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Activity of Pradofloxacin against *Porphyromonas* and *Prevotella* spp. Implicated in Periodontal Disease in Dogs: Susceptibility Test Data from a European Multicenter Study▽

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Collaborating veterinarians from five European countries collected subgingival bacterial samples from dogs exhibiting clinical periodontal disease. Sterile endodontic paper points were used for collection of the samples, which were transported to a central laboratory for susceptibility testing. Anaerobic bacteria were isolated and *Porphyromonas* and *Prevotella* isolates identified to the species level; susceptibility to pradofloxacin and metronidazole was determined using the CLSI agar dilution methodology. A total of 630 isolates, 310 of *Porphyromonas* spp. and 320 of *Prevotella* spp., were isolated. Pradofloxacin MIC data for all isolates were in the range of ≤0.016 to 1 μg/ml, the overall MIC_{50} was 0.062, and the overall MIC_{90} was 0.25 μg/ml. There were no differences in activity against *Porphyromonas* and *Prevotella* isolates or in the pradofloxacin susceptibility distributions from the different European countries. All isolates were within the wild-type distribution and were fully susceptible to pradofloxacin. Metronidazole was also highly active against these strains: 316 of 320 *Prevotella* strains (98.8%) and 309 of 310 *Porphyromonas* strains (99.7%) were susceptible (MICs of ≤8 μg/ml). However, three *Prevotella* strains had intermediate metronidazole susceptibility (MICs of 16 μg/ml), while one *Prevotella* and one *Porphyromonas* strain were metronidazole resistant (MICs of 128 and 256 μg/ml, respectively). Pradofloxacin, a novel broad-spectrum fluoroquinolone, demonstrates a high degree of antianaerobic activity against strains isolated from clinical cases of periodontal disease and shows activity against metronidazole-resistant isolates. The broad-spectrum activity of pradofloxacin makes it a suitable candidate for the treatment of periodontal disease in dogs.

Periodontal disease is a chronic, multifactorial disease of the tissues supporting the teeth (28, 35), and the significance of microorganisms in the development of all types of periodontal disease is indisputable. It is microbial density that is considered critical for the development of gingivitis and some types of chronic periodontitis, while the types of the microorganisms may be of greater importance in the initiation of aggressive periodontitis (41). Indeed, it is now well accepted within the dental research community that periodontal disease results not just from simple accumulation of volume of dental plaque but from the growth and dominance of specific pathological organisms following the development of complex plaque. This thinking has arisen from studies of human disease, and indeed, much of the published data concerning the etiology of periodontitis comes from the human arena, where one of the primary periodontal pathogens is considered to be *Porphyromonas gingivalis* (7, 34, 35).

Periodontitis in companion animals is a disease almost identical to that in humans in terms of disease course and clinical presentation (17). It has been estimated that approximately 80% of dogs and cats demonstrate some degree of periodontal disease by 4 years of age (19). It is a serious condition that threatens all dogs and is among the most common disorders seen in veterinary medicine (31). The accelerated disease progression observed in companion animals compared to that observed in humans may be due to a relative lack of routine dental care (18, 30). Companion animal periodontitis is a serious infection that can have medical consequences such as anorexia and weight loss, chronic pain, sore or loose teeth, swollen gums, tooth decay, breakage or loss of teeth, and breakage of the maxillary or mandibular bone (18). If left untreated, periodontal bacteria may spread to other sites in the body via bacteremia (5, 32) and lead to renal, coronary, or hepatic diseases (9, 33, 39). As virtually all cases of periodontal disease are bacterial disorders, they can be prevented or effectively treated by controlling pathogenic microbes residing in subgingival and supragingival plaque (41). For humans and dogs, the dental practitioner has relied heavily upon mechanical debridement in combating periodontal infections (18, 41). There is evidence, however, that additional strategies, including the use of antimicrobials, are necessary to effectively combat periodontal infection, especially in the cases of sites with probing depths exceeding 5 mm (18, 36, 44). For periodontal therapy to be effective, it must at minimum be able to target and effectively control microorganisms capable of destroying periodontal connective tissue. It is well established that the microbial flora associated with periodontitis in humans and dogs is complex (8, 18), and in this context, it has been established for a number of years that the absence of black-pigmented anaerobic indicator bacterial species, such as *P. gingivalis* and *Prevotella intermedia*, was a better predictor of

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cessation of further loss of attachment than the presence of these species was for further disease progression (18). On this basis, it has been concluded that antimicrobial therapy can be of great use in the treatment of periodontal disease (38).

Pradofloxacin is a broad-spectrum fluoroquinolone and, like moxifloxacin, has enhanced activity against gram-positive bacteria relative to narrow- and extended-spectrum compounds and good activity against anaerobes (40). It has been exclusively developed for use in veterinary medicine but has not yet received regulatory approval, and it is distinguished from enrofloxacin, the first veterinary fluoroquinolone, by two structural elements: a bicyclic amine, *S*,*S*-pyrrolidino-piperidine, replacing the ethyl-piperazine moiety located at position C-7 of enrofloxacin, and a cyano group which is attached to the C atom at position 8. The increased potency of pradofloxacin is mainly attributed to the *S*,*S*-pyrrolidino-piperidine moiety at C-7, but the cyano group at C-8 extends activity to first- and second-step fluoroquinolone-resistant strains. Early data showed its potential for use against anaerobes (40), and in this paper, we report susceptibility data from a European multicenter study of strains isolated from cases of periodontal disease in dogs.

MATERIALS AND METHODS

Sample collection. Canine periodontal pockets were sampled by veterinarians in France, Germany, Italy, Poland, Sweden, and the United Kingdom in the period of 2004 to 2006. Each veterinary practice was provided with sterile endodontic paper points for collection of periodontal pocket samples and airtight (screw-cap) polypropylene microcentrifuge tubes containing approximately 1.5 ml of sterile anaerobic VMGA III medium (46). The tubes were filled in an anaerobic workstation, and minimal headspace was left at the top of each tube prior to dispatch.

Participating veterinary surgeons were all experienced with periodontal disease. Dogs showing periodontal disease of grade 2 or grade 3 (moderate to severe periodontal disease) were defined as suitable for collection of samples. All participating animals had not been pretreated with antimicrobials for any disease, by any route of administration, for at least 4 weeks prior to sampling. It was recommended that samples were to be collected during routine mechanical scaling or dental surgery while the dog was under general anesthesia. Only one sample was collected per tooth, but samples from more than one tooth from the same dog were acceptable, as periodontal disease is considered to be a disease of individual periodontal pockets rather than a generalized disease (18). However, the maximum number of samples per dog was restricted to one per quadrant of the dentition, giving a maximum of four samples per dog. Samples were shipped to a central laboratory for isolation, identification, and MIC determination of anaerobic bacteria and were processed within 24 to 48 h after sampling.

Sample processing and identification. Each transport medium containing a paper point was transferred unopened into an anaerobic workstation (Don Whitley Scientific Limited, Shipley, United Kingdom) at 35 ± 1°C. All culture media used for sample processing and bacterial subculture were prereduced by overnight storage in the anaerobic workstation.

With sterile forceps, paper points were removed from the transport medium and used to inoculate the surface of fastidious anaerobe agar (FAA) (LabM, LAB103; Bury, United Kingdom) and then placed into fastidious anaerobe broth (LabM, LAB071; Bury, United Kingdom). Agar plates and broth were incubated in an anaerobic workstation at 35 ± 1°C for up to 7 days and inspected on each working day. The inclusion of fastidious anaerobe broth was a precaution to ensure that the incubated liquid medium would provide an "enriched" sample for recovery of bacteria if no growth was obtained on FAA. In all cases, however, FAA plates yielded growth. Most samples yielded three or more distinct bacterial colony types, and from each plate, one colony of each discernible type was subcultured to fresh anaerobic FAA to obtain a pure culture for further identification. Only obligate anaerobes were selected for identification; the specimen collection and transport system allowed for recovery of genera other than *Porphyromonas* and *Prevotella*, but as the focus of this study was the latter two genera, isolation of other anaerobes was not pursued.

Gram-negative rods, or coccobacilli, were identified to the species level by using the Biolog AN MicroPlate system (Biolog Inc., Hayward, CA), containing a panel of 95 biochemical tests. The plates were used in conjunction with the Biolog MicroStation system and MicroLog 3 software. The designated quality control strains (*Bacteroides fragilis* ATCC 25285 and *Porphyromonas gingivalis* ATCC 33277) were used at all times, and Biolog results were considered valid only if the correct identification of each control strain was achieved.

Bacterial strains. In total, 310 strains of *Porphyromonas* spp. and 320 strains of *Prevotella* spp. were isolated and identified. Isolates were stored at −80°C in brain heart infusion broth plus 10% glycerol prior to testing. The *Porphyromonas* strains identified to the species level were *P. circumdentaria*/*P. endodontalis* (n = 126), *P. levii* (n = 49), *P. asaccharolytica* (n = 39), *P. macacae* (n = 39), *P. salivosa* (n = 33), and *P. gingivalis* (n = 24). The *Prevotella* strains were *P. heparinolytica* (n = 77), *P. corporis* (n = 34), *P. nigrescens* (n = 26), *P. oris* (n = 9), *P. disiens* (n = 8), *P. intermedia* (n = 6), *P. oralis* (n = 6), *P. denticola* (n = 4), *P. loescheii* (n = 3), *P. oulorum* (n = 2), *P. buccae* (n = 2), and *P. zoogleoformans* (n = 1). There were a number of *Prevotella* isolates for which species names could not be defined; these were referred to as *Prevotella* spp. (n = 142).

Susceptibility testing. The test compounds, pradofloxacin and metronidazole, were supplied with a certificate of analysis detailing purity. The MICs of both compounds were determined using the agar dilution methodology described by the Clinical and Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards), in complete accordance with the procedures detailed in document M11-A6, using brucella blood agar (D0964-17; Difco) supplemented with hemin and vitamin K, with incubation for up to 48 h. *Bacteroides fragilis* ATCC 25285 and *Eubacterium lentum* ATCC 43055 were used as quality control organisms. All susceptibility testing was carried out using an automated multipoint inoculator with 25 inoculating pins, under strict anaerobic conditions, within an anaerobic workstation (Don Whitley Scientific Limited, Shipley, United Kingdom).

RESULTS AND DISCUSSION

The samples yielded totals of 310 strains of *Porphyromonas* spp. and 320 strains of *Prevotella* spp. from cases of periodontal disease. There was good geographic distribution of the isolates (France, 26.8%; Poland, 23.9%; Germany, 18.3%; Sweden, 13.7%; Italy, 11.3%; and the United Kingdom, 6%). Within any one country, there was an almost equal distribution between the two genera, although in Germany, strains of *Prevotella* spp. made up only 41.7%, whereas in Italy, they predominated (64.8%) relative to the *Porphyromonas* strains. Clearly, both of these bacterial groups are implicated in periodontal disease. It is important to make the point that the identification of the isolates was carried out using the Biolog AN MicroPlate system, which is unable to distinguish between *P. gingivalis* and *Porphyromonas gulae*. *P. gulae* sp. nov. was proposed as a new species to include strains isolated from the gingival sulci of animal hosts, which were distinct from related strains of *P. gingivalis* of human origin (11). Irrespective of this finding, it is clear from the MIC distribution data (Fig. 1) that there are no differences in pradofloxacin susceptibility between these two species.

The summary MIC data are presented by country in Table 1, from which it can be seen that there are no differences in pradofloxacin susceptibility between the different countries. It is for this reason that the overall susceptibility distributions for the two genera are presented in Fig. 1, which clearly demonstrates that both genera are equally susceptible to pradofloxacin and exhibit the same wild-type distribution. On the basis of this distribution, there are no strains obviously carrying resistance determinants, and the respective populations are clearly fully susceptible to pradofloxacin. MIC data for the respective species of the tested isolates are shown in Table 2. As breakpoints have yet to be assigned to pradofloxacin, resistance rates

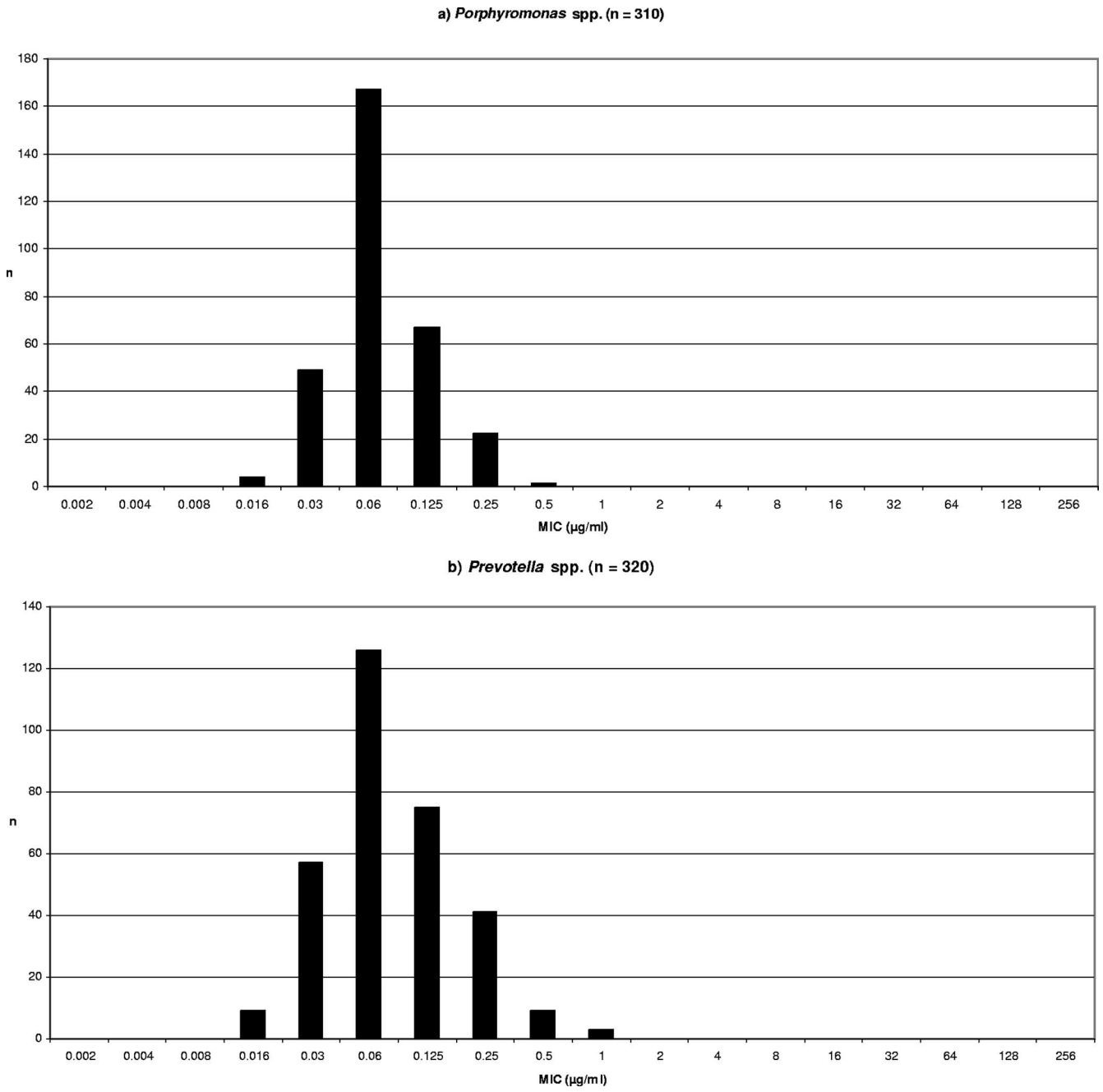

FIG. 1. MIC distribution for pradofloxacin of 310 *Porphyromonas* (a) and 320 *Prevotella* (b) strains isolated from cases of periodontal disease in dogs from a European multicenter study.

have not been reported, although as stated above, population distributions suggest an absence of resistance determinants.

While the metronidazole MIC data are similarly consistent for all countries and for both of the tested genera, strains that were outside the wild-type distribution were identified (Fig. 2). Three *Prevotella* strains had intermediate metronidazole susceptibilities (MICs of 16 μg/ml), while one *Prevotella* and one *Porphyromonas* strain were fully metronidazole resistant (MICs of 128 and 256 μg/ml, respectively). The fully resistant isolates were from Sweden, and those of intermediate susceptibility were from Sweden and Poland.

There is clearly a challenge in such multicenter studies in ensuring that the methodology for isolating anaerobes is appropriate and that isolation rates are not adversely affected by sampling. VMGA III transport medium has been evaluated (3, 8, 46) specifically for anaerobic organisms associated with periodontal disease. VMGA III is a gel-based transport medium, and it has been argued that this results in maintenance of a low redox potential, thereby contributing to the enhanced survival of obligate anaerobes. The medium has been evaluated in clinical studies as well as with pure cultures and has been shown to be suitable for longer periods of transport by mail as

TABLE 1. Susceptibilities of oral anaerobes by genus and country and results for all strains combined

Country(ies)	Value for[a]:																	
	Porphyromonas spp.							*Prevotella* spp.						All strains				
	No. of isolates	MIC (μg/ml)						No. of isolates	MIC (μg/ml)					No. of isolates	MIC (μg/ml)			
		PRA			MTZ				PRA			MTZ			PRA			MTZ	
		50%	90%	Range	50%	90%	Range		50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range
France	87	0.062	0.125	≤0.016–0.25	0.125	0.5	≤0.016–0.5	82	0.062	0.25	≤0.016–1	0.25	0.5	≤0.016–1	169	0.062	0.25	≤0.016–1	0.125	0.5	≤0.016–1
Poland	72	0.062	0.125	≤0.016–0.25	0.062	0.25	≤0.016–0.5	79	0.062	0.25	≤0.016–0.5	0.25	0.5	≤0.016–16	151	0.062	0.25	≤0.016–0.5	0.125	0.5	≤0.016–16
Germany	67	0.062	0.125	0.03–0.25	0.125	0.25	0.03–0.25	48	0.062	0.25	≤0.016–1	0.125	0.25	≤0.016–2	115	0.062	0.125	≤0.016–1	0.125	0.25	≤0.016–2
Sweden	43	0.062	0.125	≤0.016–0.25	0.125	0.25	≤0.016–256	43	0.062	0.25	≤0.016–0.5	0.25	0.5	≤0.016–128	86	0.062	0.25	≤0.016–0.5	0.125	0.5	≤0.016–256
Italy	25	0.062	0.25	0.03–0.25	0.25	0.25	0.03–0.5	46	0.125	0.25	0.03–1	0.25	0.5	0.03–2	71	0.062	0.25	≤0.016–1	0.25	0.5	0.03–2
United Kingdom	16	0.062	0.25	0.03–0.5	0.062	0.125	≤0.016–0.25	22	0.062	0.25	0.03–0.5	0.125	0.5	≤0.016–2	38	0.062	0.25	0.03–0.5	0.125	0.5	≤0.016–2
All	310	0.062	0.125	≤0.016–0.5	0.125	0.25	≤0.016–256	320	0.062	0.25	≤0.016–1	0.25	0.5	≤0.016–128	630	0.062	0.25	≤0.016–1	0.125	0.5	≤0.016–256

[a] PRA, pradofloxacin; MTZ, metronidazole.

necessitated by the logistics of this study. The majority of the received samples (>400) were processed on the day of receipt, 49 samples were processed within 24 h of receipt, and only 2 samples were processed 48 h after receipt in the laboratory. Initial validation work with the transport media had demonstrated that the test organisms remained viable for at least 4 days (unpublished observations).

The availability of anaerobic isolates from periodontal infections in dogs across Europe is extremely limited. This is in part because of the need for specialist facilities and expertise, not just for isolation of strict anaerobes but also for their identification. Anaerobes are not routinely cultured within veterinary medicine, especially as their presence can be determined generically from clinical symptoms and treatment is usually empirical. This arises in part because susceptibility test results for anaerobes are often not available for up to 5 days (24).

Periodontal disease is probably the most common disease in dogs, and almost all dogs over 5 years of age are affected by periodontitis (18). The overall prevalence of periodontal disease has been investigated by several authors and reported as 53% to 97% (4, 12, 13, 16, 20). The frequency and severity of periodontal disease increase significantly with increasing age (12, 16, 20) and decrease significantly with increasing body weight (16, 20). The initial colonization of the dental pellicle is mainly caused by *Streptococcus* spp. and *Actinomyces* spp. (22). With extension of the supragingival plaque into the gingival sulcus, aerobes consume the available oxygen, thereby creating a low redox potential, particularly at the bottom of the gingival sulcus. These environmental conditions favor the growth of anaerobic organisms. As the disease progresses, deeper periodontal pockets develop with heavy accumulation of bacteria that further lower the oxygen levels. Anaerobes take over and constitute approximately 95% of the subgingival flora in periodontitis (22). During this development, there is also a shift from the predominantly nonmotile, gram-positive flora found in the supragingival plaque and the healthy gingival sulcus to a flora of gram-negative motile anaerobic rods found in periodontal pockets (10). This change can occur within 2 weeks when plaque is allowed to accumulate (6). The shift of the bacterial flora in periodontitis has been demonstrated by Isogai et al. (25) for dogs and is a well-known phenomenon in human dentistry. Hence, the isolation of a predominant gram-negative flora from dental pockets can be viewed as an indicator of periodontal disease. For humans, *Porphyromonas* and *Prevotella* spp. are firmly implicated as periodontal pathogens, and there is an increasing amount of evidence that this is the case in canine periodontal disease (18). It should be noted here that the canine biotype of *P. gingivalis* should now be referred to as a different, new species, *P. gulae* (11). Further new *Porphyromonas* spp. have been isolated from dogs and cats; these include *Porphyromonas canoris*, *P. salivosa*, *P. cangingivalis*, *P. cansulci*, *P. crevioricanis*, and *P. gingivicanis* (18). In the study of Harvey et al. (21), *Porphyromonas* spp. and *Prevotella* spp. were the anaerobes most frequently isolated from subgingival plaque samples of dogs.

Although periodontal disease can be controlled by mechanical periodontal therapy in the majority of dogs and humans, the literature clearly supports the use of antimicrobial intervention in the treatment of periodontal disease. Slots and

TABLE 2. In vitro activities of pradofloxacin and metronidazole against *Porphyromonas* and *Prevotella* spp. isolated from canine periodontal pockets

Bacterial group	No. of isolates	MIC (μg/ml)[a] for:					
		Pradofloxacin			Metronidazole		
		Range	50%	90%	Range	50%	90%
Porphyromonas asaccharolytica	39	0.031–0.125	0.062	0.062	≤0.016–0.5	0.031	0.125
Porphyromonas circumdentaria/Porphyromonas endodontalis	126	≤0.016–0.5	0.062	0.25	≤0.016–0.5	0.125	0.25
Porphyromonas gingivalis	24	≤0.016–0.25	0.031	0.125	≤0.016–0.5	0.062	0.25
Porphyromonas levii	49	0.031–0.125	0.062	0.125	≤0.016–0.5	0.062	0.125
Porphyromonas macacae	39	≤0.016–0.25	0.062	0.125	≤0.016–0.5	0.125	0.25
Porphyromonas salivosa	33	0.031–0.25	0.125	0.125	0.031–256	0.25	0.5
Prevotella buccae	2	0.25–1	NC	NC	0.5–2	NC	NC
Prevotella corporis	34	≤0.016–0.5	0.062	0.125	≤0.016–16	0.062	0.25
Prevotella denticola	4	0.062–0.125	0.062	NC	0.062–0.25	0.125	NC
Prevotella disiens	8	0.031–0.25	0.062	NC	≤0.016–1	0.062	NC
Prevotella heparinolytica	77	≤0.016–1	0.062	0.25	≤0.016–128	0.25	0.5
Prevotella intermedia	6	≤0.016–0.125	0.031	NC	≤0.016–0.25	≤0.016	NC
Prevotella loescheii	3	0.062–0.125	NC	NC	0.062–0.5	NC	NC
Prevotella nigrescens	26	0.031–0.5	0.062	0.25	≤0.016–2	0.125	0.5
Prevotella oralis	6	0.062–0.25	0.062	NC	0.031–16	0.25	NC
Prevotella oris	9	0.031–0.062	0.062	NC	0.125–1	0.5	NC
Prevotella oulorum	2	0.031–0.125	NC	NC	0.25–0.5	NC	NC
Prevotella zoogleoformans	1	0.125	NC	NC	0.125	NC	NC
Prevotella spp. (undefined)	142	≤0.016–0.5	0.062	0.25	≤0.016–1	0.25	0.5
All *Porphyromonas* strains	310	≤0.016–0.5	0.062	0.125	≤0.016–256	0.125	0.25
All *Prevotella* strains	320	≤0.016–1	0.062	0.25	≤0.016–128	0.25	0.5
All strains	630	≤0.016–1	0.062	0.25	≤0.016–256	0.125	0.5

[a] MIC_{50} calculated for groups with ≥4 isolates and MIC_{90} for groups with ≥10 isolates. NC, not calculated.

Rams (42) concluded that appropriate systemic antimicrobial therapy in human periodontitis enhances clinical outcomes in patients who have recently sustained or are at high risk for periodontal breakdown, and Nielsen et al. (31) demonstrated that in combination with scaling, root planning, and polishing, clindamycin dosed at 2.5 mg/lb of body weight twice a day for 8 days had a significant effect on plaque and pocket depth measures of periodontal disease in dogs but not on gingivitis. Zetner and Thiemann (47) had previously shown that clindamycin given 5 days in dogs and cats before ultrasonic scaling reduces plaque bacteria by 97.6%. It is clear from the literature that systemic antibiotic therapy, when properly prescribed to patients with aggressive periodontitis, can give rise to very good clinical outcomes (43), although its value in patients with chronic periodontitis is not as clear. In a recent review, Slots and Ting (43) made the point that the current method of periodontal therapy strongly emphasizes the suppression or eradication of specific periodontal pathogens yet mechanical debridement may fail to remove pathogens because of their locations. The rationale for systemic antimicrobial use is that mechanical debridement may not adequately detoxify the periodontium and that the host immune system may not be capable of eradicating periodontal pathogens (18). Mechanical treatment alone cannot remove invasive bacteria located in the gingiva and the periodontal ligament or bacteria residing in confined spaces, such as the dentinal tubules and the alveolar bone; these bacteria constitute a reservoir for postprophylaxis reinfection (18).

Single-drug therapies with penicillins, tetracyclines, metronidazole, or clindamycin have been used frequently in periodontal practice. Since periodontal disease usually harbors a mixture of pathogenic organisms, drug combination therapies can be important (43). Combination therapies are needed to provide the necessary spectrum of activity that was not previously available until the recent availability of broad-spectrum fluoroquinolones, active against aerobes and anaerobes.

While much emphasis is given to the role of anaerobes in periodontal disease, aerobes should not be ignored. Hennet and Harvey (23) reviewed the role of aerobes in periodontal disease in dogs and, on the basis of the mixed aerobic-anaerobic flora commonly found in the early stages of periodontal disease, suggested that aerobic bacteria may have an important role in disease development. They showed that as the pathology changes from gingivitis to periodontitis the total number of viable aerobes does not change but the anaerobe/aerobe ratio increases as anaerobes predominate. Studies since then have substantiated the involvement of aerobic, gram-positive organisms (*Staphylococcus* and *Streptococcus* spp.) in gingivitis (21, 47). The aerobes constitute most of the supragingival plaque that causes gingivitis and that subsequently develops into periodontal disease. A reduction of oxygen tension caused by proliferation of the aerobic flora creates favorable growth conditions for the anaerobes (18). In this way, it is thought that the aerobic flora plays a crucial role in development of disease. It consequently follows that for antimicrobial therapy to be effective it is advantageous for the active agent to have activity against the anaerobic and aerobic flora. Goldstein (14, 15) emphasized that due to increasing development of resistance of anaerobic bacteria to all antimicrobial agents there is a need to find new broad-spectrum agents active against both aerobes and anaerobes. Currently available fluoroquinolones in veterinary medicine have only modest activity against anaerobes. It

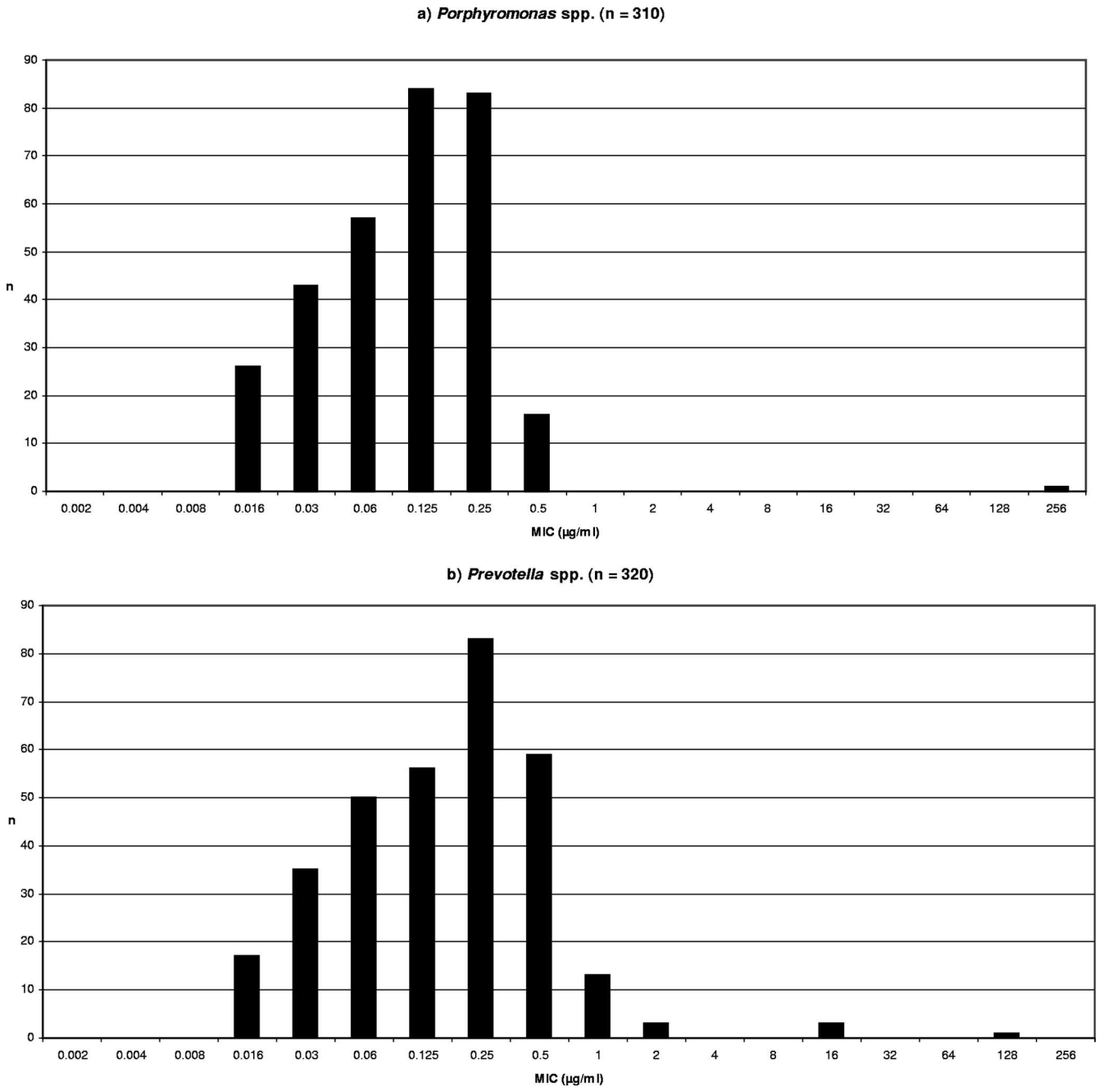

FIG. 2. MIC distribution for metronidazole of 310 *Porphyromonas* (a) and 320 *Prevotella* (b) strains isolated from cases of periodontal disease in dogs from a European multicenter study.

is clear that pradofloxacin has enhanced anaerobic activity (40) and further provides broad-spectrum coverage against aerobic organisms (45).

There is a wealth of literature from the human clinical sector that supports the use of broad-spectrum fluoroquinolones under conditions where anaerobes are implicated (1, 2, 29, 37, 44). The utility of broad-spectrum fluoroquinolones against bacteria associated with dental infections has also been demonstrated by King et al. (26) in a large pan-European study investigating the in vitro activities of a range of antianaerobic antimicrobials against gram-negative bacilli. Two of the participating laboratories in this study were indeed dental laboratories, one from the United Kingdom and one from central Europe. The majority of the isolates submitted by these laboratories were strains of *P. intermedia*, *Porphyromonas* spp., and *Fusobacterium nucleatum*, all groups implicated in periodontal disease. Of particular interest are the results for clinafloxacin, which is structurally similar to pradofloxacin and which shows MIC_{90} values of 0.06 and 0.125 μg/ml for *P. intermedia* and *Porphyromonas* spp., respectively.

In conclusion, the literature clearly supports the use of broad-spectrum fluoroquinolones for use against anaerobes. This study is the first report to show that a fluoroquinolone being developed for use in veterinary medicine has the potential to be used to treat anaerobes implicated in periodontal disease; this has been substantiated in clinical studies yet to be published. Pradofloxacin demonstrates a high degree of anti-anaerobic activity against strains isolated from clinical cases of periodontal disease and shows activity against metronidazole-resistant isolates. The broad-spectrum activity of pradofloxacin makes it a suitable candidate for the treatment of periodontal disease in dogs.

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