Enterococcal infective endocarditis following periodontal disease in dogs

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INTRODUCTION

Association between periodontal disease (PD) and several systemic medical conditions, including infective endocarditis (IE), is well established in humans. It is often caused by bacteria that colonize the oral cavity. *Enterococcus faecalis* is amongst the most frequent pathogens associated with valve endocarditis, but the role of enterococcal-PD in endocarditis evolution still remains unclear. Data extrapolated from human medicine indicate that IE in dogs can develop due to chronic PD. We investigated the possible association between periodontitis and IE, by evaluating the presence and diversity of *Enterococcus* in the gum and heart of dogs with PD.

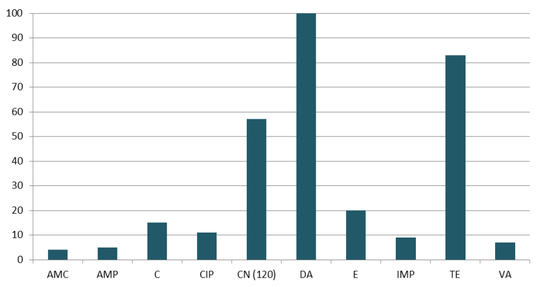
Table 1 – Sampled animals (F - female; M - male; NB - No breed; MoP - moderate periodontitis; MiP - mild periodontitis; SP - severe periodontitis; G – gengivitis).

Animal	Gender	Age (years)	Breed	PD Stage
A	F	7	NB	MoP
B	M	13	NB	MiP
C	F	17	x Pekingese	SP
D	F	7	Boxer	G
E	M	10	NB	MiP
F	M	17	NB	MoP
G	M	17	NB	MoP
H	F	13	NB	MoP
I	M	16	x Poodle	SP
J	F	13	NB	MiP
K	F	9	Husky	MiP
L	F	15	Labrador Retriever	G
M	F	15	NB	MiP
N	F	14	NB	SP
O	M	13	Labrador Retriever	SP
P	M	12	Labrador Retriever	SP
Q	M	14	Yorkshire Terrier	SP
R	F	14	NB	SP
S	M	8	German Shepard	MiP
T	M	12	NB	MiP
U	F	7	NB	MiP
V	M	16	Teckel	MoP
W	M	17	NB	MoP
X	F	9	Boxer	MiP
Y	F	14	NB	MoP
Z	M	10	Basset Hound	MiP
AA	M	12	NB	MiP
AB	M	12	NB	MiP
AC	M	14	x Poodle	MoP
AD	M	12	Labrador Retriever	G
AE	M	7	NB	MiP
AF	F	12	NB	MoP

METHODS

Samples were collected during necropsy of 32 dogs (Table 1), with a PD diagnose, which died of natural causes or euthanasia. Enterococci were isolated using Slanetz and Bartley medium. Phenotypic and molecular methods were used for identification, yielding a total of 117 isolates, further characterized by PCR-fingerprinting. Representative isolates were selected (n=46, 39 *E. faecalis*, 7 *E. faecium, 2 Ent*. sp.) and genomic diversity assessed by macrorestriction analysis, using *Sma*I. Isolates pathogenicity potential was evaluated, including: susceptibility to antimicrobial agents by the disk diffusion method according to CLSI guidelines; presence of virulence traits by plate assays (hemolysin, gelatinase) and PCR screening (*gelE*, *efaAfs*, *efaAfm*, *ebpABC*, *gls24*, *agg*, *esp*, *cylA*, *acm*, *ace*).

Figure 1 – Antimicrobial resistance profiles of the enterococcal clinical isolates (%).

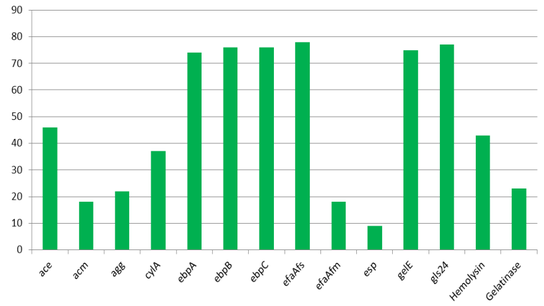


Legend: AMC- amoxycillin/clavulanate; AMP - ampicillin; C - chloramphenicol; CIP – ciprofloxacin; CN (120) - gentamicin (120 mg); DA – clindamycin; E – erythromycin; IMP – Imipenem; TE – tetracycline; VA - vancomycin.

RESULTS

All isolates were resistant to clindamycin; for tetracycline and gentamicin resistance-values were above 50% and for the remaining antimicrobials below this value (Figure 1). In search of virulence factors 43% of the isolates proved to be β-hemolytic and 23% gelatinase positive. Regarding virulence-determinants, percentages observed were above 50% for *gelE*, *efaAfs*, *ebpABC* and *gls24* and below this value for *agg*, *esp*, *efaAfm*, *cylA*, *acm* and *ace* (Figure 2).
Macrorestriction-profiles were analyzed using Bionumerics software, which allowed the comparison of enterococci recovered from both sites of isolation. In 7 of the 32 sampled animals, high similarity levels (above 90%) were observed between mouth and heart enterococci obtained from the same dog (Figure 3).

Figure 2 – Virulence profile of the enterococcal clinical isolates (%).

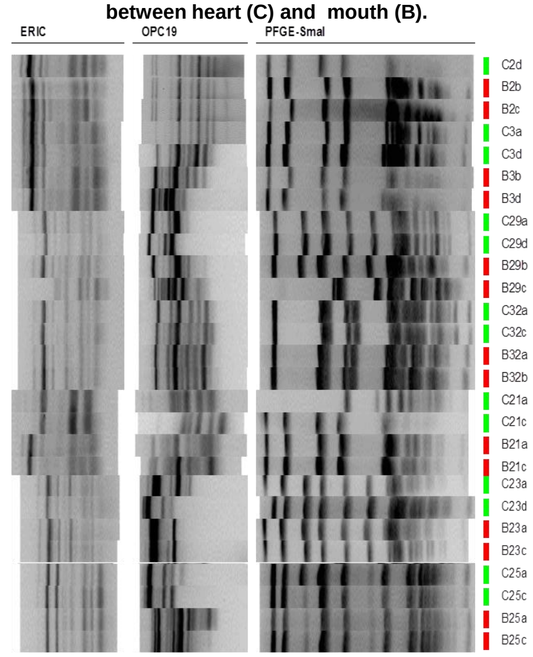


Legend: *ace* - *E. faecalis* adhesin; *acm* - functional collagen adhesin gene; *agg* - aggregation substance; *cylA* - cytolysin activator; *ebpA, ebpB* - minor pilus gene; *ebpC* - major pilus gene; *efaAfs* - *E. faecalis* antigen A; *efaAfm* - *E. faecium* antigen A; *esp* - enterococcal surface protein; *gelE* – gelatinase; *gls24* – general stress-inducible gene.

CONCLUSIONS

Results allowed us to establish an association between periodontal disease and bacterial-endocarditis, representing a major step towards understanding the pathogenesis of enterococcal-PD-driven IE. Results are extremely relevant, not only for veterinary medicine, but for the establishment of dogs as animal models for human IE.
Furthermore, frequent enterococci resistance to common therapeutics, coupled with the disinvestment in the development of new antibiotics, increases the need to intensify research aiming at testing novel antimicrobial strategies to prevent and control IE due to enterococcal PD.

Figure 3 – PCR-fingerprinting and macrorestriction profiles of the enterococci presenting identical patterns between heart (C) and mouth (B).



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