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# Evaluation of a monovalent companion animal periodontal disease vaccine in an experimental mouse periodontitis model

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**Abstract**

Periodontal disease in companion animals is clinically similar to that of human periodontal disease. Despite the usage of veterinary procedures and antibiotic therapy, the disease still remains as one of the most highly prevalent disorders seen by veterinarians. The goal of this study was to evaluate the immunogenic properties and vaccine performance of a monovalent canine periodontal disease vaccine in the mouse oral challenge model of periodontitis. Mice vaccinated subcutaneously with inactivated, whole-cell bacterin preparations of *Porphyromonas gulae* displayed both high titers of anti-*P. gulae* specific antibodies and significantly reduced alveolar bone loss in response to homologous, heterologous, and cross-species challenge. Based on the results of these studies, a periodontal disease vaccine may be a useful tool in preventing the progression of periodontitis in animals.


*Keywords:* Periodontitis; *Porphyromonas*; Veterinary

## 1. Introduction

The study of periodontal disease in humans has been of considerable interest throughout the past decade. Much progress has been made in the understanding of the disease etiology and the intricate interrelationships between the host and periodontal pathogens [1,2]. In addition, there has been steady progress in elucidating the disease mechanisms and virulence factors of the prime periodontal pathogen, *Porphyromonas gingivalis* [3–5]. Several laboratories have utilized a variety of small animal and non-human primate periodontal disease models to demonstrate efficacy of subunit- or bacterin-based vaccines [6,7]. In contrast, there is comparatively little information available regarding periodontal disease in companion animals. It has been estimated that approximately 80% of dogs and cats demonstrate some degree of periodontal disease by four years of age [8]. One contributing factor to this high percentage is the lack of routine oral care. Both the progression and etiology of periodontal disease in companion animals appears to roughly parallel the disease path in humans [9]. Black pigmenting anaerobic bacteria (BPAB) have been isolated from the periodontal pockets of dogs and cats [10–15], sheep [16,17], and several wild animals [18]. While little has been done to definitively speciate many of these isolates, they appear to be predominantly *Porphyromonas* spp. [10,15]. However, distinct differences have been noted between human and canine *Porphyromonas* spp. Isogai et al. [15] and Harvey et al. [12] utilized biochemical testing to identify *P. gingivalis*-like organisms in the gingival crevicular spaces of dogs with periodontal disease. Harvey et al. [12] noted that "human" *P. gingivalis* isolates were catalase-negative whereas "veterinary" *P. gingivalis* isolates were catalase-positive. Other *Porphyromonas* spp. of veterinary origin have been identified, including *Porphyromonas gulae*, *Porphyromonas canoris*, *Porphyromonas gingivicanis*, and *Porphyromonas crevioricanis* [18–20]. Fournier et al. [18] recently suggested that many of the previously iden-

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tified veterinary *P. gingivalis*-like bacteria should be reclassified as *P. gulae*. Our laboratory has recently isolated bacteria from other genera from dog periodontal pockets. *Bacteroides denticanium* was recently identified and was found to be capable of inducing periodontal disease in mice [21]. In addition, we have identified a novel genospecies in the Porphyromonadaceae family, tentatively classified as *Porphyromonas denticanis* [21]. Data generated in our laboratory has indicated that the most frequently isolated BPAB in dog and cat periodontal pockets are *P. gulae*, *Porphyromonas salivosa*, and *P. denticanis* [21]. Each of these isolates was demonstrated to be pathogenic in the mouse model of periodontal disease [21]. As also noted by Fournier et al. [18], *P. gingivalis* was not frequently isolated from dog and cat periodontal pockets.

The high incidence of periodontal disease in companion animals, the low compliance rate of oral care, the clinical difficulties associated with periodontal disease treatment, and potential systemic complications warrant the development of an efficacious vaccine. In this report, we assessed the performance of a *P. gulae* bacterin in the mouse oral challenge model of periodontal disease.

## 2. Materials and methods

### 2.1. Bacteria

*P. gulae* strain B43, *P. salivosa* strain B104, *P. denticanis* strain B106, and *B. denticanium* strain B78 were isolated from the gingival crevicular fluid of dogs with periodontal disease (Table 1) [21]. *P. gulae* strain B69 was isolated from the gingival crevicular fluid of a cat with periodontal disease [21]. Broth cultures of these bacteria were prepared by inoculating a 1 ml seed stock into 200 ml of modified Phytone Yeast Glucose (PYG) medium [21] followed by 48 h of incubation at 37 °C in a Bactron IV anaerobic chamber maintained in an anaerobic state (5% $H_2$, 5% $CO_2$, 90% $N_2$).

Challenge material was prepared by inoculating half-strength brain heart infusion (1/2BHI) agar plates (containing 10 mg/ml (w/v) yeast extract (Becton Dickinson), 0.15 mg/ml (w/v) hemin (Sigma)), and 0.075 ml/ml (v/v) menadione (Sigma) with 100 μl of the appropriate frozen stock and incubating for 48 h at 37 °C in an anaerobic environment. Following incubation, 1 ml of supplemented (1/2) BHI medium was applied to the surface of the plates and the cells were suspended using a disposable spreader. The cell concentration was then adjusted to approximately $2 \times 10^{10}$ cells/ml with supplemented (1/2) BHI medium. An equal volume of 2% (w/v) carboxymethyl cellulose (CMC; Sigma) was added to yield a final cell concentration of approximately $1 \times 10^{10}$ cells/ml in 1% CMC.

### 2.2. Bacterial fermentation

Bacteria used to formulate the vaccines were propagated using New Brunswick BioFlo 3000 fermentors with vessels having a 5-l working volume. Batch or partial fed-batch fermentations utilized modified PYG medium. The fermentation vessels were poised pre-inoculation by sparging sterile medium with anaerobic gases to ensure a reduced environment. Fermentors were seeded either directly with thawed vials of seed stock or with liquid cultures grown in a Bactron IV anaerobe chamber. Volumetric rates of inoculum varied from 0.02 to 8% (v/v). Sparging was continued during fermentation to both ensure reduced medium as well as a means of pH control. Dilute ammonium hydroxide was added via a pump-assisted delivery mechanism to further control the pH. The fermentation vessels were temperature controlled at 37 °C under moderate agitation. Fermentations were terminated between 42.75 and 87 h post-inoculation.

### 2.3. Bacterial inactivation

Fermentor grown *P. gulae* B43 broth cultures were inactivated using formalin-formaldehyde, heat, or aeration. Inactivation with formalin-formaldehyde was accomplished by the addition of 0.4% (v/v) formalin-formaldehyde (J.T. Baker; Phillipsburg, NJ) to a freshly harvested bacterial culture followed by incubation at room temperature for 24 h. Heat inactivation was carried out by incubating the freshly harvested culture at 60 °C for 30 min with gentle agitation. Since *P. gulae* is an obligate anaerobe, exposure to oxygen for extended periods can kill the bacterial cells. A freshly harvested bacterial culture was sparged with air at 0.1 standard liters per minute at room temperature for 48 h with gentle agitation.

Fermentor cultures at time of inactivation were determined to have optical density readings, taken at 600 nm, between 2.02 and 3.20. Total cell counts per milliliter of inactivated fermentor cultures were between $3.6 \times 10^9$ and $4.4 \times 10^9$ cells/ml.

Table 1
Bacterial strains utilized in this study

| Bacterial strain | Host | Tooth | Pocket depth |
|---|---|---|---|
| *P. gingivalis* ATCC 53977 | Human | NA[a] | NA |
| *P. gulae* B43 | Dog | NA | NA |
| *P. gulae* B69 | Cat | Upper left canine | 2 |
| *B. denticanium* B78 | Dog | Upper left pre-molar | 5 |
| *P. salivosa* B104 | Dog | Lower left first molar | 4 |
| *P. denticanis* B106 | Dog | Lower left first molar | 4 |

[a] NA, not available.