3]
	OMZ314 ^{a)}	II	1044	D17798	[3]
	OMZ409	II	1047	D17799	[3]
	ATCC 49417	III	1053	D17800	[3]
	6/26	III	1062	D17801	[3]
	HG564	IV	1083	D17802	[3]
	HNA99	V	1104	AB027294	[11]
	HG1691	Ib	1044	AB058848	[12]

a) Strains analyzed in the mouse abscess model.

b) Type strains.

c) Only a partial sequence corresponding to the *fimA* of *P. gulate* is available.

Franklin Lakes, NJ, U.S.A.) supplemented with 5% horse defibrinated blood (TS) or TS with hemin (50 mg/ml) and menadion (5 mg/ml) (mhTS), and cultured under anaerobic conditions for 7 to 10 days. Four representative colonies selected at random from each plate were pure-cultured for 4 to 7 days. The strains were stored in TS broth with 10% glycerine at -80°C until use.

The isolated strains were confirmed as *P. gulate* based on molecular biological analyses described previously [9]. Briefly, genomic DNA of each strain was extracted using a commercial kit (Puregene Yeast/Bact. Kit B, QIAGEN Inc., Valencia, CA, U.S.A.), and a broad-ranging PCR was performed using TaKaRa Ex Taq (Takara Bio. Inc., Otsu, Shiga, Japan) with the primer fD1 and rP2 targeting the 16S rRNA gene (Table 2) [18]. PCR products were directly sequenced and the 16S rRNA sequences obtained were compared with those available in databases from the National Center for Biotechnology Information server (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Identification at the species level was defined as a 16S rRNA sequence similarity of more than 99% with

that of *P. gulate* type strain ATCC 51700.

The *P. gingivalis* strains with various *fimA* genotypes used in the present study are also listed in Table 1. *P. gingivalis* and *P. gulate* strains were cultured in mhTS broth under anaerobic conditions for 2 days when the extraction of genomic DNA and preparation of the bacterial suspension for animal experiments were performed.

Sequencing and characterization of *fimA* genes: The entire *fimA* gene was sequenced as follows. First, PCR was performed with genomic DNA extracted from *P. gulate* strains using the six *P. gingivalis* *fimA*-specific sets of primers listed in Table 2 [3, 11, 12]. Since the *P. gulate* strains tested showed positive reactions to the type I and III sets, we designed primers (33277-F and 33277-R; 6/26-F and 6/26R) based on the adjacent sequence of ATCC 33277 (*fimA* type I) and of 6/26 (*fimA* type III). PCR was performed using these primers to amplify the entire *fimA* gene of *P. gulate* by TaKaRa ExTaq under the following conditions: an initial denaturation at 95°C for 4 min and then 30 cycles at 94°C for 30 sec, 50°C for 30 sec, and 72°C for 30 sec with a final

Table 2. Primers used for PCR

Specific primer set	Sequence (5' to 3')	References
Amplification of 16S rRNA		
fD1 ^{a)}	AGA GTT TGA TCC TGG CTC AG	[18]
rP2 ^{a)}	ACG GCT ACC TTG TTA CGA CTT	
Detection of <i>P. gulae</i>		
<i>P. gulae</i> 16S rRNA	TTG CTT GGT TGC ATG ATC GG GCT TAT TCT TAC GGT ACA TTC ACA	[9]
Determination of <i>fimA</i> sequence		
33277-F ^{a)}	TTC ATA CGT CGA CGA CTG CG	This study
33277-R ^{a)}	TTG AGG GTT GAT TAC CAA GT	
6/26-F ^{a)}	AAC TAC GAC GCT ATA TGC AA	This study
6/26-R ^{a)}	TAG ACA AAC TAT GAA AGT T	
Detection primers for <i>P. gulae fimA</i> types		
type A <i>fimA</i>		
Pgufim-AF ^{a)}	TGA GAA TAT CAA ATG TGG TGC AGG CTC ACG	This study
Pgufim-AR ^{a)}	CTT GCC TGC CTT CAA AAC GAT TGC TTT TGG	
type B <i>fimA</i>		
Pgufim-BF ^{a)}	TAA GAT TGA AGT GAA GAT GAG CGA TTC TTA TGT	This study
Pgufim-BR ^{a)}	ATT TCC TCA GAA CTC AAA GGA GTA CCA TCA	
Detection primers for <i>P. gingivalis fimA</i> types		
Type I <i>fimA</i>	CTG TGT GTT TAT GGC AAA CTT C AAC CCC GCT CCC TGT ATT CCG A	[3]
Type II <i>fimA</i>	ACA ACT ATA CTT ATG ACA ATG G AAC CCC GCT CCC TGT ATT CCG A	[3]
Type III <i>fimA</i>	ATT ACA CCT ACA CAG GTG AGG C AAC CCC GCT CCC TGT ATT CCG A	[3]
Type IV <i>fimA</i>	CTA TTC AGG TGC TAT TAC CCA A AAC CCC GCT CCC TGT ATT CCG A	[3]
Type V <i>fimA</i>	AAC AAC AGT CTC CTT GAC AGT G TAT TGG GGG TCG AAC GTT ACT GTC	[11]
Type Ib <i>fimA</i>	CAG CAG AGC CAA AAA CAA TCG TGT CAG ATA ATT AGC GTC TGC	[12]

a) Names of primers are indicated.

extension at 72°C for 7 min. Then, electrophoresis was done on a 0.7% agarose gel, and the amplified DNA fragments were extracted from the gel, using a QIAEX gel extraction kit (Qiagen, Düsseldorf, Germany). The DNA was directly cloned into a pGEM-T Easy vector (Promega, Madison, WI, U.S.A.), which was transformed into *Escherichia coli* XL-2. The plasmid containing the target gene was extracted using a FastGene Plasmid Mini Kit (NIPPON Genetics, Tokyo, Japan) and the nucleotide sequence was determined using a dye-terminator reaction with a DNA sequencing system (373–18 DNA sequencer; Applied Biosystems, Foster City, CA, U.S.A.) and an ABI PRISM kit. Data analyses were performed using Gene Works software (IntelliGenetics, Mountain View, CA, U.S.A.). The deduced amino acid sequences of the FimA proteins of all *P. gulae* strains tested and of *P. gingivalis* strains with *fimA* genotypes I through V and Ib listed in Table 1 were compared using the neighbor-joining method to construct a phylogenetic tree using CLUSTAL W (DNA Databank of Japan; <http://clustalw.ddbj.nig.ac.jp/top-j.html>) and Tree View software (<http://taxonomy.zoology.gla.uk/rod/treeview.html>).

Construction of a PCR method to classify fimA types: Table 2 lists the PCR primers used. Those for the classification of *fimA* type A and B were designed based on the *fimA* sequence of *P. gulae* determined in the present study. The specificity of these primers was tested with the program Amplify [6], based on information on the DNA sequence of *P. gulae* strains obtained in the present study as well as that of *P. gingivalis* in the GenBank database. The specificity of the primers was also confirmed using GenBank data. The amplification was performed in a total volume of 20 µl with 1 µl of template solution using TaKaRa Ex Taq. The PCR was performed in a thermal cycler (iCycler; Bio-Rad, Hercules, CA, U.S.A.) with the following parameters: an initial denaturation at 95°C for 4 min and then 30 cycles of 95°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, with a final extension at 72°C for 7 min. The PCR products were subjected to electrophoresis in a 1.5% agarose gel-Tris-acetate-EDTA buffer. The sensitivity of the PCR assay was determined using serial dilutions of titrated *P. gulae* cultures in sterile distilled water.

Distribution of fimA types in P. gulae-positive oral swab

specimens: All study protocols were approved by the Animal Research Committee of Azabu University. Prior to collection of the specimens, the owners of all the dogs were informed of the contents of the present study and gave approval for their participation. Oral specimens were previously collected from one specific location, the gingival margin of the right fourth maxillary premolar, in 26 dogs using swabs (Seed-Swab^R γ -1 or 2) [9]. In the present study, 102 additional specimens were collected to give a total of 128. Mean age was 9.62 (2–16) years. These dogs came to the clinic for health checkups, vaccinations, coxarthropathy, external otitis, external disc disease, trauma, and diarrhea. All were reported to be kept indoors. First, bacterial DNA was extracted from each specimen and PCR was performed using *P. gulae*-specific sets of primers [9], as described above. Then, types of *fimA* were determined by a method established in the present study. For *P. gulae*-specific PCR positive but types A and B *fimA*-specific PCR negative specimens, the amplified *P. gulae*-specific PCR products were sequenced to confirm

that they were indeed 16S rRNA gene sequences of *P. gulae*.

Mouse abscess model: All procedures and protocols involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of Osaka University Graduate School of Dentistry prior to the experiments. The virulence of 6 *P. gulae* strains including ATCC 51700 as compared to *P. gingivalis* OMZ314 was evaluated using a mouse abscess model constructed for the evaluation of *P. gingivalis* strains with some modifications as follows [14]. Female BALB/c mice (5 weeks old) were maintained in horizontal-flow cabinets and provided with sterile food and water *ad libitum*. In all, 80 mice were randomly divided into 8 groups (6 *P. gulae*, 1 *P. gingivalis* and 1 phosphate-buffered saline (PBS) groups) to examine inflammatory changes caused by the infection. At 40 days of age, a single site approximately 1 cm lateral from the midline on the dorsal surface was depilated, and 0.1 ml of bacterial suspension (1×10^9 colony-forming units (CFU) of a test strain) or PBS (control group) was injected subcutaneously. For quantita-

			↓		
ATCC51700-A	1	MKKTFFLLGLAALAMTACNKDNEAPVVEGNATISVVLKTSNPNRAFGV		50	
D067-A	1			50	
D077-B	1	I.....		50	
ATCC51700-A	51	ADDEAKVAKLTVMVYNGEQEAIESAENATKIKCGAGSRTLVMANT		100	
D067-A	51			100	
D077-B	51	K.....I.V.....		100	
ATCC51700-A	101	GTMDLTGKTLADV KALTTeltaENQEAAGLIMTAEPKAIVLKAGKNYIGY		150	
D067-A	101			150	
D077-B	101	.G.E.A.....E.....T.....VDVT.V..N..Y..		150	
ATCC51700-A	151	NGAGEGNHIEND-PLKIKRVHARMAFTEIKVQMSAAYDNIYTFAPKIKYIG		199	
D067-A	151			199	
D077-B	151	D.TQG..Q.SQGT.....I...K.E.K..DS.V.K.N.T..N..A		200	
ATCC51700-A	200	LIAKKQSNLFGATLVNADANYLTGSLTTFNGAYTPANYANVPWLSRNYVA		249	
D067-A	200			249	
D077-B	201	.V...K.....TS.A.S.DA.....TH.A..G.G.T.		250	
ATCC51700-A	250	PTANAPQGFYVLENDYSANGGTIHPTILCVYGLQKN-GADLAGADLAAA		298	
D067-A	250			298	
D077-B	251	.SND.....SA.AQ.A.-LR.....K...T.HD.TP.SSEEMT..		299	
ATCC51700-A	299	QAANWV--DAEGKTYYPVLVNFNSNNYTYDNGYTPKNKIERNHKYDIKLT		346	
D067-A	299			346	
D077-B	300	FNVG.IVANNDPT.....E.....TGDAVE.G..V...F..N..		349	
ATCC51700-A	347	ITGPGTNNPENPITESAHLNVQCTVAEWVLVGQNATW		383	
D067-A	347			383	
D077-B	350	N...N.V..A.KG.V..VI.		386	

Fig. 1. Multiple alignments of deduced amino acid sequence of the FimA encoded by type A and B *fimA* genes of *P. gulae* compared to reference strain ATCC 51700. Only amino acid residues different from ATCC 51700 are presented. Identical amino acids are indicated by dots. The arrow indicates the cleavage site estimated by the SignalP 3.0 Server (<http://www.cbs.dtu.dk/services/SignalP/>).

tive evaluation of the infectious inflammatory change, serum C-reactive protein (CRP) was measured as follows. Blood specimens (0.1 ml) were collected from an orbital vein on days 0, 1, 2, 4, 7 and 14 after bacterial infection, and then centrifuged at 3,000 rpm. for 10 min to separate the serum. CRP concentrations in sera were colorimetrically quantified using a commercial kit (MOUSE C-REACTIVE PROTEIN (CRP) ELISA TEST KIT; Life Diagnostics, Inc., West Chester, PA, U.S.A.) according to the manufacturer's instructions. The mice were also monitored for signs and symptoms of infection, i.e. ruffled hair, abscess formation and emergence of erosion, as described previously [14]. Two weeks after infection, all mice were killed under ether anesthesia, and the spleens were extirpated and weighed. Spleen weight values were standardized by body weight.

Statistical analyses: Statistical analyses were performed using the computational software package Prism 4 (Graph-Pad Software Inc., La Jolla, CA, U.S.A.). Intergroup differences were estimated using Bonferoni's method after an analysis of variance (ANOVA). A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Diversity of *fimA* sequences in clinical strains: The *fimA* genes of 17 *P. gulae* strains (deposited in the DNA Database of Japan under accession nos. AB663087-AB663103) were sequenced, and found to mainly fall into 2 *fimA* types. One type had a sequence of 1152 bp similar to ATCC 51700 and was designated type A. The length of the *fimA* gene in the other type, type B was 1161 bp. Figure 1 shows the deduced amino acid sequence of strains D067 (type A) and D077 (type B) as compared to that of *P. gulae* ATCC 51700 (type A). The FimA in D067 and that in *P. gulae* ATCC 51700 showed 100% identity. The FimA in D077 and that in *P. gulae* ATCC 51700 showed 73% identity. The phylogenetic tree showed that type A FimA of *P. gulae* was close to type I FimA of *P. gingivalis* (86–93% identity), whereas type B FimA of *P. gulae* was close to types II and III FimA of *P. gingivalis* (86–92% identity) (Fig. 2).

PCR system to identify *fimA* types of *P. gulae*: The primers used to identify type A and B *fimA* genes of *P. gulae* are listed in Table 2. Figure 3A illustrates the positions of those primers. The estimated size of the amplified fragments of *fimA* type A and B was 190 bp and 357 bp, respectively.

Fig. 2. Evolutionary relationship based on deduced amino acid sequences of FimA in *P. gulae* and *P. gingivalis*. The neighbor-joining method was used to construct a phylogenetic tree with CLUSTAL W (DNA Databank of Japan) and Tree View software. Pgu and Pgi indicate *P. gulae* and *P. gingivalis*, respectively. The roman numerals following Pgi give the *fimA* genotypes of *P. gingivalis*. The numbers shown in the tree are bootstrap values.

Fig. 3. Construction of the primers specific for type A and B *fimA* of *P. gulae*. (A) The position of the primers for each *fimA* type. (B) Specificity of the PCR method used in the present study. W, sterile water. M, molecular size marker (100-bp DNA ladder). (C) Sensitivity of the PCR method examined using titrated cultures of *P. gulae* strains ATCC 51700 (type A) and D040 (type B). W, sterile water. M, molecular size marker (100-bp DNA ladder).

The specificity of this PCR system was confirmed using the strains listed in Table 1, which demonstrated that primers designed for type A and B *fimA* genes of *P. gulae* did not generate positive bands from *P. gingivalis* strains tested. In addition, the sensitivity was shown to be approximately 100 CFU per reaction (Fig. 3B and 3C).

Prevalence of *fimA* types in clinical specimens: PCR analyses of 128 specimens using *P. gulae*-specific primers revealed that 115 were positive. PCR for typing of *fimA* of *P. gulae* showed that 49 (42.6%) and 26 (22.6%) specimens were positive for *fimA* type A and B, respectively. In addition, 30 specimens (26.1%) were positive for both *fimA* types. As for the remaining 10 specimens (8.7%), which were negative for *fimA* type A and B, the sequence of 16S rRNA gene fragments amplified by *P. gulae*-specific PCR had 100% homology with that of *P. gulae* type strain ATCC 51700.

Evaluation of virulence in the mouse abscess model: Table 3 summarizes the results of the experiment with the mouse abscess model. Subcutaneous injections of strains with type B *fimA* (D040 and D044) caused large abscesses to form similar to OMZ314. Serum CRP values at 2 days post-infection were significantly higher than for PBS ($P<0.05$), and the spleens extirpated at 14 days after infection were also significantly heavier ($P<0.05$). As for the mice inoculated with strains with type A *fimA*, the intensity of inflammation tended to be higher than that of PBS-inoculated mice, but the difference was not significant.

DISCUSSION

This is the 1st study to demonstrate the presence of 2 major *fimA* types of *P. gulae* using clinical isolates from dogs. The FimA of *P. gulae* was first demonstrated by Hamada *et al.* [7] who reported 96.8% identity between the FimA of ATCC 51700 and *P. gingivalis* ATCC 33277. Hamada *et al.* [7] analyzed one more strain, B43 [5], in a phylogenetic tree based on deduced amino acid sequences of FimA. They translated the *fimA* sequence of B43 into amino acids based on data in GenBank (accession number CS228034). The sequence was 16% incomplete, however, it belongs to *fimA* type B.

Analyses of *fimA* in *P. gulae* strains from our laboratory

Table 3. Infectious inflammation by *P. gulae* as compared with *P. gingivalis* OMZ314 evaluated with the mouse abscess model

Inoculated strains	<i>fimA</i> types	CRP values at 2 days after infection [$\mu\text{g/ml}$ (mean $\pm$ SE)]	Symptom (no. of mice /total)	Spleen weight at 14 days after infection [mg/g body weight (mean $\pm$ SE)]
OMZ314	II	5.59 $\pm$ 0.35 ^b	Abscess formation (9/10)	5.06 $\pm$ 0.27 ^a
D044	B	11.63 $\pm$ 0.69 ^c	Abscess formation (7/10)	5.33 $\pm$ 0.28 ^a
D040	B	5.35 $\pm$ 0.25 ^a	Abscess formation (10/10)	5.04 $\pm$ 0.30 ^a
ATCC 51700	A	4.63 $\pm$ 0.36	Ruffled hair (10/10)	4.35 $\pm$ 0.14
D035	A	4.33 $\pm$ 0.23	Ruffled hair (4/10)	4.26 $\pm$ 0.18
D036	A	4.56 $\pm$ 0.53	Ruffled hair (4/10)	4.09 $\pm$ 0.10
D034	A	4.68 $\pm$ 0.51	Ruffled hair (6/10)	3.67 $\pm$ 0.09
PBS	-	3.21 $\pm$ 0.35	None	3.79 $\pm$ 0.20

Statistically significant difference with the PBS group (^a $P<0.05$, ^b $P<0.01$ and ^c $P<0.001$) by Bonferonni's method after ANOVA.

stock showed the prevalence of *fimA* type A and B to be 72.3% and 27.3%, respectively. Among the 115 *P. gulae*-positive oral swab specimens from dogs, approximately 70% had type A FimA and approximately 50% type B. Furthermore, there were several specimens positive for both *fimA* types. These results suggest that type A *fimA* could be more prevalent in dogs and that dogs carrying both strain with type A *fimA* and that with type B *fimA* are present. In addition, there are several *P. gulae*-positive specimens negative for type A and type B, indicating the presence of *P. gulae* strains without FimA. Alternatively, there might be additional *fimA* types. Further investigation of the *P. gulae* strains isolated from these specimens and determination of *fimA* sequences will be needed.

In *P. gingivalis*, the genotypic variation of *fimA* was suggested to be related to the severity of human periodontitis. *P. gingivalis* has been isolated from both periodontally healthy individuals and patients with periodontitis, though the distribution of *fimA* types was very different between the two groups [2]. In fact, strains with type II *fimA* were more virulent than those with type I *fimA* in a mouse abscess model [14]. Earlier studies obtained *P. gingivalis*-like isolates (likely *P. gulae*) from both healthy and diseased gingival pockets in dogs [1, 16]. In our previous study, *P. gulae* was detected in approximately 90% of oral swab specimens from Japanese dogs, which came to veterinary clinics for health checkups and vaccinations [9]. This evidence suggested the variation of *fimA* types in *P. gulae* to be associated with the virulence of periodontitis in dogs. The experiment using the mouse abscess model demonstrated that strains with type B *fimA* caused more severe systemic inflammation than those with type A. However, the mouse model may not necessarily reflect the virulence of periodontitis in dogs. Further studies should focus on elucidating the association between *fimA* type and periodontitis in dogs.

In summary, this is the first study to classify clinical isolates of *P. gulae* based on FimA. The distribution of the different *fimA* types in subjects with periodontitis should be analyzed further using large-scale studies.

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