

## RESEARCH REPORTS

## Clinical

A.K.L. Wan<sup>1</sup>, W.K. Seow<sup>1\*</sup>,  
D.M. Purdie<sup>2</sup>, P.S. Bird<sup>3</sup>,  
L.J. Walsh<sup>1</sup>, and D.I. Tudehope<sup>4</sup>

<sup>1</sup>University of Queensland, School of Dentistry, 200 Turbot Street, Brisbane, Queensland 4000, Australia; <sup>2</sup>Queensland Institute of Medical Research, Population & Clinical Sciences Division, Herston Rd., Brisbane, Queensland 4006, Australia; <sup>3</sup>Oral Biology Laboratory, University of Queensland, St Lucia, Brisbane, Queensland 4072, Australia; and <sup>4</sup>Mater Misericordiae Hospitals, Raymond Tce., South Brisbane, Queensland 4105, Australia; \*corresponding author, [k.seow@uq.edu.au](mailto:k.seow@uq.edu.au)

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## ABSTRACT

We previously reported that, before tooth eruption, over one-half of infants aged 6 mos were already infected with *Streptococcus mutans*. The aim of this investigation was to determine the colonization of *S. mutans* after tooth eruption in the same cohort of 111 infants (35 pre-term, 76 full-term). Our results showed that *S. mutans* colonization increased with increasing age, so that by 24 mos of age, 84% harbored the bacteria ( $p < 0.01$ ). The mean and median ages of *S. mutans* colonization in dentate infants were 15.7 mos and 16.0 mos, respectively. Factors associated with *S. mutans* colonization were sweetened fluids taken to bed ( $p < 0.01$ ), frequent sugar exposure ( $p < 0.03$ ) and snacking ( $p < 0.03$ ), sharing of foods with adults ( $p < 0.03$ ), and maternal *S. mutans* levels of  $> 10^5$  CFU/mL ( $p < 0.02$ ). In contrast, non-colonization of *S. mutans* was associated with toothbrushing ( $p < 0.03$ ) and multiple courses of antibiotics ( $p < 0.001$ ). Analysis of our data establishes the timing of *S. mutans* colonization in children from birth to 24 mos of age.

**KEY WORDS:** *Streptococcus mutans*, pre-term, full-term, oral colonization.

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# A Longitudinal Study of *Streptococcus mutans* Colonization in Infants after Tooth Eruption

## INTRODUCTION

*Mutans* streptococci, comprised of the species *Streptococcus mutans* and *Streptococcus sobrinus*, are the principal bacteria responsible for dental caries in humans (Masuda *et al.*, 1979; Loesche, 1986). Traditionally, *mutans* streptococci are believed to colonize the mouth only when teeth are present (Loesche, 1986), and most studies reported that initial colonization occurred only after eruption of the primary teeth (Masuda *et al.*, 1979; Caufield *et al.*, 1993; Li and Caufield, 1995; Karn *et al.*, 1998). However, our recent investigations showed that colonization of *S. mutans* can occur in preterm infants as young as 3 mos of age (Wan *et al.*, 2001a,b), and that in six-month-old children without teeth, over 50% of pre-term and 60% of full-term infants were already infected with these bacteria. In this study, we followed up the same cohort of children to determine the ages when the remainder of the subjects became colonized with *S. mutans*. Therefore, we aimed to establish the infection rate and median/mean ages of *S. mutans* colonization after tooth eruption in infants, from 6 to 24 mos of age, as well as factors which contribute to colonization. To the best of our knowledge, no similar longitudinal studies have been published.

## MATERIALS &amp; METHODS

## Subjects

Ethical clearance was obtained from relevant human research ethics committees. Signed informed consent was obtained from the mothers. Pre-term ( $< 37$  wks gestation with birthweights  $< 2000$  g) and full-term infants with normal birthweights ( $> 2500$  g) were randomly recruited at birth from the Mater Mothers' Hospital, South Brisbane, Australia. The population of this study is well-represented, as indicated by even distribution of different levels of parental education and income. The total study cohort consisted of 312 (93 pre-term and 219 full-term) infants, followed from birth until 24 mos old at three-monthly intervals.

## Data Collection

The subjects were recalled every 3 mos from birth until they were 24 mos of age. Interviews and dental examinations were conducted by one investigator (AKLW). Medical, dental, social, and feeding histories were recorded and updated at each recall visit, with the use of previously validated questionnaires (Seow *et al.*, 1999). In addition, medical information provided by the mothers during interviews was verified by hospital records. Dental examinations of infants and their mothers were performed at the University Dental School, with the use of dental mirrors. Intra-examiner consistency for dental examination was established at over 95% (Wan *et al.*, 2001b).

## Collection of Microbiological Samples

We obtained microbiological samples from mothers and infants by swabbing the dorsum of the tongue and all surfaces of the teeth with sterile cotton tips. Each swab, which held 0.1 mL of saliva, was placed into a sterile vial containing

phosphate-buffered saline, transported to the laboratory at 4°C, and processed within 4 hrs.

**Isolation of *Streptococcus mutans* from Saliva**

The methods used for *S. mutans* isolation and enumeration have been published previously (Wan *et al.*, 2001a,b, 2002). In brief, after samples were vortexed for 30 sec to disperse bacteria, 50-µL aliquots of ten-fold dilutions were plated onto *S. mutans*-selective tryptone-yeast-cysteine-sucrose-bacitracin agar (TYSCB; Microdiagnostic, Brisbane, Australia). Plates in triplicate were incubated at 37°C under anaerobic conditions for 72 hrs. Colonies were enumerated by means of a colony-counter, and the mean colony-forming units/mL saliva (CFUs/mL) were calculated for *S. mutans*. Control plates with known concentrations of *S. mutans* (NCTC 10449) were incubated with the sample plates. Bacterial identification was verified on random colonies by Gram stains and biochemical analyses (Rapid Strep ID32 API, bioMerieux Vitek, Marcy-l'Etoile, France). Colonization was considered positive when 2 consecutive saliva samples showed *S. mutans*.

**Statistical Analysis**

All statistical analyses were performed with SPSS version 10.0.1. Non-parametric statistical analyses were performed with Chi-square, Mann-Whitney, Kruskal-Wallis, and Friedman tests and Spearman's rank-order correlation. We used logistic regression to determine independent predictors for *S. mutans* colonization.

**RESULTS**

**Demography**

Only infants who were not previously colonized by *S. mutans* at the time of first tooth eruption were included in the study. Of the 312 infants in the study cohort, 111 infants (35 pre-term and 76 full-term) met the inclusion criteria (Table). The overall mean birthweight and gestational age of pre-term infants were 1.8 ± 0.7 kg and 32.7 ± 3.6 wks compared with 3.6 ± 0.5 kg and 39.8 ± 1.0 wks in full-term infants (p < 0.01).

**Overall Prevalence of *S. mutans***

Fig. 1 shows the overall prevalence of *S. mutans* in the study cohort of 312 infants. By the end of the study at age 24 mos, 79% of the 312 infants were colonized by *S. mutans*. The cumulative prevalence of *S. mutans* colonization in all children was 5%, 18%, 49%, 53%, 62%, 68%, 70%, 74.5%, and 79% at 0, 6, 9, 12, 15, 18, 21, and 24 mos of age, respectively. As reported in our previous paper, the overall prevalence of *S. mutans* colonization in infants without teeth was 64% (153/238) (Wan *et al.*, 2001b). The prevalence increased to 84% (93/111) after the eruption of teeth in this part of the study.

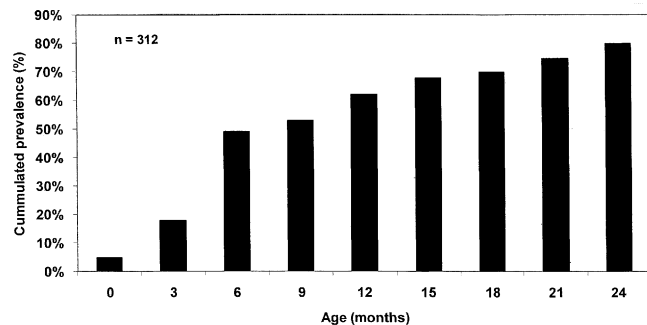
**Prevalence of Oral *S. mutans* Infection in Pre-term and Full-term Infants**

As shown in Fig. 2, the overall prevalence of *S. mutans*

**Table.** General Demography of Subjects with *S. mutans* Present/Absent at 24 Mos of Age (data as at end of study period)

	<i>S. mutans</i> Present				<i>S. mutans</i> Absent				P value <sup>c</sup>
	Pre-term	Full-term	p value <sup>a</sup>	Total	Pre-term	Full-term	p value <sup>b</sup>	Total	
N	33/35	60/76	0.0421 <sup>d</sup>	93/111	2/35	16/76	0.042d	18/111	0.042 <sup>d</sup>
%	94	79		84	6	21		16	0.042 <sup>d</sup>
<b>Age at end of study</b>									
Mean age ± SD (mos)	27.4 ± 4.5	26.7 ± 3.9	n.s. <sup>e</sup>	26.9 ± 4.1	34.0 ± 2.8	26.1 ± 3.6	n.s. <sup>e</sup>	27.2 ± 4.3	n.s. <sup>e</sup>
<b>Dental caries present at 24 mos of age, N (%)</b>	4 (12)	4 (7)	n.s. <sup>d</sup>	8 (9)	0	0	n.s. <sup>d</sup>	0	n.s. <sup>d</sup>
<b>Gender, N (%)</b>									
Female	16 (48)	29 (48)	n.s. <sup>d</sup>	45 (48)	0	10 (63)	n.s. <sup>d</sup>	10 (56)	n.s. <sup>d</sup>
Male	17 (52)	31 (52)		48 (52)	2 (100)	6 (38)		8 (44)	
<b>Ethnicity, N (%)</b>									
Caucasian	24 (73)	46 (77)	n.s. <sup>d</sup>	70 (75)	2 (100)	16 (100)	n.s. <sup>d</sup>	18 (100)	0.018 <sup>d</sup>
Other <sup>f</sup>	9 (27)	14 (23)		23 (25)	0	0		0	
<b>Birth details</b>									
Gestational age (mean ± SD), wks	32.7 ± 3.6	39.8 ± 1.0	< 0.001 <sup>e</sup>	37.3 ± 4.1	27.5 ± 2.1	39.7 ± 1.9	< 0.001 <sup>e</sup>	38.4 ± 4.1	n.s. <sup>e</sup>
Birthweight (mean ± SD, Kg)	1.9 ± 0.7	3.6 ± 0.5	< 0.001 <sup>e</sup>	2.95 ± 0.99	0.9 ± 0.2	3.5 ± 0.5	< 0.001 <sup>e</sup>	3.23 ± 0.98	n.s. <sup>e</sup>
<b>Tooth eruption</b>									
Mean age at eruption of first tooth (mos) ± SD	8.3 ± 1.8	7.7 ± 1.7	n.s. <sup>e</sup>	7.9 ± 1.8	9.8 ± 3.2	7.2 ± 2.1	n.s. <sup>e</sup>	7.5 ± 2.3	n.s. <sup>e</sup>
Mean age at eruption of first molar (mos) ± SD	15.4 ± 3.2	14.9 ± 2.4	n.s. <sup>e</sup>	15.3 ± 2.7	15.5 ± 3.5	14.0 ± 2.1	n.s. <sup>e</sup>	14.2 ± 2.2	n.s. <sup>e</sup>
Mean no. of teeth at age 12 mos ± SD	9.0 ± 3.0	9.0 ± 4.0	n.s. <sup>e</sup>	9.1 ± 4.0	4.0 ± 0.0	5.9 ± 3.5	0.001 <sup>e</sup>	5.4 ± 3.6	0.001 <sup>e</sup>
Mean no. of teeth at age 24 mos ± SD	16.6 ± 3.0	16.0 ± 2.5	n.s. <sup>e</sup>	16.0 ± 2.5	15.0 ± 1.0	16.0 ± 3.0	n.s. <sup>e</sup>	15.5 ± 3.0	n.s. <sup>e</sup>

<sup>a</sup> Comparing pre-term vs. full-term infants colonized by *S. mutans*.  
<sup>b</sup> Comparing pre-term vs. full-term infants not colonized by *S. mutans*.  
<sup>c</sup> Comparing infants with and without *S. mutans*; n.s. = not statistically significant, p > 0.05.  
<sup>d</sup> Chi-square test.  
<sup>e</sup> Mann-Whitney U test.  
<sup>f</sup> Other = non-Caucasians, mostly Asians.



**Figure 1.** Cumulative prevalence of *Streptococcus mutans* colonization for 0 to 24 mos of age in all subjects. This Fig. shows the cumulative prevalence of *Streptococcus mutans* colonization from birth to 24 mos of age at each three-month interval for all infants (pre-term and full-term) within the study. It represents a combined prevalence of pre-dentate and dentate colonization over the entire duration of the study. "n value" is the total number of infants in the study, which is 312, at each three-month interval.

colonization in all children after tooth eruption was 1%, 12%, 37%, 54%, 58%, 71%, and 84% at 6, 9, 12, 15, 18, 21, and 24 mos of age, respectively. Cumulative colonization is represented by these prevalence rates. At 12 mos of age, the prevalence of *S. mutans* infection was similar between pre-term and full-term infants ( $p > 0.1$ ) (Fig. 2). Before 12 mos of age, there was a higher prevalence in full-term compared with pre-term children, and after 12 mos of age, a higher prevalence in the pre-term infants, but the difference was not statistically significant ( $p > 0.1$ ) (Fig. 2). In contrast, at 24 mos of age, the prevalence in pre-term infants was significantly higher, at 94%, compared with that in full-term infants, at 79% ( $p < 0.042$ ; OR = 4.4) (95% CI = 1.0-20.3) (Fig. 2). The pre-term infants had 4.4 times (95% CI = 1.0-20.3) higher odds of being colonized by *S. mutans* compared with full-term infants.

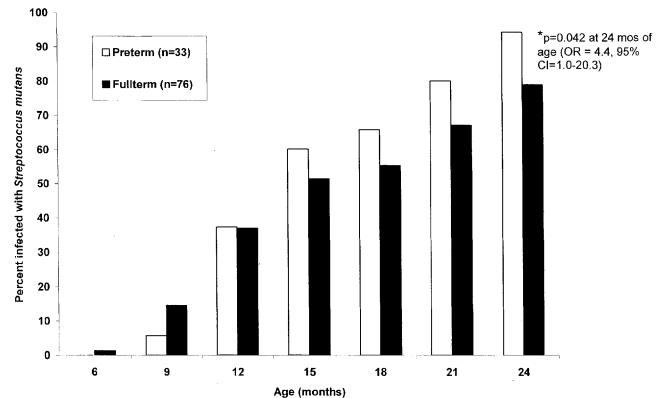
### Mean and Median Ages of *S. mutans* Colonization

Overall, the mean chronological age of colonization after tooth eruption was  $15.7 \pm 5.1$  mos. Although pre-term infants showed a higher mean chronological colonization age ( $16.1 \pm 4.8$  mos) compared with that of full-term infants ( $15.4 \pm 5.3$  mos), the difference was not significant ( $p > 0.1$ ). In contrast, the median age of colonization was significantly lower in full-term compared with pre-term infants (15.0 mos vs. 16.5 mos,  $p < 0.017$ ).

The differences in colonization ages between pre-term and full-term infants disappeared when comparisons with pre-term infants were made by corrected age rather than by chronological age. The mean corrected age of colonization for pre-term infants was  $15.0 \pm 4.7$  mos vs.  $15.4 \pm 5.3$  mos in full-term infants ( $p > 0.1$ ), and the median corrected age of colonization in pre-term infants was 14.3 mos vs. 15.0 mos in full-term infants ( $p > 0.1$ ).

### Mean Levels of *S. mutans* in Saliva

At all ages when colonization was first detected, the mean *S. mutans* levels in pre-term infants ( $707 \pm 1400$  CFU/mL) were similar to those in full-term infants ( $716 \pm 1695$  CFU/mL) ( $p > 0.1$ ). However, the levels in both groups of children increased significantly with increasing numbers of erupted teeth ( $p < 0.001$ ).



**Figure 2.** Prevalence of *Streptococcus mutans* colonization after tooth eruption in pre-term and full-term infants at ages 6 to 24 mos. This Fig. shows the cumulative prevalence of *Streptococcus mutans* colonization after tooth eruption in infants who were not previously colonized during the pre-dentate period. The black columns represent the cumulative prevalence of *Streptococcus mutans* colonization after tooth eruption in full-term infants, while the white columns represent the pre-term infants. "n value" for pre-term infants is 33 and for full-term infants is 76, at each three-month interval. The Fig. illustrates prevalence rates, and therefore, estimate of variability is not included.

### Dental Caries

By 24 mos of age, 9% of all infants (four pre-term, four full-term) colonized with *S. mutans* showed dental caries. In contrast, none of the infants without the bacteria had dental caries (Table). The mean age of colonization in infants who developed dental caries was higher ( $19.7 \pm 5.4$  mos) compared with that of those who remained caries-free at 24 mos ( $15.3 \pm 5.0$  mos,  $p < 0.03$ ).

### Factors Associated with *S. mutans* Colonization in Pre-term Infants

Two major factors were identified in pre-term children which contributed to their increased predisposition to *S. mutans* colonization. These include a 26.2 times increased risk of enamel hypoplasia (95% CI = 7.8-88.1), and a 7.9 times (95% CI = 3.4-18.7) higher risk of frequent sugar consumption (> 3 times/day) in pre-term compared with full-term infants. Other factors associated with *S. mutans* colonization were generally similar in all infants, and are listed below.

### General Infant Factors Associated with *S. mutans* Colonization

The risk factors associated with *S. mutans* colonization are summarized in Web appendix ([www.dentalresearch.org](http://www.dentalresearch.org)).

#### Demographic Factors

*S. mutans* colonization was higher in infants from families of low total annual income, and infants with mothers who have only a primary school education, at 9 mos ( $p < 0.01$ ), 12 mos ( $p < 0.03$ ), and 15 mos of age ( $p < 0.05$ ). However, at 24 mos of age, this trend was reversed, and the *S. mutans* colonization rate was higher in infants of high socio-economic status ( $p < 0.03$ ).

#### At 9 Mos of Age

The most significant factors associated with colonization of *S. mutans* at 9 mos of age were being habitually kissed on the lips

(OR = 6.4, 95%CI = 3.0-13.4) and having food pre-tasted (OR = 6.4, 95%CI = 2.9-14.5). Other significant factors were foods or utensils shared with other individuals (OR = 4.6, 95%CI = 2.3-9.5) and total sugar exposures of > 3/day (OR = 4.6, 95%CI = 2.3-9.2).

#### At 12 Mos of Age

At 12 mos of age, the most significant factors were: irregular toothbrushing habits of  $\leq$  1 day (OR = 2.1, 95%CI = 1.8-2.6), spending > 10 hrs *per week* at care facilities (OR = 3.7, 95%CI = 1.2-7.3), presence of enamel hypoplasia (OR = 5.0, 95%CI = 2.8-9.1), snacking > 3 times/day (OR = 5.6, 95%CI = 2.8-11.1), sharing food with others (OR = 5.7, 95%CI = 2.4-13.4), sugar exposure from fluids or solids > 3 times/day (OR = 6.1, 95%CI = 2.7-13.5), and formula feeding on-demand (OR = 8.9, 95%CI = 1.9-41.6).

#### At 15 Mos of Age

Sharing utensils with others (OR = 15.5, 95%CI = 4.1-58.4), plaque covering > 50% of teeth (OR = 8.1, 95%CI = 1.7-38.2), use of child care facilities before 12 mos of age (OR = 6.0, 95%CI = 1.4-26.2), sweetened fluids taken to bed (OR = 5.2, 95%CI = 1.9-14.3), presence of enamel hypoplasia (OR = 4.8, 95%CI = 2.7-8.7), and drinking only from bottles (OR = 4.8, 95%CI = 1.6-14.7) were the most significant risks associated with *S. mutans* colonization at 15 mos of age.

#### At 18 Mos of Age

Regular consumption of sticky snacks (OR = 10.4, 95%CI = 3.7-29.2), snacking > 3/day (OR = 9.3, 95%CI = 3.1-28.1), total sugar exposure > 3/day (OR = 6.2, 95%CI = 1.2-5.3), taking sweetened fluids to bed (OR = 4.4, 95%CI = 1.5-12.4), use of care facilities before 12 mos of age (OR = 3.9, 95%CI = 2.1-7.0), and sharing foods with others (OR = 3.1, 95%CI = 1.2-8.0) were the most significant risk factors associated with *S. mutans* colonization at 18 mos of age.

#### At 21 Mos of Age

At 21 mos of age, significant factors associated with *S. mutans* colonization were total sugar exposure of > 3/day (OR = 26.0, 95%CI = 6.1-111.5), sharing food or utensils (OR = 15.2, 95%CI = 3.9-58.7), feeding at night and sleeping beside mother (both OR = 7.3, 95%CI = 2.7-20.1), and snacking > 3/day (OR = 10.3, 95%CI = 2.1-50.3).

#### At 24 Mos of Age

The most significant factors at 24 mos of age were feeding at night (OR = 62.5, 95%CI = 7.8-94.9), drinking only from bottles (OR = 14.1, 95%CI = 4.4-45.3), on-demand feeding (OR = 11.6, 95%CI = 3.1-43.3), sleeping beside mother (OR = 7.8, 95%CI = 2.9-20.9), and total sugar exposure from > 3/day (OR = 3.6, 95%CI = 1.3-10.4).

### Maternal Factors Associated with Infant *Streptococcus mutans* Colonization

Presence of *S. mutans* at levels > 10<sup>5</sup> CFU/mL (OR = 2.1-8.5, 95%CI = 1.2-27.6), snacking > 3/day (OR = 3.3-30.3, 95%CI = 1.2-233.2), plaque covering > 50% of dentition (OR = 3.8-18.8, 95%CI = 0.9-84.0), and periodontal pocketing/CPI > 2 (OR = 1.6-5.3, 95%CI = 0.3-30.1) were identified as important maternal factors associated with *S. mutans* colonization in infants aged 9-24 mos.

### Factors Associated with Non-colonization of *S. mutans* in Dentate Infants

Non-colonization of *S. mutans* was associated with parent-assisted toothbrushing (OR = 1.3-2.7, 95%CI = 1.1-5.6) and multiple courses of antibiotics taken between 13 and 24 mos of age (OR = 1.4-2.6, 95%CI = 1.1-11.6).

## DISCUSSION

Since dental caries is an infectious disease, a plausible method of prevention is the removal of cariogenic bacteria from the mouth. In this regard, knowledge of the time when *S. mutans* colonizes the mouths of infants is important for determination of the optimal period for preventive and interceptive treatments (Seow, 1998). In the first part of our longitudinal studies, we found that before tooth eruption, 50% of pre-term and 60% of full-term infants were colonized by *S. mutans* (Wan *et al.*, 2001b). The present study followed the same cohort of infants to determine the times of colonization in the remainder of the study population who became infected after tooth eruption. Analysis of our data thus showed that, at 24 mos of age, 84% of all dentate infants harbored *S. mutans*. In contrast to earlier age periods, when there were no differences in infection rate between pre-term and full-term infants, we found that at 24 mos of age, 94% of pre-term infants were colonized with *S. mutans* compared with only 79% of full-term infants. The present study of dentate children found that pre-term infants had 4.4 times higher odds of being colonized by *S. mutans* than did full-term infants. This increased infection rate after tooth eruption in pre-term infants can be explained by their predisposition to enamel hypoplasias resulting from the medical complications of prematurity (Seow *et al.*, 1987). Enamel hypoplasia presents irregular and retentive surfaces for increased bacterial adhesion, so that infants who have enamel hypoplasia have increased levels of *S. mutans* (Li *et al.*, 1994), as well as a higher risk of dental caries (Pascoe and Seow, 1994; Lai *et al.*, 1997).

In contrast to the concept of a discrete "window of infectivity" for the timing of *S. mutans* colonization, we found instead a steady increase in the rate of infection as the infants' ages and numbers of erupted teeth increased. The cumulative prevalence of *S. mutans* colonization in all infants was 5%, 18%, 49%, 53%, 62%, 68%, 70%, 74.5%, and 79% at 0, 6, 9, 12, 15, 18, 21, and 24 mos of age, respectively. Colonization after tooth eruption showed the same trend: At 6 mos of age, the infection rate was approximately only 1%, rising to 12% at 9 mos, 37% at 12 mos, 54% at 15 mos, 58% at 18 mos, 71% at 21 mos, and 84% at 24 mos of age in those infants who were colonized by *S. mutans* after tooth eruption. Compared with previous studies, colonization rates at the various ages in this study are generally higher than those reported by other investigators (Caufield *et al.*, 1993; Karn *et al.*, 1998). The differences are likely to be related to cohort population differences, or to the relatively large numbers of subjects in the present study. In addition, increased recovery potential of *S. mutans*-selective TYCSB agar used in the present investigation compared with other media for isolation of *S. mutans* (Wan *et al.*, 2002) may have increased the culture sensitivity of the samples in the present study.

As is the case before tooth eruption, *S. mutans* colonization after tooth eruption is influenced by both maternal and infant factors. It is now well-recognized that the mother is usually the primary source of *S. mutans* for infection of her child (Kohler and Bratthall, 1978; Li and Caufield, 1995), and poor maternal oral hygiene and dietary habits increase the likelihood of transmission of the infection from mother to child. Hence, in this study, greater numbers of mothers of infected infants showed higher *S. mutans* levels, less frequent toothbrushing, greater plaque levels, and higher daily frequencies of snacking and sugar exposures compared with mothers who had uninfected infants.

Furthermore, since successful colonization by *S. mutans* probably requires repeated exposures, our data showed that child-rearing habits which facilitate saliva transfer from adults to the child, such as sharing of food and utensils, and habits which involve close contact, such as breast feeding and sleeping beside the mother, were also significantly associated with colonization of *S. mutans*.

Several infant factors contribute significantly to the colonization of *S. mutans*. First, although *S. mutans* may colonize the mouth before tooth eruption, the emergence of teeth increases the non-shedding surfaces for adherence of *S. mutans*. Thus, with tooth eruption, the colonization rate of the infants increases as their ages increase. Second, infant dietary and oral hygiene habits also facilitate the colonization of *S. mutans*. As in our previous studies on pre-dentate infants, frequent exposure to sugars, especially from snacking and sweetened pacifiers, correlated significantly with *S. mutans* colonization (Wan *et al.*, 2001b). In the case of pre-term infants, this habit is worsened by the increased sugar content in diets that are recommended to boost calorie intake (Sauve and Geggie, 1991).

The importance of regular toothbrushing in the prevention of *S. mutans* infection in infants is also demonstrated in this study. Our data thus support and extend the work of others (Habibian *et al.*, 2002), who reported that infants who have their teeth brushed by 12 mos of age are less likely to harbor *S. mutans* compared with those who had not commenced brushing.

Previous studies suggested that the earlier the colonization of *S. mutans*, the higher the caries risk (Kohler *et al.*, 1988). In the present investigation, we found that in the eight infants who developed caries, *S. mutans* was first detected at a median age of 18 mos. Although these results relating age of colonization to caries development are preliminary, they suggest that age of colonization on its own may not be the most important aspect in caries development, since other factors, such as sugar consumption and oral hygiene, are also likely to play significant roles in determining caries risk (Loesche, 1986).

In conclusion, data from our longitudinal studies have established the timing of *S. mutans* colonization from birth to 24 mos of age, and provided insight into infant and maternal factors which facilitate the colonization. These data would be highly relevant in the institution of preventive strategies for early childhood caries.

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