Age and Prevalence of *Porphyromonas gingivalis* in Children

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The acquisition of *Porphyromonas gingivalis* was examined in a cross-sectional study of 198 subjects from 0 to 18 years of age using a PCR-based assay. *P. gingivalis* was detected in the oral cavities of 37% of subjects and at similar frequencies among subjects of all ages. These data indicate that *P. gingivalis* may be acquired in the first days of life.

Periodontitis is marked by irreversible loss of the attachment of teeth to supporting structures. It is widespread, with more than 90% of persons 13 years of age or older exhibiting some loss of attachment (4). Although the etiology of periodontitis is not completely understood, it is generally accepted to be an infectious process, probably caused by specific bacteria (20). Of the several species that have been investigated as potential periodontal pathogens, the presence of *Porphyromonas gingivalis* has been shown to be among the best risk indicators of periodontitis (11, 19), and in a longitudinal trial has been shown to be a risk factor for periodontitis (3).

The reported prevalence of *P. gingivalis* in young subjects (1, 2, 6, 7, 9, 12, 14, 16, 23–25) varies widely, probably because of differences among studies in the disease status of populations examined, site selection, sampling strategy, and the detection methods used. Although *P. gingivalis* has been found in young subjects, the age of acquisition and the prevalence in young children have not been determined. In order to determine the relationship of age to colonization, we carried out a cross-sectional study to determine the presence of *P. gingivalis* in subjects between birth and 18 years of age. A DNA assay based on PCR using *P. gingivalis*-specific primers (17) provided the necessary sensitivity and efficiency to accurately determine the age of acquisition and the prevalence of *P. gingivalis* in children.

One hundred ninety-eight children between the ages of 0 and 18 years, including at least 10 subjects for each year of age, were recruited from Columbus Children's Hospital Dental Clinic, Columbus, Ohio. Persons in orthodontic treatment, those reporting antibiotic use in the previous 30 days, and those with early-onset forms of periodontal disease were excluded. Verbal consent was obtained from the parent or guardian of each subject for this institutionally approved protocol. The study population consisted of 111 (56%) males and 87 (44%) females ranging in age from 20 days to 18.9 years. The racial composition of the population was 126 (64%) Caucasian, 64 (32%) African-American, 3 (2%) Asian-American, 1 (1%) Hispanic, and 4 (2%) other individuals. All subjects were examined and samples were obtained by a single investigator (D.L.M.) in the Columbus Children's Hospital Dental Clinic. Saliva was removed from the sampling sites with sterile gauze pads to minimize detection of transient contaminating bacteria

from an exogenous source. A sterile medium paper point was inserted into the mesial sulcus of every tooth and was removed 10 s after placement. Paper points were dragged across the buccal mucosa and the fissures of the dorsum of the tongue. The paper points from a single subject were pooled and stored frozen in a sterile 2-ml microcentrifuge tube until they were transported to the laboratory for processing.

Bacteria were eluted from the paper points, and DNA was isolated and purified by using GeneClean (Bio 101, Inc., La Jolla, Calif.) as previously described (17). A *P. gingivalis*-specific DNA fragment was generated by using nested PCR by a modification of the method described previously (17). A schematic representation of the bacterial rRNA operon, the relative locations and orientations of the primers used, and the resulting DNA fragments is shown in Fig. 1. Primer sequences are listed in Table 1. The spacer region located between the 16S and 23S ribosomal genes was first amplified by using the universal procaryotic primers 785 and 422. This was followed by a second, nested PCR using a *P. gingivalis*-specific primer, Pg8R, and the universal procaryotic primer 241. Pg8R is complementary to oligonucleotide Bg8 described by Dix et al. (8). Reactions were performed as described by Leys et al. (17), with the following exceptions: 0.03 µg of each primer was added to each reaction mixture, and for the second amplification primers were annealed at 52°C. DNA fragments were separated by electrophoresis in 1% agarose in TBE (0.1 M Tris-borate [pH 8.3], 2 mM EDTA). Gels (Fig. 2) were stained with ethidium bromide and photographed by using UV light. The presence of *P. gingivalis* was scored as positive or negative on the basis of the presence of a clear band of the expected size. All second amplifications were repeated and scored a second time. If the results were not in agreement, the amplification was repeated again. To eliminate the possibility of false negatives resulting from failure to obtain or amplify DNA from the plaque sample, the presence of DNA in all negative samples was confirmed by amplifying the product from the first amplification using the universal procaryotic primers 3-17R and 241.

Of the 111 males, 44 (40%) were positive for *P. gingivalis*, and 30 (34%) of the 88 females were positive. *P. gingivalis* was detected in 47 (37%) of 126 Caucasian subjects and 24 (38%) of 64 African-American subjects. Chi-square analysis did not show a significant relationship between race or sex and colonization.

Overall, *P. gingivalis* was detected in 37% of the 198 subjects examined. When assays that require culturing have been used, investigators have seldom detected *P. gingivalis* in children of any age (7, 9, 12, 16, 24). However, in studies of older children that have employed more sensitive DNA-based detection methods (1, 14), the prevalence of *P. gingivalis* is comparable to

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FIG. 1. Schematic representation of the procaryotic ribosomal operon and the locations and orientations of the primers and resulting DNA fragments. Universal primers 785 and 422 were used to generate a DNA fragment in a first nonspecific amplification step, and species-specific primer Pg8R and the universal primer 241 were used in a second amplification. Universal primers 3-17R and 241 were used in an alternative second amplification to confirm the presence of DNA in *P. gingivalis*-negative samples.

that reported here. The PCR-based detection methodology used for the present study has a limit of detection of 10 bacterial cells or less (17), while the detection limits for the most sensitive DNA hybridization assays are reported to be on the order of 10^2 to 10^3 cells (5). In comparison, culturing has been demonstrated to be less sensitive than DNA probes (18). On the basis of this evidence it appears likely that *P. gingivalis* is present in children in amounts below the threshold of detection for culture methods.

In addition to the method of detection, sampling strategy can also affect detection rates. In a comparison of sampling strategies, a high percentage of false negatives was obtained for *P. gingivalis* when a limited number of sites were sampled (13). To maximize the probability of detection in the current study, an intensive sampling strategy including the mesial sulcus of every tooth present plus the tongue and buccal mucosa was employed.

It is possible that the detection of *P. gingivalis* in children by a sensitive method may be attributed to a transient, low-level inoculation with bacteria from, for example, a heavily colonized parent. However, the removal of saliva from the sampling site and the similar prevalence observed by previous investigators using less sensitive methods (1, 14) suggest that this is not the case.

The prevalence of *P. gingivalis* by age is shown in Fig. 3. *P. gingivalis* was detected in all age groups, even among children less than 1 year of age. The youngest child in whom *P. gingivalis* was identified was 20 days old, and three of the six predentate infants in the sample population were positive for *P. gingivalis*. Logistic regression analysis showed no association between age and colonization status for any of the following age groups: all ages, age 3 years and below, age 2 years and below, or age 1 year and below. These data suggest that *P. gingivalis* is acquired rapidly upon exposure, even in the first days of life, and before what has been assumed to be its primary ecological niche, the gingival sulcus, has developed. The acquisition of *P. gingivalis* before the eruption of the teeth indicates that colonization at other mucosal sites may precede establishment in the sulcus. *P.*

FIG. 2. *P. gingivalis*-specific DNA fragments amplified directly from plaque samples. The markers in the far left lane are *Eco*RI and *Hind*III digestion products of bacteriophage lambda DNA. Lane 1, DNA amplified from *P. gingivalis* ATCC 33277, which appears at approximately 2.0 kb; lanes 2 through 7, DNA amplified from plaque samples. A *P. gingivalis*-specific fragment was detected in the first three of the six samples shown. The image was captured with a model 4910 CCD camera (Cohu, Inc., San Diego, Calif.) and an LG-3 Frame Grabber Board (Scion Corp., Frederick, Md.). Analysis was performed on a Power Macintosh 7100 computer with the public-domain NIH Image program (developed at the National Institutes of Health and available from the Internet by anonymous FTP from zippy.nimh.nih.gov or on floppy disk from the National Technical Information Service, Springfield, Va., part number PB95-500195GE1).

gingivalis has previously been cultured from the tonsils, tongue, buccal mucosa, and saliva of dentate children. Extracellular and sulcular colonization were observed both independently and together (21). Previous investigators (9, 15) did not detect *P. gingivalis* in pooled swab samples collected from the tongue, lips, cheeks, or palate of edentulous infants, but this is very likely due to the insensitivity of the culture-based assays that they used. Their results suggest, however, that *P. gingivalis* is not present in high numbers until the development of a sufficiently deep gingival pocket. Future studies in which bacterial levels are quantitated would be informative.

Because *P. gingivalis* does not survive exposure to oxygen, it is likely that this species is usually transmitted via intimate contact. Recent studies indicate that the colonization status of children is dependent upon that of their parents (10, 21, 22). This, together with the results of the study reported here, suggests that contact with infected individuals may be a much better predictor of colonization than the age of the child.

FIG. 3. Percentage of subjects colonized with *P. gingivalis* at each year of age. The number at the top of each bar indicates the number of subjects (the total number of subjects was 198).

TABLE 1. Sequences of oligonucleotide primers

Primer	Sequence
785	GGATTAGATACCCTGGTAGTC
422	GGAGTATTTAGCCTT
Pg8R	TGTATATGACTGATGGTGAAACC
3-17R	GGCTGGATCACCTCCTT
241	TTCGCTCGCCGCTACT

The overall colonization rate of 37% observed among children in this cross-sectional study is very similar to that observed among adults for which the same detection methodology and sampling strategy were used: *P. gingivalis* was detected in 46% of 380 adults over 20 years of age (10). The similar prevalence observed irrespective of age is consistent with the maintenance of colonization throughout the life of an individual. Longitudinal studies are needed to determine if these data reflect a subset of individuals who are stably colonized or if colonization is transient and dependent upon local factors. If *P. gingivalis* acquired early in childhood is maintained, it may be a long-term risk factor for the development of periodontal disease.

In conclusion, *P. gingivalis* was detected in 37% of children and was seen at similar frequencies throughout infancy, childhood, and adolescence. These data indicate that *P. gingivalis* may be acquired immediately upon exposure, possibly in the first days of life.

This study was supported by NIH grant DE10467.

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