In vitro antimicrobial susceptibilities of three *Porphyromonas* spp and in vivo responses in the oral cavity of cats to selected antimicrobial agents

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Objectives To determine in vitro susceptibility of *Porphyromonas gingivalis*, *P salivosa* and *P circumdentaria* to seven antimicrobial agents by agar dilution and Epsilometer test methods and to assess the effectiveness of these antimicrobial agents in reducing the numbers of each *Porphyromonas* spp in the oral cavity of 16 domestic cats.

Design A two-part prospective study involving in vitro antimicro-bial studies using *Porphyromonas* spp obtained from naturally occurring feline infections and in vivo antimicrobial response studies using client-owned cats with naturally occurring periodontal disease.

Procedure Isolates (n = 25) of three feline *Porphyromonas* spp from the oral cavity and oral-associated disease were tested for their in vitro susceptibility to amoxycillin, amoxycillin-clavulanate, benzylpenicillin, clindamycin, doxycycline, erythromycin and metronidazole, using agar dilution and Epsilometer test methods. Digoxigenin-labelled whole chromosomal DNA probes directed against *P gingivalis* VPB 3492, *P circumdentaria* NCTC 12469^T and *P salivosa* VPB 3313 were used to quantify organisms taken from two sample sites at the gingival margins of these cats prior to, and 5 days after, treatment with one of four commonly used antimicrobial products (amoxycillin-clavulanate, clindamycin, doxycycline or spiramycin-metronidazole). The response to treatment was assessed clinically for each cat.

Results All isolates were susceptible in vitro to all seven antimicrobial agents using both methods. The numbers of *P gingivalis* were not reduced at the gingival sample sites by administration of amoxycillin-clavulanate for 5 days, although this treatment reduced the numbers of *P salivosa* and *P circumdentaria* to below detection levels in six of eight and two of three of sample sites, respectively; clinical improvement was not observed in cats treated with amoxycillin-clavulanate. Treatment with clindamycin, doxycycline or spiramycin-metronidazole resulted in clinical improvement and a marked reduction of all *Porphyromonas* isolates at the sample sites.

Conclusion The Epsilometer test is a simple and accurate method for determining the minimum inhibitory concentration for *P gingivalis*, *P salivosa* and *P circumdentaria*. All strains were susceptible in vitro to all the antimicrobial agents tested ,although clinical improvement of gingival disease was not noted with amoxycillin-clavulanate when given for 5 days at usual doses. This appears to be the first report of the disparity between the in vivo and in vitro susceptibility of oral bacterial strains to amoxycillin-clavulanate in the veterinary dental literature. This also appears to be the first report in which clinical and microbiological responses to commonly used antimicrobial agents for periodontal disease in cats has been documented and quantified. It was shown that treatment with clindamycin, spiramycin-metronidazole or doxycycline not only produced a substantial reduction in the number of *Porphyromonas* spp (in the majority of cases to below detection levels), but also resulted in substantial clinical improvement. This would indicate that these antimicrobial agents are useful adjunctive therapy to mechanical debridement in domestic cats.

Aust Vet J. 2000, 78:533-537
Keywords: *Porphyromonas* spp, antimicrobial susceptibility, periodontal disease, antimicrobial agents, clinical responses.

Although the pathogenesis of periodontal disease in humans and other animals is not understood completely, certain bacteria and their products in the dental plaque are amongst several important determinants of the onset and progression of periodontal disease. The feline oral *Porphyromonas* spp have been established as numerically prominent and clinically prevalent in feline periodontal disease[1] and subcutaneous abscesses[2] and the presence of putative virulence factors has been demonstrated.[3-4] The susceptibility of these organisms to antimicrobial drugs is essential information for the effective management of oral and oral-associated diseases in veterinary practice and in human medicine, where these organisms are encountered in cat-bite wounds.[5]

Optimal periodontal therapy in humans has been described as the total elimination of periodontal pathogens with complete regeneration of periodontal tissue and return to normal function.[6] The importance of antimicrobial therapy as part of a therapeutic plan has long been recognized in the treatment of destructive periodontal diseases in humans[7] and several antimicrobial agents have been tested extensively for their usefulness as adjunctive treatment for destructive periodontal disease. These have included tetracyclines, clindamycin, penicillins, metronidazole, cephalosporins and erythromycin. Antimicrobial therapy may be used to decrease bacterial counts before or after mechanical debridement and to reduce bacterial reservoirs in periodontal pockets and other intra-oral sites.

Dental scaling, gingival crevicular lavage and periodontal surgery have been the mainstay of periodontal therapy in veterinary dentistry.[8] In veterinary practice over the past 10 years, increased recognition of the importance of periodontal disease in domestic cats and greater emphasis on maintaining good dental hygiene have been seen. In veterinary dentistry, the necessity for antimicrobial agents as an adjunct to mechanical debridement has been debated.[9] It was commonly stated in older texts that antimicrobial agents were not required prior to mechanical debridement, with the justification that normal dogs clear oral bacteria from the blood within 20 min of bacter-

ATCC American-type culture collection
CFU Colony forming units
E-test Epsilometer test
MIC Minimum inhibitory concentration
NCCLS National committee for clinical laboratory standards
NCTC National collection of type cultures
Pc Porphyromonas circumdentaria
PgF feline strain Porphyromonas gingivalis
PO Per os
PRBHIB Prereduced brain heart infusion broth
Ps Porphyromonas salivosa
PT Total Porphyromonas spp
SBA Sheep blood agar
SBLA Supplemented Brucella laked blood agar
VPB Veterinary pathology and bacteriology

aemia.[10] However, the need for antimicrobial agents in animals with moderate to severe destructive periodontal disease and with other systemic illness (such as renal, hepatic or cardiovascular disease) has since been recognized.[11]

In vitro testing has been the mainstay for determining the susceptibility of organisms to antimicrobial agents. The agar dilution method and more recently the E-test have proven effective and reliable methods to evaluate antimicrobial susceptibility of human isolates of *Porphyromonas*.[11-13] There have been relatively few quantitative studies on drug susceptibility of anaerobic bacteria of animal origin because of the labour-intensive and time-consuming nature of the agar dilution method. Because the agar dilution method is seldom practicable for testing individual clinical isolates, interest has been aroused in the reliability of the E-test. Antimicrobial susceptibility testing of *Porphyromonas* isolates of animal origin has shown these organisms to be highly susceptible in vitro to many antimicrobial agents, with the obvious exception of the aminoglycosides.[14-17]

The current study aimed to provide veterinarians with antimicrobial susceptibility data for *Porphyromonas* spp in order to assist with the management of periodontal disease and oral-associated diseases and was presented in part at the Anaerobe Society of the Americas Conference, Buenos Aires, May 1998. The study used the in vitro methods of agar dilution and the E-test and compared those results with the response in vivo to four commonly prescribed antimicrobial preparations.

Materials and methods

In vitro antimicrobial susceptibility studies

For the agar dilution method, 25 feline-origin *Porphyromonas* spp were used, comprising 11 isolates of PgF, 10 of Ps and 4 of Pc. For the E-test, 19 feline-origin *Porphyromonas* spp were used comprising 9 PgF, 7 Ps and 3 Pc. All organisms had been isolated from naturally occurring subcutaneous abscesses, pyothorax or the oral cavity of cats[18-20] and had been identified by phenotypic characteristics and DNA-DNA hybridization by in solution methods[21] or whole chromosomal probes labelled with digoxigenin (Boehringer-Mannheim).[22] *Bacteroides fragilis* ATCC 25285 and *B thetaiotaomicron* ATCC 29741 were used as quality control strains.

Inocula for each sensitivity test were prepared by subculturing to 5 mL PRBHIB (Oxoid) organisms taken from a 5 day culture grown on SBA supplemented with haemin and formate-fumarate[23] and growing them anaerobically overnight at 37°C. The following morning, each culture was diluted with PRBHIB to the turbidity of a 0.5 McFarland density tube standard. SBLA plates prepared (containing a range of concentrations of each antimicrobial agent) were inoculated with 1 μL of each diluted culture before incubation for 4 days at 37°C. All antimicrobial agents used in the agar dilution method were free-acid standards of known potency and supplied as indicated: benzylpenicillin (Sigma), amoxycillin (Pfizer), clavulanic acid (Pfizer), erythromycin (Sigma), doxycycline (Pfizer), clindamycin (Sigma) and metronidazole (Sigma).

The MIC values were determined using the NCCLS reference agar dilution method[13] and Brucella agar (Difco Laboratories) supplemented with haemin-menadione, formate-fumarate[23] and 5% laked sheep blood. The MIC was determined after 4 days' incubation at 37°C as the concentration at which there was a marked change in growth compared with the control. MIC readings were compared with the NCCLS-approved breakpoints[13] for antimicrobial agents to enable interpretation of the antimicrobial susceptibility for each microorganism.

One E-test strip (Epsilometer AB Biodisk, Solna, Sweden) containing amoxycillin, amoxycillin-clavulanate, benzylpenicillin, erythromycin, clindamycin, doxycycline or metronidazole was applied to each 90 mm plate containing SBLA, which had been inoculated in accordance with manufacturers' instructions. Plates were incubated as described above and the MIC value was read at 4 days, as recommended by the manufacturer, as the point of intersection between the inhibition ellipse edge and the E-test strip.

In vivo antimicrobial response studies

Cats that had not been treated with any antimicrobial agent in the previous 12 months and with grade 2 or 3 periodontal disease[24] were chosen for the study from cats presented to a busy suburban veterinary practice for a variety of procedures. Owner consent was obtained for involvement in the trial. Within these limits, only cats with oral disease clearly unrelated to gingival/periodontal disease were excluded from the study. The oral cavity was examined as part of a full physical examination and an evaluation made of the general status of the periodontal tissue. A grade was assigned to the periodontium over the upper right canine and third upper right premolar teeth. Following antimicrobial therapy, the periodontal tissue was assessed again for the degree of gingival erythema and oedema and degree of halitosis.

Cats in the four treatment groups were allocated by sequential rotation and received the following antimicrobial preparations for 5 days at dose rates recommended by the manufacturers: amoxycillin-clavulanate (4:1;Clavulox, Pfizer Animal Health) at 12.5 mg/kg PO twice daily; doxycycline (Vibravet, Pfizer Animal Health) at 5 mg/kg loading dose then 2.5 mg/kg every 12 hours for two doses then once daily PO; clindamycin (Antirobe, Pharmacia Upjohn) at 5.5 mg/kg twice daily PO, and spiramycin 75 000 units/kg and metronidazole 12.5 mg/kg (Stomorgyl, Rhone Merrieux) at 12.5 mg/kg PO once daily. Some animals were lost to the study between the first sample and the postantimicrobial-administration sample leaving the four unequal groups as indicated in Table 2. The cats that comprised the study ranged in age from 3 to 18 years (median 6 years) and consisted of eight males and eight females.

Immediately before antimicrobial therapy was instituted, and again 24 h after treatment ceased, samples for bacteriological investigation were taken from each cat from the gingival margin of the buccal surface of the right upper canine tooth (canine site) and the right upper third premolar tooth (premolar site). Sampling method and bacteriological procedures were routine and have been described elsewhere.[1-3] Samples collected onto swabs from each site were placed into PRBHIB and 5 μL of well-mixed aliquots were grown anaerobically for 7 days on SBA supplemented with haemin and formate-fumarate; digoxigenin-labelled whole chromosome DNA probes of PgF VPB 3492, Ps NCTC 11632 and Pc NCTC 12469^T were used to enumerate each species from colony lifts taken from replicate plates 25 and these counts were used also to derive total CFU of PT per sample. Successful treatment was defined as a reduction of greater than one standard deviation of the mean CFU present prior to treatment.

Clinical Assessment

The oral cavity was assessed for gingival erythema (marginal, +, ++, +++), gingival oedema (+, ++, +++), bleeding on probing

Table 1. Agar dilution and E-test determinations of antimicrobial susceptibility of isolates *Porphyromonas gingivalis*, *P salivosa* and *P circumdentaria* from oral cavity and oral-associated diseases of cats.

Antimicrobial agent	Minimum inhibitory concentration ranges for isolates tested (μg/mL)						Break point[a] (μg/mL)
	P gingivalis		*P salivosa*		*P circumdentaria*		
	Agar dilution (n = 11)	E-test (n = 9)	Agar dilution (n = 10)	E-test (n = 7)	Agar dilution (n = 4)	E-test (n = 3)	
Amoxycillin	< 0.008 -0.064	< 0.016 - 0.032	1.024 - 2.048	2.0	0.008 - 1.024	< 0.016 - 1.0	4
Amoxycillin/clavulanic acid	< 0.00 - 0.064	< 0.016	0.064 - 0.256	0.050 - 0.075	0.008 - 1.024	< 0.016 - 1.0	4
Benzylpenicillin	0.004 - 0.512	0.002 - 0.125	0.512 - 1.024	0.50 - 0.75	0.004 - 1.024	0.002 - 0.75	4
Clindamycin	0.002 - 0.004	< 0.016	0.004 - 0.016	< 0.016	0.002 - 0.008	< 0.016	4
Doxycycline	0.032 - 0.256	0.064 - 0.125	0.128 - 0.256	0.094 - 0.125	0.008 - 0.512	< 0.016 - 0.25	8
Erythromycin	0.128 - 1.024	0.25 - 0.50	0.512 - 1.024	0.25 - 0.50	0.016 - 1.024	0.023 - 1.0	NA
Metronidazole	0.001 - 0.064	< 0.002 - 0.023	0.002 - 0.064	< 0.002 - 0.032	0.032 - 0.064	0.023 - 0.032	16

[a]Break points approved by the NCCLS[13]
NA Not available

(±, +, ++, +++) and halitosis (nil, mild, moderate, foul) as outlined by West-Hyde and Floyd.[24]

Results

In vitro antimicrobial susceptibility studies

The MIC values obtained by both methods for PgF, Ps and Pc are shown in Table 1. Agreement between E-test and agar dilution MIC values within ± $\log_2$ dilution was 100% for all antimicrobial agents tested against all three species. PgF, Ps and Pc were susceptible in vitro to all antimicrobial agents tested using both methods.

In vivo antimicrobial response studies

As can be seen from Table 2, there was a successful response to treatment with doxycycline, clindamycin and spiramycin-metronidazole, as indicated by reduction in PT. There was similarly a reduction in total numbers of PgF from treatment with these three antimicrobial products but not with amoxycillin-clavulanate. Similarly, as indicated for individual cats in Table 3, there was a successful response as indicated by reduction in numbers of Ps and Pc after treatment with doxycycline, clindamycin and spiramycin-metronidazole but not with amoxycillin-clavulanate.

Table 2. Mean colony forming units of total *Porphyromonas* spp and *P gingivalis* at canine and premolar sample sites pre and postantimicrobial treatment.

Treatment group (n)	Colony forming units at canine site (mean ± SD)			
	Total Porphyromonas		*P gingivalis*	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment
Amoxycillin-clavulanate (6)	2.59×10^5 ($\pm 1.11 \times 10^5$)	1.83×10^5 ($\pm 8.97 \times 10^4$)	2.09×10^5 ($\pm 7.13 \times 10^4$)	1.77×10^5 ($\pm 8.39 \times 10^4$)
Doxycycline (4)	1.50×10^5 ($\pm 7.21 \times 10^4$)	1.13×10^4 ($\pm 1.40 \times 10^4$)	9.35×10^4 ($\pm 3.51 \times 10^4$)	1.05×10^4 ($\pm 1.28 \times 10^4$)
Spiramycin-metronidazole (3)	2.54×10^5 ($\pm 1.72 \times 10^5$)	0	1.57×10^5 ($\pm 7.34 \times 10^4$)	0
Clindamycin (3)	1.75×10^5 ($\pm 2.26 \times 10^5$)	0	1.37×10^5 ($\pm 1.59 \times 10^5$)	0
	Colony forming units at premolar site (mean ± SD)			
Amoxycillin-clavulanate (6)	2.40×10^5 ($\pm 2.05 \times 10^5$)	2.48×10^5 ($\pm 1.65 \times 10^5$)	1.53×10^5 ($\pm 8.69 \times 10^4$)	2.09×10^5 ($\pm 1.01 \times 10^5$)
Doxycycline (4)	2.08×10^5 ($\pm 1.27 \times 10^5$)	3.15×10^4 ($\pm 3.12 \times 10^4$)	1.47×10^5 ($\pm 1.04 \times 10^5$)	3.0×10^4 ($\pm 3.04 \times 10^4$)
Spiramycin-metronidazole (3)	3.35×10^5 ($\pm 1.29 \times 10^5$)	0	1.91×10^5 ($\pm 9.42 \times 10^4$)	0
Clindamycin (3)	1.59×10^5 ($\pm 1.23 \times 10^5$)	6.67×10^2 ($\pm 1.15 \times 10^3$)	1.47×10^5 ($\pm 1.17 \times 10^5$)	6.67×10^2 ($\pm 1.15 \times 10^3$)

Amoxycillin-clavulanate (Clavulox), doxycycline (Vibravet), spiramycin and metronidazole (Stomorgyl), clindamycin (Antirobe)
SD standard deviation

Clinical assessment after antimicrobial therapy

On examination 24 h after cessation of antimicrobial therapy, cats in the doxycycline, clindamycin and spiramycin-metronidazole, but not in the amoxycillin-clavulanate, treatment groups showed a marked decrease in halitosis, gingival oedema and erythema (at least one grade reduction in each parameter).

Discussion

The in vitro antimicrobial susceptibility studies reported here have shown that recent isolates of PgF, Ps and Pc were susceptible to benzylpenicillin, amoxycillin, amoxycillin-clavulanic acid, erythromycin, doxycycline, clindamycin and metronidazole. These results were comparable to previous studies that showed similar susceptibility patterns of feline oral *Porphyromonas* spp.[15] The additional information provided here is the susceptibility of these organisms to antimicrobial preparations currently used in veterinary practice. Other recent studies have found unspeciated members of the genus *Porphyromonas* from dogs and cats[17] or *P gingivalis* from cats[16] to be susceptible to antimicrobial agents including metronidazole, chloramphenicol, amoxycillin-clavulanate, ampicillin, cefadroxil and clindamycin. The results of in vitro antimicrobial susceptibility testing were also comparable to those found in previous studies with human strains of *P gingivalis* and other species[14, 26-27] that showed that the strains tested were very susceptible to benzylpenicillin, ampicillin, cefaclor, cefoxitin, cefuroxime,

Table 3. Colony-forming units (x10³) of *Porphyromonas* spp isolated from the canine and premolar sample sites pre and postantimicrobial treatment.

Cat Number	Antimicrobial agent	Grade[a] at C/P	Canine sample site						Premolar sample site					
			P gingivalis		*P salivosa*		*P circumdentaria*		*P gingivalis*		*P salivosa*		*P circumdentaria*	
			Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	Amoxycillin-clavulanate	3/3	232	64	0	0	84	0	148	300	0	0	0	0
2	Amoxycillin-clavulanate	3/3	120	128	0	0	0	0	148	100	80	0	48	36
3	Amoxycillin-clavulanate	3/3	264	232	68	36	20	0	70	136	8	0	28	0
4	Amoxycillin-clavulanate	3/3	280	300	0	0	104	0	320	360	0	0	320	200
5	Amoxycillin-clavulanate	2/2	120	140	0	0	20	0	120	156	0	0	40	0
6	Amoxycillin-clavulanate	3/3	240	200	0	0	0	0	110	200	0	0	0	0
7	Doxycycline	3/3	124	26	80	3	30	0	176	40	64	8	92	6
8	Doxycycline	2/2	80	16	0	0	0	0	46	0	0	0	0	0
9	Doxycycline	3/3	120	0	0	0	64	0	280	68	0	0	0	0
10	Doxycycline	2/2	50	0	30	0	20	0	86	12	46	0	40	0
11	Spiramycin-metronidazole	3/3	240	0	200	0	0	0	300	0	180	0	0	0
12	Spiramycin-metronidazole	3/3	132	0	90	0	0	0	142	0	136	0	12	0
13	Spiramycin-metronidazole	3/3	100	0	0	0	0	0	132	0	90	0	12	0
14	Clindamycin	2/2	42	0	0	0	0	0	60	0	0	0	14	0
15	Clindamycin	2/2	48	0	0	0	0	0	102	2	0	0	0	0
16	Clindamycin	3/3	320	0	116	0	0	0	280	0	132	0	20	0

[a]Grade of periodontal disease[24]

Amoxycillin-clavulanate (Clavulox), doxycycline (Vibravet), spiramycin and metronidazole (Stomorgyl), clindamycin (Antirobe)
C/P canine/premolar sample site
Pre pretreatment
Post posttreatment

cefotaxime, imipenem, erythromycin, tetracycline, doxycycline, metronidazole and clindamycin by either the agar dilution or E-test methods. We have also shown good agreement between the results obtained by agar dilution method and E-test methods for these feline oral *Porphyromonas* spp. While the agar dilution method remains the reference method for anaerobes, the E-test has considerable advantages by being simpler and less labour-intensive. The agreement between these methods has made the E-test a feasible option for use in commercial laboratories to aid medical and veterinary practitioners in the correct choice of antimicrobial agent to treat oral-associated infections involving these organisms. While antimicrobial susceptibility testing of clinical isolates is not always required due to the frequently favourable clinical response to empirical therapy and the inevitable delay in growth of fastidious obligate anaerobes such as *Porphyromonas*, it is valuable to know that the E-test may be used if required.

This appears to be the first report in which clinical and microbiological responses to commonly used antimicrobial agents for periodontal disease in cats has been documented and quantified. It was shown that treatment with clindamycin, spiramycin-metronidazole or doxycycline not only produced a substantial reduction in the number of *Porphyromonas* spp (in the majority of cases to below detection levels), but also resulted in substantial clinical improvement. This would indicate that these antimicrobial agents are useful adjunctive therapy to mechanical debridement in domestic cats.

In vivo findings raise some interesting questions about the correlation between in vitro antimicrobial susceptibility testing and in vivo responses of microbes in complex polymicrobial infections such as periodontal disease. Treatment with amoxycillin-clavulanate did not reduce PgF numbers at the canine and premolar sample sites by greater than one standard deviation from the mean in any of the cats tested, while the results of the in vitro tests showed all strains were susceptible to amoxycillin and amoxycillin-clavulanic acid. This appears to be the first report of a disparity between the in vivo and in vitro susceptibility of oral bacterial strains to amoxycillin-clavulanate in the veterinary dental literature. There does not appear to be a simple explanation for these findings but several factors may have played a role. These include the possibility that the total bacterial load in the periodontal pocket may have been too large in relation to the maximal achievable antibiotic concentration. This has also been described as the 'inoculum effect'. The determination of MIC uses a standard inoculum of approximately 10^5 CFU/mL and it has been shown that the periodontal pocket and supragingival tissue harbour many more bacteria than this. Also, the subgingival microbiota can be considered an adherent layer of bacterial microcolonies (biofilms). The production of glycocalyx by plaque organisms may induce a biofilm phenomenon whereby the microbes are more resistant to the bactericidal actions of antimicrobial agents.

While it may be argued that these factors should have affected all the antimicrobial agents used, the concentration of amoxycillin-clavulanate reached in the gingival crevicular fluid and the rest of the oral cavity may not have been sufficient to cope with a large bacterial inoculum existing in the biofilm of plaque. In the human dental literature it is reported that a single oral dose of 500 mg amoxycillin produces a peak serum concentration of 8 μg/mL and a peak gingival crevicular fluid concentration of 3 to 4 μg/ml.[28] The NCCLS-approved breakpoint MIC for amoxycillin against anaerobes is 2 μg/mL. In a complex environment, such as the oral cavity, in which the inoculum exceeds 10^5 CFU/ml, this concentration of amoxycillin may be insufficient to reduce the numbers of all bacteria present. Further investigations are required to examine the concentration of amoxycillin achieved in the gingival crevicular fluid of animals with various grades of periodontal disease to determine whether

larger doses achieve favourable results in the treatment of these and other similar disorders.

There have been a number of cases in the human dental literature in which bacterial isolates from clinical cases have been sensitive to antimicrobial agents in vitro but the condition failed to improve and the organisms were still isolated after these agents had been administered parenterally.[29-31] Most recently, a randomised double-blind placebo-controlled study of the clinical and microbiolgical effects of initial periodontal therapy in conjunction with systemic amoxycillin-clavulanate, found no advantages in using these agents.[32] The in vivo effect of numerous periodontal pathogens were analysed, amongst them human strains of *P gingivalis*; the four patients positive for this organism remained positive after treatment with amoxycillin-clavulanate. It is clear that bacterial in vitro susceptibility to antimicrobial agents should be regarded as a guide to potential activities in vivo but the clinical utility of each antimicrobial agent should be based on its pharmacological properties and the results of clinical trials.

Reports on the efficacy of amoxycillin-clavulanate in periodontal disease in small animals have focused on the in vitro response of commonly isolated oral organisms rather than an assessment of changes in clinical and microbiological variables in vivo.[17] In one study,[33] 92% of cats (11 of 12) and 77% of dogs (17 of 22) treated with amoxycillin-clavulanate were clinically 'cured' of gingivitis. In that study, oral isolates were cultivated prior to treatment and antimicrobial susceptibility was assessed in vitro, but isolation of organisms after treatment was not performed to verify the clinical response to treatment. In addition, an undisclosed proportion of these 34 animals were given additional undisclosed medical or surgical treatments but were still included in the calculations of those responding to amoxycillin-clavulanate.

Our findings have established the E-test as an acceptable method of in vitro antimicrobial testing for the feline oral species of *Porphyromonas* and confirmed the in vitro susceptibility of these organisms to commonly used antimicrobial agents. However, important questions remain about the correlation between the clinical response to treatment of a disease with complex aetiology and the antimicrobial susceptibility of individual organisms within the complex. Further studies are required to investigate the reasons for such clinical failure.

Acknowledgments

This work was supported in part by a grant from the Australian Research Council. D Wigney, L Patoka and F Taeker provided valuable assistance throughout. The staff of Inner West Veterinary Hospital were helpful with access to clinical cases.

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(Accepted for publication 9 December 1999)