Bacterial, behavioral and environmental factors associated with early childhood caries

Francisco J. Ramos-Gomez* / Jane A. Weintraub** / Stuart A. Gansky*** /
Charles I. Hoover**** / John D. B. Featherstone*****

The goals of this cross-sectional study were to characterize and compare demographic, behavioral, and environmental factors potentially associated with early childhood caries (ECC) and to assess salivary levels of mutans streptococci (MS) and lactobacilli (LB) in underserved, predominantly Hispanic children. One hundred forty-six children aged 3 to 55 months with a range of caries experience were identified and examined. ECC was primarily associated with the presence of MS and lack of access to dental care. Salivary MS levels among young children with ECC were higher than would be expected in a dentally healthy population, but lower than levels reported among older children at high risk for caries. After adjustment for age, children with log10 MS ≥ 3.0 or log10 LB ≥ 1.5 were about five times as likely (OR=4.9, 95%CI=2.0,12.0) to have ECC than those with lower bacterial levels. This study demonstrated a significant association between relatively low cariogenic bacterial levels and dental caries in infants and toddlers. Antibiotic use, exposure to lead, and anemia were not significantly associated with the number of decayed and filled surfaces or decayed and filled teeth. ECC correlated significantly with child's age and lack of dental insurance of the children, as well as inversely with both family income and the educational level of the mother of the child.
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INTRODUCTION

Dental caries in preschool children remains a significant public health problem in the United States. The Third National Health and Nutrition Examination Survey (NHANES III, 1988-1994) found that 8.4% of 2-year-old children and over 40% of 5-year-old children have at least one decayed or filled tooth.[1] The prevalence of caries is especially high among low-income children, particularly Native Americans, Mexican-Americans, and African-Americans. In these groups, early childhood caries (ECC) poses a serious threat to child welfare.

ECC is a preventable condition characterized by decay of primary teeth that may begin as soon as an infant's teeth erupt. In its severe form, manifestations include rampant decay, pain, infection, abscesses, chewing difficulty, malnutrition, gastrointestinal disorders, and low self-esteem. ECC can lead to caries in the permanent dentition and malocclusion and may be associated with poor speech articulation, retarded growth, and social ostracism.[2-5]

ECC is a chronic, transmissible, infectious disease whose etiology is complex and multifactorial. While some risk factors for ECC have been identified, the effects on specific ethnic groups or very young preschool children have not been adequately investigated. Preliminary analyses of NHANES III data found that Mexican-American children had the highest relative frequency of ECC.[6] In a study of rural Mexican-American children of migrant workers, the mean age at weaning from breast or bottle feeding and patterns of bottle use during sleep did not differ significantly between children with ECC and those without, and there were no clear patterns of cariogenic food frequency and disease status.[7] Davies[8] characterized ECC as a serious public health problem of disadvantaged

* Francisco J. Ramos-Gomez, Growth and Development (Division of Pediatric Dentistry), School of Dentistry, University of California, San Francisco, CA 94143.
** Jane A. Weintraub, Preventive and Restorative Dental Sciences, School of Dentistry, University of California, San Francisco, CA 94143.
*** Stuart A. Gansky, Preventive and Restorative Dental Sciences, School of Dentistry, University of California, San Francisco, CA 94143.
**** Charles I. Hoover, Stomatology, School of Dentistry, University of California, San Francisco, CA 94143.
***** John D. B. Featherstone, Preventive and Restorative Dental Sciences, University of California, San Francisco, CA 94143.

Send all correspondence to Dr. Francisco Ramos-Gomez, Department of Growth and Development, University of California, Box 0753, 707 Parnassus Avenue, San Francisco, CA 94143-0753.

Tel.: (415) 476-6826
Fax: (415) 502-1450
E-mail: ramos@itsa.ucsf.edu.

communities in both developing and industrialized countries and identified dietary habits and early MS infection as important predisposing factors. Horowitz[9] suggested the importance of assessing prenatal and perinatal histories, nutritional status, and microbiologic factors in the development of ECC, especially how and when infants and young children are colonized by MS. A possible role of antibiotics in preventing the transmission of MS from mother to child has been suggested, but not confirmed.

We conducted a cross-sectional study of predominantly Hispanic preschool children with a wide range of caries experience. The goals of the study were to characterize and compare demographic, behavioral, and environmental factors potentially associated with dental caries and to characterize cariogenic bacterial and chemical markers in saliva, specifically levels of mutans streptococci (MS), lactobacilli (LB), calcium, phosphate, and fluoride, which are potentially associated, either positively or negatively, with ECC. Our approach was deliberately multifactorial in order to develop a comprehensive risk assessment model for ECC in economically disadvantaged children.

METHODS

Study population

Study subjects were recruited from three sites based at San Francisco General Hospital (the Family Dental Center, the Women, Infant and Children Program (WIC), and the Well Child Clinic), both serve primarily low-income Hispanic and African-American families. At each site, an outreach worker invited parents with children <60 months of age to participate in the study. Written informed consent was obtained from all parents who agreed to answer a detailed questionnaire and to allow their children to undergo a dental examination and saliva collection.

Parental interview

A trained research assistant conducted a structured interview of the parent or guardian in English or Spanish. The interview was based on a standardized, 69-item questionnaire designed to collect demographic data and information on the education and income of the parents, feeding practices, oral health and hygiene, and exposure to antibiotics and lead. Some questions were specifically targeted to gain information about the child and others about the caregiver. Response categories were on ordinal or dichotomous scales.

Clinical examination

After the interview, one of the authors (FRG) conducted a complete dental assessment of each child in the dental operatory at the chair or by a knee-to-knee examination. Decayed and filled primary teeth (dft) and surfaces (dfs) and incipient lesions (idfs) were assessed according to the dental caries diagnostic criteria of the National Institute of Dental and Craniofacial Research.[10]

ECC was diagnosed based on the presence of at least one primary tooth affected by one or more carious lesions. Caries on smooth surfaces or in pits and fissures of the tooth had to reveal loss of tooth structure, with clinically obvious undermining and opacity. Children with only incipient lesions (white spot lesions with a chalky appearance, but no loss of or breakdown of enamel tissue) were included in the study, but they were analyzed as a separate intermediate disease category.

Data recording

All data from the interviews and the clinical examinations were recorded on a web-based, electronic survey form accessed from a computer workstation. Before it was used in the study, this system was pilot tested in conjunction with the pilot testing of the interview questionnaire and clinical exams.

Saliva collection and analysis

Unstimulated saliva (~3 ml) was collected from each child with a sterile disposable polyethylene Pasteur pipette and transferred to a sterile 5-ml transport tube. Unstimulated samples were obtained because of the difficulty of obtaining stimulated samples from very young children. Saliva samples were immediately placed on ice and transported from the clinic to the laboratory, where they were cultured within 24 hours of collection.

Quantitative microbiological culture techniques were used to determine the number of mutans streptococci (MS) (*Streptococcus mutans*, *S. sobrinus*) and *Lactobacillus* species (LB), and total bacteria per milliliter of saliva. In brief, 0.1-ml portions of unstimulated saliva and 0.1-ml portions of 10-fold serial dilutions (10^{-1} through 10^{-5}) of unstimulated saliva prepared in reduced transport fluid (RTF)[11] were plated on selective and nonselective media for culture. This facilitated accurate determination of target microbial concentrations in the range of 10^{1} to $>10^{6}$ colony-forming units (CFU)/ml. All plates were incubated anaerobically (85% N_2, 10% H_2, and 5% CO_2) at 37°C for 48–72 hours.

MS were cultured on Mitis Salivarius Sucrose Bacitracin (MSSB) agar.[12] MSSB agar is a selective differential medium for isolating MS.[13] After inoculation, the MSSB plates were examined under a dissecting microscope, and colonies that conformed to the typical colony characteristics of MS were enumerated as described elsewhere.[12,14,15] On MSSB agar, MS form blue/black, convex to raised colonies that usually have a granular or etched surface resembling burnt sugar or frosted glass. Frequently the MS colonies are topped by a drop or surrounded by a pool of polysaccharide.

LB were cultured on Rogosa tomato juice (RTJ) agar.[12] RTJ agar is a selective medium for isolating LB.[16]

Table 1. Potential caries risk factors and caries experience

Risk Factor	ECC (n = 63)*	Incipient Lesions (n = 23)	Caries Free (n = 60)	Overall (n = 146)	Adjusted for age and child's dental insurance: Odds Ratio ECC vs. Caries Free	Adjusted for age and child's dental insurance: 95% CI
Female (%)	49	35	45	45	1.18	[0.55–2.53]
Age (months, mean ± SD)	34 ± 11	30 ± 12	27 ± 12	30 ± 12	1.06	[1.02–1.10]‡
Ethnicity (%)†						
Mexican	27	13	10	18	1.32§	[0.27–6.40]
Mexican-American	43	57	40	44		
Central American	25	30	3831			
Other	5	0	12	7		
Single-parent family (%)	8	22	20	15	0.40	[0.12–1.30]
Parents' ages (years, mean ± SD)						
Mother	27 ± 4	28 ± 6	28 ± 5	28 ± 5	0.99	[0.91–1.07]
Father	30 ± 6	30 ± 5	31 ± 5	30 ± 5	1.01	[0.93–1.08]
Child w/o dental insurance (%)†	43	22	22	31	2.31	[1.01–5.28]
Parents w/o dental insurance (%)	90	65	82	83	1.86	[0.59–5.82]
Mothers ≥HS graduate (%)†	16	35	35	27	0.5	[0.20–1.26]
Fathers ≥HS graduate (%)	28	38	22	27	1.15	[0.47–2.84]
Family Income ≥$15,000 (%)	29	26	32	29	0.72	[0.31–1.68]
Parents w/current caries (%)	84	77	75	79	1.37	[0.49–3.87]
No chance to find dentist (%)	57	65	65	62	0.87	[0.39–1.91]
Sweetened beverage (%)	35	40	30	34	1.50	[0.66–3.40]
Lead exposure (%)	0	6	4	3	Inestimable	
Antibiotics during pregnancy (%)	5	32	15	13	0.30	[0.07–1.27]
Antibiotics during 1st year (%)	52	68	55	56	0.96	[0.44–2.06]
Iron deficiency (%)	21	30	21	22	0.73	[0.27–1.99]

*Includes one child with dfs = 0 who had extractions from ECC.
†Spearman rank correlation $p \leq 0.05$.
‡Adjusted only for child's dental insurance.
§Partial (age-adjusted) Spearman rank correlation $p \leq 0.05$ (Latinos vs. others).
¶Adjusted only for age.

After incubation, colonies were enumerated, as above, by using a dissecting microscope. On RTJ agar, LB form smooth, white colonies that are usually convex.

The total CFU of bacteria per milliliter of unstimulated saliva was determined by culture on Trypticase soy agar supplemented with 5% defibrinated sheep blood. After incubation, the plates were examined as above, and every colony was enumerated. The colony counts were determined, and the corresponding dilution factors were then used to calculate the CFU/ml of unstimulated saliva for each of the target bacteria.

Whenever a sufficient volume of saliva (0.5 ml) was available after bacterial analyses were performed, fluoride, calcium, and phosphate levels were assessed as described by Leverett *et al*.[17]

Statistical analysis

The study was originally designed as a balanced case-control study. However, because some children had incipient lesions upon examination, the actual study design was that of a cross-sectional convenience sample. As in a traditional (the planned) case-control study, logistic regression was used to compare cases (ECC) versus controls (no caries), excluding the intermediate group (incipient lesions). Odds ratios and their 95% confidence intervals (CIs) were calculated for these comparisons.

The originally planned sample size of 60 cases and 60 controls would have had at least 80% power to detect an odds ratio of 3.0 for factors with one proportion between 0.25 and 0.75 inclusive, with a two-sided chi-square test (alpha = 0.05). A power analysis of the revised study design (before study completion), which was projected to include 61 participants with ECC, 24 with incipient lesions only, and 35 with no caries, demonstrated at least 84% power resulting from two-sided trend tests (alpha = 0.05), with varying proportions of the three groups between 0.225 and 0.550. Thus, the actual sample size of 63 participants with ECC, 23 with incipient lesions, and 60 with no caries, should provide adequate power to detect important differences.

Associations among caries experience (ECC, incipient lesions only, and no caries) and characteristics of the children and their families were assessed with Spearman rank correlations, partial Spearman rank correlations adjusting for age, Kruskal-Wallis tests, median ANOVA tests, age-adjusted Kruskal-Wallis tests, and logistic regression. Rank correlations were used to compare ordinal caries status groups (ECC, incipient lesions only, or no caries) with dichotomous,

Table 2. Caries experience (mean ± SD)

	ECC (n = 63)	Incipient lesions (n = 23)	Caries free (n = 60)	Overall (n = 146)
No. of teeth	18.9 ± 2.2	17.8 ± 3.6	14.9 ± 5.6	17.1 ± 4.5
ds*	4.5 ± 5.3	—	—	2.0 ± 4.2
dfs	5.0 ± 5.7	—	—	2.2 ± 4.5
idfs	6.0 ±5.7	3.1 ± 1.8	—	3.1 ± 4.7
dt	3.3 ± 2.9	—	—	1.4 ±2.5
dft	3.4 ± 2.9	—	—	1.5 ± 2.5
idft	4.2 ± 2.8	3.1 ±1.8	—	2.3 ± 2.8

*Abbreviations: ds, decayed surface; dfs, decayed or filled surface; idfs, incipient, decayed, or filled surface; dt, decayed tooth; dft, decayed or filled tooth; idft, incipient, decayed, or filled tooth.

ordinal, and continuous factors. Odds ratios (adjusted for age and dental insurance) and 95% confidence intervals were calculated for comparisons of the ECC group with the no caries group with logistic regression analyses. Receiver operating characteristic (ROC) curves[18] were used to determine cutoffs to characterize patients as having high or low levels of bacteria and then to assess the association between these bacterial levels and the presence or absence of ECC. ROC analysis yields the cutoffs that have the best sensitivity and specificity for predicting ECC. For practicality of future use, we limited final cutoffs of $\log_{10}$ values to 0.5 increments. These cutoffs will allow other researchers or clinicians to use bacterial categories for diagnostic purposes.

RESULTS

Demographic characteristics of children and parents

One hundred forty-nine children, all from San Francisco, were enrolled in the study. One child was excluded from this analysis because an adequate saliva sample could not be obtained, and two children were excluded because they were predentate. Of the remaining 146 children, 45% were female and 55% were male. Demographic information and potential caries risk factors are shown in Table 1. The median age was 30 months (range, 3 to 55 months). One hundred thirty-seven children (94%) were of Hispanic origin (Mexican, Mexican-American, Central American, or Puerto Rican), two were African-American, and four were non-Hispanic whites. Fifteen percent of the children were from single-parent families. At the interview, 95% of caregivers were mothers, 4% were fathers, and 1% were other relatives. The median age of the mothers was 27 years (range, 18–45 years), and the median age of the fathers was 28 years (range, 21 to 49 years). Among both mothers and fathers, 73% had less than a high-school education, and 71% of families earned less than $15,000 annually. Except for age of the child, none of these factors were significantly different between ECC and caries-free children.

Diet and feeding practices

Seventy-two percent of the children had at least three snacks/day other than regular meals. Among primary caregivers, 82% (120), believed that a child's teeth were harmed when sleeping with a bottle. Nevertheless, when their children were 1 year old, 30% (43) had put them to sleep with a bottle containing sweetened liquid. At the time of the interview, 75% (111) normally put the child to sleep with a bottle of milk or formula (sweetened or unsweetened) or sweetened liquid or fed them at the breast before putting them to sleep; 30% (43) always used a bottle of sweetened liquid. However, there were no statistically significant differences in the diet and feeding practices of children with and without ECC.

Access to dental care

Overall, 69% of children, but only 17% of parents, had dental insurance. Most (63%) of the children had never seen a dentist before. When asked why, 86% of parents said the child was too young, 73% said there was no need for the child to see a dentist, and 62% said they had been unable to find dentists to examine their children. The majority of parents (55%) had not seen a dentist in the previous two years, but 79% reported that they currently had tooth decay. There was a statistically significant correlation between ECC and lack of dental insurance of the children, even after adjustment for age. Children without dental insurance were more than twice as likely to have ECC (age-adjusted odds ratio, 2.31) as children with dental insurance (public or private). For each year of age, children were 1.82 times more likely to have ECC [95% CI: 1.24–2.67].

Table 3. Bacterial levels as assessed in saliva (colony-forming units/ml)

	Caries Experience Group			
	ECC (n = 63)	**Incipient Lesion (n = 21)**	**Caries Free (n = 59)**	**Overall (n = 143)***
Mean $\log_{10}$ MS (±SD)†	4.0 ± 0.2	3.0 ± 0.3	2.4 ± 0.3	3.2 ± 0.2
Median $\log_{10}$ MS‡	4.2	3.7	2.9	3.8
% $\log_{10}$ MS > 0§	94	86	63	80
Mean $\log_{10}$ LB (±SD)†	0.9 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.1
Median $\log_{10}$ LB¶	0	0	0	0
% $\log_{10}$ LB > 0§	40	10	7	22

Log salivary bacterial levels were significantly associated with caries experience group and with dfs, idfs and idft (partial Spearman rank correlation (r_s), 0.33–0.51; $p < 0.001$).
*Three participants had insufficient saliva collection for analysis.
†$p < 0.001$(Kruskal-Wallis test comparing the three caries groups).
‡$p = 0.018$ (median ANOVA test comparing the three caries groups).
§$p < 0.001$ (chi-square test comparing the three caries groups).
¶$p < 0.001$ (median ANOVA test comparing the three caries groups).

Clinical findings

Caries experience is summarized in Table 2. Forty-three percent of children had ECC (presence of any caries in the primary dentition), 16% had only localized enamel demineralization, and 41% had neither. The children with ECC were older than those without caries (mean ± standard deviation [SD]), 34 ± 11 vs. 27 ± 12 months). For example, only 19% of the ECC group were <2 years old, compared to 47% of the no caries group and 35% of the incipient lesions group; moreover, 46% of the ECC group were (3 years old, compared to 23% of the no caries group and 26% of the incipient lesions group. Thus, almost all subsequent analyses statistically adjust for age, to account for this age difference and compare the groups at a common age. ECC was more common in Mexican children than in children from other ethnic groups studied, but the difference was not statistically significant. Among children with caries experience, the mean number (±SD of decayed surfaces (ds) was 4.5 ± 5.3, and the mean number (±SD) of decayed and filled surfaces (dfs) was 5.0 ± 5.7. These counts were not normally distributed, but were skewed to the right (positive direction). The median for both measures was 3.0. The dfs was inversely associated with the mother's education (partial Spearman correlation, –0.21, p = 0.015) and with the mother's use of antibiotics during pregnancy (partial Spearman correlation, –0.22, p = 0.010).

Bacterial levels

Salivary bacterial levels are summarized in Figure 1 and Table 3. MS were present in the saliva of most children, including predentate children as young as 3 months of age (excluded from the study). The mean $\log_{10}$ MS count was highest in children with ECC, lowest in those without caries, and intermediate in those with incipient lesions; these differences were statistically significant. The MS levels generally increased with age; bacteria were present in very young children with incipient lesions and ECC (Figure 1).

LB were present in the saliva of children as young as 8 months of age (Figure 2). The salivary level of LB was highest in children with ECC, but these bacteria were present at lower levels in children with incipient lesions and in those with no caries. In the ECC group, the mean $\log_{10}$ LB count was higher in older children, whereas it decreased with age in the other groups (Figure 2).

ROC curves **(**Figure 3**)** show how sensitivity (% true positives) and 1 – specificity (% false positives) change for the different diagnostic cutoffs used to classify children as having high or low bacterial levels. In these young children, bacterial level cutoffs associated with caries determined by ROC analyses were as follows: $\log_{10}$ (MS) of 3 gave 84% sensitivity (Sn) and 53% specificity (Sp); $\log_{10}$ (LB) of 1.5 gave Sn of 33% and Sp of 97%. Several combinations of MS an LB cutoff values were also evaluated. They yielded favorable sensitivity (a cutoff of 3 for $\log_{10}$ MS or 1.5 for $\log_{10}$ LB gave Sn of 86% and Sp of 51%), favorable specificity (4.5 for $\log_{10}$ MS or 1.5 for $\log_{10}$ LB gave Sn of 57% and Sp of 81%), or intermediate values of both measures (4 for $\log_{10}$ MS or 1.5 for $\log_{10}$ LB gave Sn of 68% and Sp of 69%). The sensitivity and specificity of the various cutoff values are summarized in Table 4.

Elevated bacterial levels ($\log_{10}$ MS ≥3 or $\log_{10}$ LB ≥1.5) were associated with 6.2 times the odds that a child had

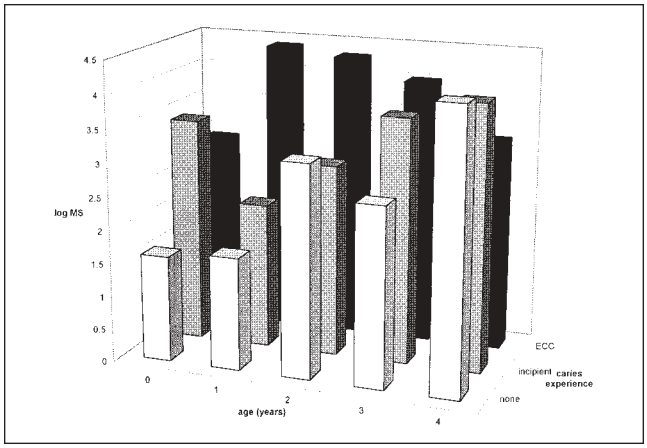

Figure 1. Salivary levels of MS plotted against age and cariogenic experience.

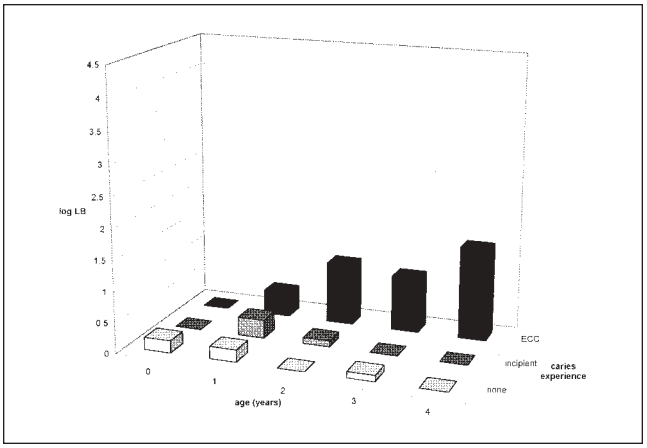

Figure 2. Salivary levels of LB plotted against age and cariogenic experience.

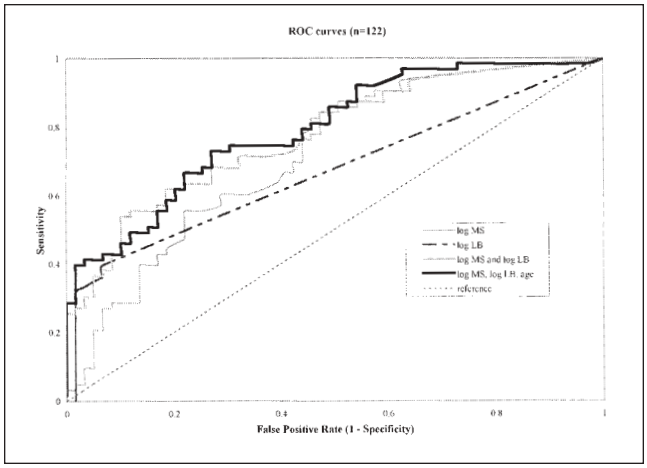

Figure 3. Receiver operator characteristics curves showing sensitivity and specificity of different log10 MS and LB cutoff levels alone, in combination, and in combination with age.

ECC (95% CI, 2.6–14.8). After adjustment for age, the odds ratio was 4.9 (95% CI, 2.0–12.0). In Figure 3, he 45-degree line is a reference for a diagnostic test that is the same as flipping a coin. Curves farthest from the reference line and closest to the upper left corner (x,y coordinates of 1,0) are best. Although the $\log_{10}$ MS curve was better than the $\log_{10}$ LB curve, combining $\log_{10}$ MS with $\log_{10}$ LB produced a better diagnostic test; using age as well produced the best curve.

When log MS and log LB were added for a final multivariable logistic regression model of ECC versus no caries, age and child's dental insurance were significant, in addition to log MS and log LB. Children with no dental insurance were 2.90 [95% CI: 1.12–7.54] times more likely to have ECC than those with any form (public or private) of dental insurance. For each year of age children were 1.64 times more likely to have ECC [95% CI: 1.05–2.54]. For each log of MS, children were 1.49 times [95% CI: 1.16–1.90] more likely to have ECC; for each log of LB, they were 2.04 times [95% CI: 1.17–3.54] more likely to have ECC.

Salivary fluoride, phosphate, and calcium levels

Sufficient quantities of saliva for measurement of salivary fluoride, phosphate, and calcium levels were obtained from 66 subjects; in the other subjects, only enough saliva was collected for bacterial analysis. The fluoride levels were lower than expected from previous studies in older children,[17] and calcium and phosphate levels were normal (Table 5). There were no significant differences in these levels between children with ECC or incipient lesions or those without caries.

DISCUSSION

This study of predominantly Hispanic preschool children with varied caries experience showed significant associations between caries and bacterial levels at a very young age. MS were detected in infants as young as 3 months of age and were present in almost two-thirds of children under 24 months of age. The MS level associated with caries in these young children was much lower than that in older children aged 6 to 12 years,[17] which was two orders of magnitude higher. LB were present in 10% of children under 12 months of age, and the level of LB increased in older children with ECC. Log_{10} salivary MS and LB levels were significantly associated with dfs, idfs, decayed and filled teeth (dft), and incipient decayed and filled teeth (idft), even after adjustment for age. ECC correlated significantly with age and lack of dental insurance of the children and correlated significantly (inversely) with the mother's level of education and family income. After adjustment for age, lack of dental insurance was significantly associated with ECC; children lacking dental insurance were twice as likely to have ECC as those who had insurance. No associations between dfs or dft and calcium, phosphate, fluoride, antibiotic use, exposure to lead, or anemia was observed.

At least three categories of risk factors have been associated with caries development: microorganisms,

Table 4. Sensitivity and specificity of bacterial cutoffs for high and low levels of bacteria from roc curve analysis (n = 123)

	Log_{10} MS	Log_{10} LB	Sensitivity	Specificity
Individual	3.0	—	84%	53%
	—	1.5	33%	97%
Combined (either, or)	3.0	1.5	86%	51%
	4.0	1.5	68%	69%
	4.5	1.5	57%	81%
Combined + age (either, or)	3.0	1.5	87%	53%
	4.0	1.5	84%	56%
	4.5	1.5	76%	63%

Table 5. Salivary levels of fluoride, calcium, and phosphate

	ECC (n = 35)	Incipient Lesions (n = 8)	Caries Free (n = 24)	Overall (n = 66)
Saliva sample ≥ 0.5 ml	56%	35%	40%	45%
Fluoride (ppm)	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01
Calcium (mmo1/L)	1.4 ± 0.06	1.3 ± 0.12	1.4 ± 0.08	1.4 ± 0.05
Phosphate (mmo1/L)	5.8 ± 0.4	5.3 ± 0.5	5.3 ± 0.4	5.6 ± 0.2

There were no significant differences between groups.

substrate/oral environment, and host/teeth. Recent evidence strongly suggests that the first step in the development of ECC is by MS infection. Our study showed that MS are present much earlier than at 7 to 9 months of age, as previously reported.[19] One child who was 3.8 months old had an MS level of 700 CFU/ml without detectable LB. In another study of 199 children 6 to 31 months of age, *S. mutans* were present in 25% of predentate children.[20] A long-held assumption was that poor dietary habits or prolonged bottle-feeding provide the substrate for rapid proliferation of the bacteria. In our study, however, neither dfs nor dft correlated with exposure to a cariogenic diet, bottle use, or frequency of carbohydrate consumption (more than three snacks a day); however, cariogenic bacteria were present even before tooth eruption. This finding suggests that the bacteria initiate the colonization and demineralization of tooth enamel and that dietary factors contribute to the severity of ECC, but do not initiate the process.

As the MS pass through their life cycle, they produce acidic byproducts that penetrate the protective exterior tooth surface. LB present in the mouth can also colonize damaged areas, producing more acid byproducts and further damaging the interior of the tooth. Rapid demineralization and cavity formation ensue, resulting in rampant dental caries.[21] Caries may become clinically evident as early as 12 to 16 months of age, usually appearing first on the labial-gingival and lingual surfaces of the upper incisors. Our findings support this scenario. LB were present at a very low level in children as young as 8 months, regardless of caries status. However, only in children with ECC were LB levels progressively higher in older children.

Multiple studies have directly or indirectly focused on bacterial risk factors for ECC. Buttner[22] linked the high incidence of caries among underserved children to levels of MS and LB and suggested that early infection, particularly the oral transmission of saliva from mother to child might be the source. Other studies[23-25] have linked the MS level in the mother with the MS level in the child; Brambilla *et al*.[26] found that reducing the MS levels in mothers delayed the colonization of bacteria in their children for about 4 months. Karn *et al*.[27] found a relationship between the age at which a child becomes colonized with MS and the occurrence of ECC. In our study, the mother's use of antibiotics during pregnancy correlated inversely with dfs, suggesting reduced transmission of bacteria from mother to child. Seow[28] pointed out that while the etiology of ECC appeared similar to that of other types of caries, the role of predisposing factors still needed to be clarified. Bowen[29] questioned the role of milk and infant formulas and suggested prenatal exposure to lead as a possible factor. In our study, however, there was no association between ECC and exposure to sweetened beverages or exposure to lead. The presence of anemia and the child's exposure to antibiotics during the first year of life were also not associated with dfs or dft.

The lack of association between ECC and dietary factors or lead/antibiotic exposure could reflect inaccu-

rate self-reports, low statistical power, or a true lack of association. If exposure information cannot be accurately determined by self-reports in a research study such as ours, it is highly unlikely that it will be a clinically useful screening tool to identify high-risk children. Post hoc power analyses showed we had adequate power to detect moderate effects.

The most striking finding of our study was the low levels of MS associated with ECC in very young children. MS cutoffs associated with caries in older children studied by Leverett *et al*.[17] were two orders of magnitude higher than those in the children we studied. In that study, stimulated saliva was used to sample the whole mouth, but it was not possible to obtain stimulated saliva samples from the very young children in the present study. This difference at least partially explains the lower bacterial levels in our subjects. Nevertheless, our study shows that levels of MS colonization not previously thought to be significant may be quite harmful in the very young. Moreover, the associations of $\log_{10}$ salivary MS and LB levels with dfs, dft, idfs, and idft were significant even after adjustment for age (partial rs: 0.33–0.50; all $p < 0.001$). In addition to MS and LB levels, factors associated with ECC were lack of dental insurance for the child and low educational level of the mothers. Salivary levels of fluoride were very low in our study subjects; calcium and phosphate levels were normal. There were no significant differences in these levels between children with ECC or incipient lesions or those without caries.

In our study, several family-related factors were significantly associated with ECC. Children were more likely to have ECC if they were from a single-parent or low-income family or if the caregiver had a low level of education. A recent Finnish study showed that several family-related factors were associated with an increased likelihood of caries in 5-year-old children.[30] These factors included a teenage mother, living in a rural area, and poor oral hygiene of the parents. Our study also showed that barriers to access to dental care for very young children are a significant problem. Indeed, 63% of the children in our study had never been to a dentist before. When asked why, parents gave a variety of responses, including that the child was too young (86%), that there was no need to see a dentist (73%), and that they could not find a dentist to see the child at such a young age (62%). These findings suggest that educational and socioeconomic disparities have a profoundly adverse effect on the oral heath of very young, underserved children. The clinical finding of demineralization of enamel and the presence of cariogenic bacteria, even in the youngest subjects in our study, suggest that children should see a dentist starting at 1 year of age to allow risk assessment and preventive intervention before ECC becomes severe and extremely difficult and expensive to treat.[31,32] Children without dental insurance were 2.3 times more likely to develop ECC than children with dental insurance after adjusting for age.

Although lack of knowledge about oral health and hygiene has been identified as a risk factor for ECC,[33] educational efforts to prevent ECC have led to mixed results. Several studies have pointed to the importance of diet, particularly sugary snacks, as a risk factor,[19,28,34-36] but efforts to educate caregivers to reduce sugary snacks and increase brushing have been only modestly successful[37] or have failed entirely to prevent ECC.[38] Of the low-income parents in our study, only 32% had ever received counseling on ECC, and 83% had no dental insurance. Efforts to reduce ECC should include improving dental education and access to dental care of adult caregivers to reduce transmission of MS from adult to child.

CONCLUSION

This study shows that there is a low cariogenic bacterial threshold for the onset and progression of ECC and that there is a significant association between caries and bacterial levels in infants and toddlers. In these young children, MS and LB threshold levels associated with caries were lower than those considered significant in older children and adults. Although other demographic and behavioral risk factors undoubtedly contribute to ECC, bacterial risk factors were significantly associated with the presence and severity of ECC.

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