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# Effect of Putative Caries Risk Factors on Salivary Mutans Streptococci Levels and Caries in a 6-Month- to 2-Year-Old Children

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**THE EFFECT OF PUTATIVE CARIES RISK FACTORS ON  
SALIVARY MUTANS STREPTOCOCCI LEVELS AND CARIES  
IN 6-MONTH- TO 2-YEAR-OLD CHILDREN**

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**B.D.S., University of Madras, 1991**

**A Thesis  
Submitted in Partial Fulfillment of the  
Requirements for the Degree of  
Master of Dental Science  
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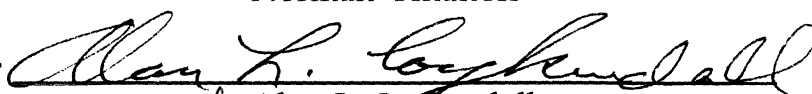
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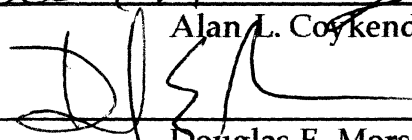
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SALIVARY MUTANS STREPTOCOCCI LEVELS AND CARIES IN  
6-MONTH- TO 2-YEAR-OLD CHILDREN

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## INTRODUCTION

Early childhood caries (ECC) is a new term that describes dental caries in infants and toddlers, often attributed to the prolonged use of a baby bottle. It generally is thought to be initiated and amplified by inappropriate feeding with a nursing bottle containing fermentable carbohydrates in liquid form. Although it is well known that mutans streptococci are the primary pathogens involved in the development of ECC, factors that affect their acquisition in the mouths of young children are not well understood. This study investigated the role of putative caries risk factors such as age, number of teeth, baby bottle content and mothers' salivary mutans streptococci on salivary mutans streptococci levels in 6- to 24-month-old children.

## REVIEW OF LITERATURE

Early childhood caries (ECC) is a new term that describes dental caries in infants and toddlers (ECC Conference, CDCP, Atlanta, Georgia, 1994), often attributed to the prolonged use of a baby bottle containing fermentable carbohydrates in liquid form (Schwartz et al., 1993). Other terms for this distinct pattern of caries include nursing caries, baby bottle tooth decay, nursing bottle syndrome, bottle mouth syndrome, baby bottle syndrome, nursing bottle caries and nursing bottle mouth. Early childhood caries generally is thought to be initiated and amplified by inappropriate feeding with a nursing bottle. Fass (1962) is credited with the first description of what he called "nursing bottle mouth", and he and others have reported that nocturnal bottle feeding is the primary etiologic factor for rampant caries in children. The condition also has been reported in children using sweetened pacifiers, in breast-fed children and in children who regularly take vitamin syrups containing sugar (James et al., 1957; Derkson and Ponti, 1982; Winter et al., 1966; Holt et al., 1982).

The primary maxillary incisors are the first teeth to be affected most likely because they erupt by the age of 6 to 7 months and are exposed to the cariogenic challenge over a prolonged period of time. Nursing caries usually begins on the labial surface of the primary maxillary incisors (James et al., 1957). The maxillary and mandibular first primary molars erupt between 12 and 18 months and may be affected if the bottle habit persists after their eruption. The canines and second

primary molars are affected to a lesser degree. The mandibular incisors remain relatively unaffected, reportedly due to the protection afforded by the tongue as it covers these teeth during bottle feeding which prevents pooling of the liquid around them. It has also been suggested that pooling of saliva under the tongue “might dilute sucrose and affect the plaque favorably” (Picton and Wiltshire, 1970). Nursing caries usually is apparent by 16 months (Kroll and Stone, 1967), but has been described as early as 9 months (James et al., 1957) and 11 months (Suher et al., 1953).

### PREVALENCE

Results from studies examining prevalence levels vary widely, in part due to the lack of a universally accepted definition of nursing caries and the difficulty in accounting for the potential interaction of the variables involved in the disease process. In general, disadvantaged children, regardless of ethnicity or culture, are more vulnerable to nursing caries. In developed countries, the prevalence is reported to vary between 1.0% and 12.0% (Milnes, 1996). For instance, caries prevalence was found to be 5.9% among 554 children surveyed by a study conducted in England (Goose, 1967). In contrast the prevalence in developing countries and within disadvantaged populations in developed countries has been reported to be as high as 85% (Milnes, 1996). A study conducted on 514 3- to 5-year-old Native American Head Start children in Alaska and Oklahoma found that the prevalence of baby bottle tooth decay at nine sites in Alaska ranged from 44% to 85%, and ranged from 17% to 60% in the sites at Oklahoma (Kelly and

Bruerd, 1987). A study comparing 426 from Connecticut Head Start programs and 199 from Beijing nursery schools found caries prevalence higher in Chinese children than Connecticut children, with 3-yr-old Chinese children having a caries prevalence of 67% and 4-yr-old children having a caries prevalence of 74% compared to 28% and 32% in Connecticut children of similar age groups (Douglass et al., 1994). In another study of Chinese children, caries prevalence was reported to be 67% in the 3-yr-olds and 84% in the 6-yr-old children (Douglass et al., 1995). Another study that examined 200 3½- to 5-year-old Head Start children in 2 Ohio communities in the USA reported a caries prevalence of 11% (Johnsen et al., 1986).

### SOCIO-ECONOMIC FACTORS

Demographic data on parents of children with nursing caries showed that the majority were high school graduates and that most claimed not to have been informed about early weaning or discontinuation of bottle use at an early age (Dilley et al., 1980). Parents of children with nursing caries were found to be younger and less educated than those of children without nursing caries (Derkson and Ponti, 1982; Johnsen, 1984). The former were more likely to be obese, and were pessimistic about their own dentition and generally unaware of the cariogenic potential of milk or sweet liquids when used in a nursing bottle at night (Johnsen, 1984).

Backgrounds of 134 3½-year-old preschool children with carious incisors were compared with those of 90 caries-free children in 4 different geographic and



practice locations. The study suggested that nursing caries may be due to overindulgence by the parents. Parents of caries-free children were more successful at substituting water in the bottle for milk or any other liquid than parents of the children with nursing caries. Additionally, a greater percentage of children with early caries were found to be second siblings (Johnsen et al., 1984). More mothers of children with nursing caries used the bottle to motivate the child, possibly indicating that they had trouble setting limits (Serwint et al., 1993).

In a study conducted in the predominantly rural area of Appalachia, West Virginia, the average number of children in a family was less where there were caries-free children when compared to families with high-caries children. The study also reported that accessibility to snack foods was significantly different between the caries-free and high-caries groups. Of the group of caries-free children, 41% were permitted free accessibility to snack foods compared with 82% of children in the high-caries group, this difference being statistically significant (Johnsen et al., 1980).

A pilot study conducted on 125 8 month- to 4-year-old children in a migrant Mexican-American farmworkers clinic reported a caries prevalence of 29.6%. The study reported that babies with nursing caries were viewed as more easy-going and less strong willed than those children without nursing caries. Interestingly, the study also reported that parents of children with nursing caries used the baby bottle less frequently and cleaned their child's teeth regularly compared with parents of children without nursing caries. Also, parents of

children with nursing caries reported to have learned about the disease only when their child was older (Weinstein et al., 1992). Although these results did not reach statistical significance, this may have been due to a small sample size and will have to be evaluated further in a larger study on the same population.

## BIOLOGIC FACTORS

### Feeding habits

While the influence of specific feeding habits on the initiation and progression of nursing caries has been well documented, infant feeding practices may be influenced by cultural, ethnic and socioeconomic factors, making comparisons between population groups difficult and often leading to conflicting results (Milnes, 1996). Conventional wisdom holds that teeth that are exposed to liquids in the bottle during naptime and nighttime use when salivary flow ceases and swallowing is infrequent, will get caries. Fass (1962) conjectured that the longer a child is on the bottle and the earlier the eruption of the primary teeth, the greater the risk of caries attack. One study remarked that there was a “clear and distinct association between the pattern of decay and nocturnal bottle feeding”. Although these authors reported a statistically significant relationship between nocturnal bottle feeding and rampant caries, the study used a small number of children and did not report their ages (Kroll and Stone, 1967).

A study compared three groups of children for the effects of early feeding habits on nursing caries prevalence. Children who were bottle-fed with milk containing sugar at night and slept with the bottle in their mouths were reported

to have a higher prevalence of nursing caries compared with children who did not sleep with the bottle or who were wholly breast fed. The higher prevalence of nursing caries in the children fed with sugar-supplemented milk was attributed to the increased duration of time that the teeth were in contact with the substrate. However, the type of "sugar" in the bottle and the amount of sugar added to milk was not discussed as a factor in explaining the results (Picton and Wiltshire, 1970).

In a study examining characteristics of children with nursing caries and their families, children with nursing caries were reported to have "a history of use of the bedtime bottle and a use of the bottle or breast beyond the normal weaning time". 97% of 75 1- to 5-year-old children in the study were bottle fed until the age of 23.4 months and weaning the child from the bottle did not occur until bedtime bottle usage was discontinued. However, there was no mention of a control group in the study to help validate these observations and no statistical analysis of the data was reported (Dilley et al., 1980). In a study that surveyed parents of 47 children with baby bottle tooth decay, 96% reported that their child slept or had slept with the bottle. Of the 20 caries-free children in the control group, none were reported to have slept overnight with the bottle but 45% had had the bottle removed after falling asleep (Johnsen, 1984).

Another study reported that 57.9% of children with nursing caries used the bottle every night and at every nap compared with 40.2% of children without nursing caries. The only explanation offered for the 40% of children who showed no evidence of caries in spite of a prolonged feeding habit was that there might

be other factors besides the baby bottle that could predispose a child to caries. In addition, the children with nursing caries reportedly had more prolonged daytime use and were weaned later (mean of 21.4 months compared with 17.7 months for children without nursing caries). The frequency of keeping the bottle nipple in the mouth all night was higher in children with nursing caries than in children without nursing caries (Derkson and Ponti, 1982). Although this study considered many variables in its analyses, the prevalence of nursing caries among study subjects was only 3.2%, since of the 595 children in the study, only 19 were found to have nursing caries; thus, the study may not have used a truly representative sample. Also, there was no statistical analysis of the reported data to show significance. The parents of one hundred and fifty six children in the age range of 14 months to 8 years also have been surveyed to relate “the manner in which the child went to sleep” to the development of nursing caries. Of those who discarded the bottle before falling asleep, 27% developed nursing caries; yet of those who fell asleep with the bottle in their mouths, 62% developed nursing caries (Schwartz et al., 1993).

In a study from South Africa, comparing 1- to 5-year-old rural and urban black children, prevalence of “labial caries” (defined as caries on the labial surface of one or more incisor or canine teeth) was found to be higher in the rural population and “rampant caries” (defined as a dmft score of 5 or more) was higher in the urban population (Cleaton-Jones et al., 1978). In a subsequent study conducted on black rural and urban children and white urban children, bottle feeding was found to start and end later in black infants than white infants. Fruit

syrup intake was high among the white children but negligible among the black children. Surprisingly, the study found the prevalence of labial caries to be similar in rural black and urban white children (approximately 12%), but significantly less in urban black children (approximately 4%). The authors concluded that bottle feeding or fruit syrup intake had “little or no association” with labial caries (Richardson et al., 1981).

Sweetened pacifiers have been shown to play a significant role as an etiological factor in the development and progression of caries (James et al., 1957; Winter et al., 1966). One of the earliest documented accounts on children using sweetened pacifiers was Struve in 1801. It has been reported that the use of pacifiers dipped in vitamin syrups resulted in significantly more caries than when plain pacifiers were used (Winter et al., 1966). In a survey performed in Camden, England, 19% of 555 children in the study had received a sweetened pacifier; yet, only 9% of these children were found to have rampant caries. Although an association between the use of sweetened pacifiers and rampant caries was reported, no significant relationship was found between the use of sweetened pacifiers and overall caries prevalence in the study (Holt et al., 1982).

A study reported that of 14 children using sugared pacifiers, half developed labial caries during the course of the study. The authors suggested that other factors, such as degree of oral hygiene and the volume and buffering capacity of saliva, in addition to the sugared pacifier influenced the development of caries (James et al., 1957). Another study, evaluating the role of the pacifier in the development of caries found that 5% percent of 100 children who had used a

sweetened pacifier for 5 to 22 months after eruption of their anterior teeth remained unaffected by caries at the end of the study. Additionally, 34% of these children showed no evidence of caries despite the prolonged use of baby bottles. Individual resistance to the disease was suggested to be a factor in these cases (Winter et al., 1966).

A recent study conducted on 110 children between the ages of 18 and 36 months, with and without nursing caries, reported no significant association between prolonged bottle feeding and nursing caries. Nursing caries was found in 22 (20%) of the children. Surprisingly, almost all children in both groups had nighttime bottles at 12 and 18 months, a finding that contradicts the prevailing thoughts on this subject (Serwint et al., 1993).

Although it is generally accepted among practitioners that prolonged bottle feeding is the important factor in the development of caries, no clear association between prolonged or nocturnal bottle feeding and nursing caries can be found in the literature. Studies that have evaluated the practice of prolonged bottle feeding show that some children bottle fed over a long period of time do not develop nursing caries. Reasons for this selective nature of the disease remain obscure.

### Substrates

Even though fermentable carbohydrates are a necessary factor in caries initiation and progression, few studies have been conducted to examine the possible association of bottle contents with nursing caries. The American Academy of Pediatric Dentistry recommends that children should not sleep with

a bottle containing “juice, milk, formula, or any other liquid sweetened with fermentable carbohydrate” (American Academy of Pediatric Dentistry Reference Manual, 1995). Yet studies suggest that milk by itself has no cariogenic effect and that it might even have some cariostatic effects, by virtue of its calcium and phosphorus content (Jenkins and Ferguson, 1966). Milk added to fermentable food was even shown to decrease caries. Additionally, it was found that chocolate milk was more destructive to the enamel than plain milk (Bibby et al., 1980). In a clinical study, children having plain milk or milk and sugar in the bottle did not have more caries than those who were not on the bottle (Goose and Gittus, 1968).

A caries-conducive environment requires the presence of microorganisms to allow acid production from fermentable carbohydrates such as milk. A drop in the pH of the environment is then an essential part of caries initiation and progression. The pH changes of dental plaque were measured after rinses with milk, fruit juices and artificially sweetened beverages. Milk was found to produce the smallest decrease in initial pH followed by natural fruit juices (e.g. orange, apple and lemon), while artificial beverages were found to be very acidogenic compared with milk and fruit juices and produced a large drop in initial pH (Frostell, 1970).

Another study showed that milk produced the least change in initial pH when compared with pH drops produced by either a combination of milk and 5% sucrose, or solutions of sucrose or lactose alone. The solutions of sucrose or lactose alone (i.e. without any added milk) produced a much greater pH change

than in any mixture containing milk (Mor and McDougall, 1977). Moreover, in a study on the effect of diet on the implantation of caries-inducing streptococci in hamsters, it was shown that sucrose and lactose highly favored implantation. When sucrose in the experimental diet was replaced by glucose, very few streptococci were recovered (Krasse, 1965).

Lactose is present in bovine and human milk in concentrations of 4% and 7%, respectively. However, data are scarce on the caries-conducive potential of lactose because animals fed on diets that contain large amounts of this sugar do not remain healthy, making assessment of cariogenic potential difficult to assess (Green and Hartles, 1969). One study on the metabolism of lactose by mutans streptococci reported the induction by lactose of the phosphoenolpyruvate-dependent phospho-transferase system in 4 strains of the organism. This induction resulted in the transport and phosphorylation of the lactose molecule. There also appeared to be a subsequent induction of the 6-phospho- $\beta$ -galactosidase enzyme, resulting in the formation of glucose and galactose 6-phosphate (G6P). This enzyme was not always induced in high levels in mutans streptococci and the G6P was found to be metabolized to triose phosphates of the glycolytic pathway via the tagatose 6-phosphate pathway (Calmes, P; Hamilton and Lebttag, 1979). Although lactose apparently can be metabolized by mutans streptococci in the oral cavity, evidence of its cariogenicity is lacking. The effects of lactose concentration in milk and formula and therefore their association with the development of dental caries clearly needs further investigation.



In order to assess the effects of consumption of different potentially cariogenic fluids on caries prevalence, one hundred and twenty Sprague-Dawley rats in 2 groups were compared. All rats were fed on a highly cariogenic diet and all were infected with *S. sobrinus*. One group consumed different fluids (2% milk, 4% lactose, lactose-reduced milk, 10% fructose, 10% sucrose, and distilled water as a control) and the other group consumed a combination of 2% milk with different concentrations of sucrose (2%, 5%, 10%) and distilled water with 10% sucrose as a control. In the first group, increased caries levels were noted in the 10% fructose and 10% sucrose groups compared with the other fluids. In the second group, although the highest caries prevalence was found among animals drinking the control fluids, the addition of even 2% sucrose to 2% milk produced an increase in caries. It was concluded that addition of sucrose to milk increased the milk's cariogenicity and that milk had no caries protective effects. It also was suggested that the amount and extent of caries promoted by both milk and water was similar (Bowen and Pearson, 1993). This indicates that milk is essentially noncariogenic unless it has sugar added to it. However, in a study using an artificial mouth, it was concluded that "milk itself without addition of extra carbohydrate has the potential to produce dental caries if left to stagnate over the tooth surface for a sufficient time" (Vianna, 1971). Clinical studies that have assessed the contents of the bottle have reported similar results (Schwartz et al., 1993; Picton and Wiltshire, 1970; Dilley et al., 1980; Derkson and Ponti, 1982).

Therefore, data differ on the effect of content of the bottle on the development of caries. This contradicts clinical anecdotes that prolonged bottle

feeding with anything other than water is directly associated with the development of nursing caries. Milk, formula, cereal and juice present the main dietary challenge in young children and the cariogenic potential of these substances need to be examined further.

### Microorganisms

The oral microflora is a complex ecosystem that contains a wide variety of microbial species. Predominant microbial species are significantly different in different sites. It appears that certain oral streptococcal species have a predilection for colonizing particular sites in the mouth. Whereas, *S. salivarius* is an early colonizer in the human mouth after birth, *S. sanguis* and mutans streptococci are found much later, only after the eruption of teeth. Mutans streptococci have been associated with human dental caries and also have a high level of pathogenicity when tested in rodent models. Clinical studies (Loesche et al., 1975; Köhler and Bratthall, 1978; Brown et al., 1985; Loesche, 1986) have implicated mutans streptococci in human caries by showing a positive correlation between caries activity and degree of infection with the organism (Berkowitz et al., 1984). A study was initiated to determine the microbiology of lesion development in nursing caries, from intact enamel to the time of lesion formation. Nine children were studied and plaque samples were used from the labial and palatal surfaces of the maxillary primary incisors. The study showed increased levels of mutans streptococci and lactobacilli in carious lesions as well as white spot areas. However, susceptible sites that were caries-free also showed

increased levels of the organisms in spite of the absence of lesion formation. In addition, veillonella was isolated regularly from the caries susceptible sites, representing about 7-10% of the total cultivable flora (Milnes and Bowden, 1985). In a study of the oral microflora of children with nursing caries, mutans streptococci averaged about 60% of the total cultivable flora of dental plaque obtained from carious lesions and white spot margins of these lesions and about 27% in plaque from clinically sound areas of the posterior teeth (van Houte et al., 1982).

#### Initial Detection of Mutans Streptococci in Infants

Many clinical studies have indicated that mutans streptococci cannot be detected in the mouths of normal predentate infants. However, some strains of cariogenic streptococci were found to make their appearance in the oral cavities of infants at about one month of age (Zinner and Jablon, 1969) and mutans streptococci were detected in 3 of 21 normal predentate infants (Edwardsson and Mejare, 1978). Two of 10 predentate infants with acrylic cleft palate obturators also were found to harbor mutans streptococci but the organism was not detected in 91 normal predentate infants (Berkowitz et al., 1975).

An extensive study on 92 children, ranging from newborns to 5-year-old children examined the prevalence and localization of mutans streptococci by the number of primary teeth. The predentate infants had no recoverable mutans streptococci while 46% of children with a full complement of primary teeth had recoverable mutans streptococci (Catalanotto et al., 1975). The absence of mutans streptococci from the mouths of predentate infants in these studies supports the

idea that the organism requires a non-shedding surface, such as tooth structure, for its establishment, but could transiently colonize the mouth in early infancy.

There are conflicting reports on the time of appearance of mutans streptococci in the mouth in relation to the eruption of teeth in children. Mutans streptococci were isolated from children who had erupted only primary incisors (Berkowitz et al., 1980), yet in another study, mutans streptococci were detected only in children with the entire complement of primary teeth present (Catalanotto et al., 1975). Twenty five infants were followed over 5 years to study the oral establishment of various species of lactobacilli and streptococci. Mutans streptococci were not recovered from the majority of infants until the age of 5, when it was found in 84% of them. Lactobacilli were recovered in low numbers, and in children younger than two, seemed to be mostly transients (Carlsson et al., 1975).

A recent study hypothesized that the initial acquisition of mutans streptococci in infants occurred during a well-delineated age range that was designated as a “window of infectivity” from 19- to 30-months of age. Forty six children were studied from birth to five years of age and the median age of initial acquisition of mutans streptococci was 26 months. It further was speculated that the discrete nature of initial mutans streptococci acquisition was directly related to the presence of newly emerged teeth (Caufield et al., 1993). This study did not, however, show testing for the initial acquisition of mutans streptococci at specified time periods, nor did it define the role of dietary factors in the

acquisition of mutans streptococci. It also did not include demographic data on the population so that comparisons with other studies would be difficult.

Sample site also appears to be an important parameter in the detection of mutans streptococci (Zinner and Jablon, 1969). A study evaluated plaque and salivary levels of mutans streptococci and showed a significant association between plaque levels of mutans streptococci and caries. However, saliva samples tended to have low levels of mutans streptococci (Loesche et al., 1975).

#### Maternal Transfer of Mutans Streptococci

There is evidence to show that mutans streptococci are transmitted from mother to child (Zinner and Jablon, 1969; Catalanotto et al., 1975; Masuda et al., 1980; Boue et al., 1987; Köhler and Bratthall, 1987). Serological characterization of 66 isolates of mutans streptococci from 9 mother-child pairs showed that serotype 'c' predominated. The possibility of maternal transfer of mutans streptococci due to isolation of the serotype 'b' strain of the organism in one mother and her child was subsequently suggested (Berkowitz et al., 1975).

Bacteriocin production and sensitivity patterns of 120 mutans streptococci isolates from 4 mother-infant pairs were studied to test the likelihood of maternal transmission of this species. Bacteriocin codes of the majority of mutans streptococci strains isolated from the 4 infants were similar to the codes of mutans streptococci isolated from their mothers, which strongly suggests maternal transfer of the species (Berkowitz and Jordan, 1975). In another study, nearly all 107 isolated mutans streptococci strains were of serotype 'c' (Milnes

and Bowden, 1985). Similar findings were reported by other investigators (Berkowitz et al., 1984; Boue et al., 1987).

Another study related maternal salivary levels of mutans streptococci and infant infection. All the infants studied had 6-8 primary incisors and it was concluded that when maternal salivary levels of mutans streptococci exceeded  $10^5$  CFU's / ml of saliva, a higher proportion of infant infection was observed. The frequency of infection was nine times greater when maternal reservoirs exceeded  $10^5$  CFU / ml of saliva, than when they were less than or equal to  $10^3$  CFU / ml of saliva (Berkowitz et al., 1981). However, another study found no association between children's mutans streptococci scores and whether they had nursing caries, nor any association between maternal mutans streptococci scores and a child's caries status (Brown et al., 1985). In 36 children, 4½ to 5 years of age, a correlation was reported between the number of mutans streptococci in the mothers and their children, and it was suggested that there is a definite risk for high caries experience if a child is infected with more than 1 million CFU / ml saliva (Köhler and Bratthall, 1978). This information along with earlier evidence of the role of mutans streptococci in human dental decay (Duchin and van Houte, 1978; Hamada and Slade, 1980; Loesche, 1986) indicates microbial specificity in nursing caries with mutans streptococci being an important etiologic factor.

Mutans streptococci are recognized as an important pathogen involved in the initiation and progression of nursing caries. Their transmission from mother to child has been shown since the 1970s and has been supported by many

investigators. Studies suggest that the older a child and the greater the number of erupted teeth, the greater the number of mutans streptococci that colonize the mouth. However, the actual time of appearance of mutans streptococci in the mouths of children remains controversial. It also is not known if the time of appearance of these microorganisms would differ in different population groups, owing to differences in cultural and socioeconomic backgrounds. Sampling of saliva in young children remains difficult and other age appropriate methods will have to be developed to better understand the efficacy of the present methods.

Although implicated repeatedly in the development of nursing caries, there are no data on any “threshold level” of mutans streptococci that would contribute to disease. The proposed “window of infectivity” (age range of 19 to 30 months) does not explain why mutans streptococci would colonize a mouth with only a full complement of teeth, but not colonize, or cause nursing caries, in a younger child with fewer numbers of erupted teeth.

#### Other Biologic Factors

Additional factors possibly contributing to early childhood caries, such as prematurity of birth and childhood illnesses, appeared to be causally related to caries. It has been suggested that changes in the oral environment such as decreased salivation that accompanies periods of pyrexia and dehydration could predispose the child to an increased caries prevalence (Winter et al., 1966; Winter et al., 1982). Other studies reported that 18% of subjects with nursing caries had had childhood illnesses (Dilley et al., 1980) and that children with baby bottle

tooth decay were more likely to have had significant medical problems (Richardson et al., 1981). The latter study had only one child in the caries-free group who had a significant medical problem and therefore does not permit a true comparison.

Linear enamel hypoplasia has been reported to be a predisposing factor in the development of early childhood caries in developing countries. Linear enamel hypoplasia of the primary maxillary incisors was found in 73% of 104 Guatemalan children who were recovering from severe malnutrition (Sweeney et al., 1969). In another study, the presence of linear hypoplasia was reported to correlate with prematurity of birth, infections in the early neonatal period and very low serum Vitamin A levels (Sweeney et al., 1971). In a study conducted on 2,192 1- to 4-year-old children in Tanzania, the prevalence of rampant caries was found to correlate with the prevalence of linear hypoplasia (Matee et al., 1994).

Oral hygiene and regular use of fluoride are important factors to be considered in studies on early childhood caries. In the few studies that have reported oral hygiene measures, no differences were detected between the group of children with nursing caries and the group of children who were caries free in the onset of tooth brushing and oral hygiene measures (Dilley et al., 1980; Winter et al., 1966; Serwint et al., 1993). Caries levels were compared in Head Start children residing in fluoridated and non-fluoridated, urban and non-urban sites in Ohio, USA. The fluoride status was, of course, of greater importance in this study than the residential status of these children. Percentages of caries-free children were higher in the urban and non-urban fluoridated sites compared



with the urban and non-urban non-fluoridated sites (Johnsen et al., 1986). Additionally, fluoride supplementation was provided more regularly by mothers in a group of children without nursing caries compared with a group of children with nursing caries (Derkson and Ponti, 1982; Johnsen et al., 1984). Yet, no differences were found in the intake of fluoride supplements between two groups of children in another study (Serwint et al., 1993).

In summary, despite many reports on baby bottle use in preschool children and its reported association with ECC, little is known about the role of bottle contents and other dietary practices that may contribute to the initiation of the caries process. The association of mutans streptococci -- specifically, its acquisition time and levels of infection -- in children of this age also is poorly understood.

## **SPECIFIC AIMS**

This study aims to:

1. evaluate the differences in the levels of salivary mutans streptococci in 6- to 24-month-old children.
2. determine the association between salivary mutans streptococci and the combination of bottle content, age and teeth.

## **HYPOTHESES**

1. There is no association between bottle content and salivary mutans streptococci in children.
2. There is no association between the number of teeth or child's age and salivary mutans streptococci in children.
3. There is no association between salivary mutans streptococci in mothers and their children.
4. There is no correlation between sampling techniques, i.e., the tongue depressor or the mouth mirror for sampling salivary mutans streptococci.

## MATERIALS AND METHODS

### I. Study population and identification of potential subjects

The study was initiated on healthy children and their mothers from the Hartford, CT Women, Infants, Children (WIC) program. The program has an average enrollment of 12,100 mothers and their children, with approximately 150 new families enrolled each month. The racial/ethnic distribution of this population is 40% Black, 54% Hispanic and 6% other. Eligibility for the program is based on welfare status and nutritional needs of the family. Parents were recruited for the study during their visits to the WIC center, to collect WIC coupons that could be used towards the purchase of food and other items.

Parents and their children were eligible for participation in this study if the child was between 6 and 24 months of age and was/was not using a nursing bottle at any time. If subjects met the eligibility criteria, informed consent for participation in the study was obtained from parents and a copy of the consent form was given to them.

Each child was examined clinically for dental caries and the number of erupted teeth were counted (see 'Clinical Examination'). Children and their mothers then were sampled for salivary mutans streptococci (see 'Microbial Sampling'). A questionnaire was administered to the parents to obtain demographic information. The survey included questions about psychosocial factors and details of baby bottle usage (see 'Questionnaire').

## II. Clinical examination

Dental examinations on the children were conducted by a dentist in the WIC center. The age of the child, as well as the number of erupted teeth was recorded. Clinical detection of caries was by visual/tactile examinations using #23 explorers, front surface mirrors, and dental light. Criteria for caries detection were those described by Radike (1972), i.e., in which fissures were considered carious if an explorer resists removal after insertion and/or there was loss of normal translucency of enamel next to the fissure. Buccal or lingual surfaces were considered carious if penetrated by an explorer or if enamel could be scraped away by the explorer. Proximal surfaces were considered carious if the marginal ridge showed opacity, or if the explorer recorded discontinuity along with other signs such as opacity or shadow by transillumination. No radiographs for caries diagnosis were taken because of human use concerns.

## III. Microbial sampling

The microbial screening test involved sampling saliva from each child and mother by placing a sterile wooden tongue blade and then a mouth mirror on the tongue until they were visibly moistened. The mouth mirror sampling technique was only used in the older age group. The tongue blade and the mouth mirror then were impressed onto plates that contained media selective for mutans streptococci i.e., mitis salivarius agar supplemented with sorbitol, kanamycin sulfate, tellurite, and bacitracin (Kimmel and Tinanoff, 1991). These plates were incubated in a candle jar environment at 37°C for 72 hours, after which time bacterial growth was assessed by counting the number of colony forming units

(CFU) resembling mutans streptococci (i.e., dark, discrete, raised colonies) that appeared within the impression (Köhler and Bratthall, 1979). Counts were obtained with the naked eye and with the use of a dissecting microscope. The number of colony forming units were recorded for statistical evaluation as low (0 CFU), moderate (1 - 50 CFU) and high (> 50 CFU) (Thibodeau et al., 1993). This uncomplicated microbial procedure to estimate mutans streptococci have been used for several years and there are numerous publications showing its value in predicting caries in young children (Edelstein and Tinanoff, 1989; Crall et al., 1990; O'Sullivan and Tinanoff, 1993; Thibodeau et al., 1993; Thibodeau and O'Sullivan, 1994).

#### IV. Questionnaire on baby bottle usage

As part of a larger study, a 13-item questionnaire on baby bottle usage was administered to parents of the children who were still using a baby bottle. The questions examined current and past baby bottle usage at night and during the day, and factors that may have been associated with this behavior. Diet analysis questions were also administered at baseline. Several of the survey questions were evaluated for their potential influence on, or association with, the clinical findings (Appendix A).

### Statistical Section

Power analyses were attempted using Phase 1 data (6 to 15 month age group) (Appendix B). However, the estimate of mutans streptococci counts were not representative of counts from the older age group (16 to 24 month age group). No other studies are available from which estimates of mutans streptococci in 16 to 24 month aged children can be obtained. Thus, 65 additional subjects were chosen for the second phase, giving a total sample size of 131.

The dependent variables in the study were (1) the number of salivary mutans streptococci present in a child and (2) the presence/absence of dental caries. The independent variables were the number of teeth present in a child, the age of the child, the number of salivary mutans streptococci present in the mother, and baby bottle content. The numbers of salivary mutans streptococci present were ranked as follows : (a) low (0 CFU), (b) moderate (1-50 CFU), and (c) high (> 50 CFU). Age was analyzed with five groups of 4-month intervals and the number of teeth were grouped into four categories by eruption status, i.e., 0 to 4, 5 to 8, 9 to 12, and 13 to 20 teeth. Subjects' bottle content data was divided into three groups: (a) children bottle-fed with milk and non-sucrose containing infant formulas; (b) children bottle-fed with sweetened beverages, and (c) children not bottle-fed. Multiple logistic regression models were used to determine the importance of the independent variables in the prediction of salivary mutans streptococci isolation.

Since preliminary data suggested that a mouth mirror may be preferable to a tongue blade for salivary mutans streptococci collection from the young

children in this population, due to the non-absorbable back surface, dual samplings were collected in the older age group. The Spearman's rank correlation coefficient was used to determine the correlation, if any, between the two methods.

## RESULTS

This study was conducted with 131 mother/child pairs. It was conducted in two phases. Initially 66 mothers and their 6- to 15 month-old children were studied. After that sample was analyzed, an additional 65 mothers and their 16- to 24 month-old children were recruited.

Analysis of the effect of the children's developmental stage on salivary mutans streptococci (SMS) isolation frequency was carried out using chronological age and the number of erupted teeth. In the 4-month age intervals studied, SMS was isolated from one child in the youngest category (6 to 9 months). The isolation frequency of SMS tended to be greater in successive age groups, with 45% of the children in the oldest age interval harboring SMS. The number of SMS recovered also increased with age. More than one quarter of children in the 22- to 24- month age interval had the highest level of SMS i.e., > 50 colony forming units (CFU) (Figure 1).

The relationship between the number of erupted teeth and level of SMS was also similar to the findings for age. While SMS was isolated from only 2 of 29 (7%) children with 0 to 4 teeth, those children with more teeth had greater numbers of SMS. The level of recovery of the highest category of SMS, i.e., >50 CFU, was greatest in those children with 20 teeth (Figure 2).

The content of the baby bottle also had an effect on the levels of SMS in children. Only 2% of children in the milk group were found to have the highest level of recovery of SMS, i.e., > 50 CFU, compared with 16% of children in the water group and 15% of children in the sweetened beverages group (Figure 3).



When the three categories of bottle content were assessed across the different age groups, it was noted that the percentage of children in the milk group were successively less with an increase in age. In contrast, the percentage of children in the sweetened beverage and water groups generally were greater with an increase in age (Table 1; Figure 4). A similar trend was seen when bottle content was assessed by different numbers of teeth (categorized as described previously) (Table 2; Figure 5).

Based upon the *a priori* assumption that age and the number of teeth present could confound the relationship between diet and the recovery and isolation frequency of SMS, it was decided to analyze levels of SMS by bottle content, while stratifying on the age of the children and the number of teeth present. For this purpose, SMS levels in children were dichotomized (infected vs. non-infected) and dietary factors were examined in each age or teeth category. The percentage of children harboring SMS was generally higher in each age interval for those consuming sweetened beverages in the baby bottle (Figure 6).

SMS levels also were assessed by bottle content in children with number of teeth present stratified into four groups based on eruption status. Similar to the results found with age stratification, there was increased isolation of SMS with advanced eruption status especially in the sweetened beverage group, among children with more than four teeth (Figure 7).

Logistic regression models were used to examine the effects of child's age, number of teeth, bottle content and mother's SMS levels on the isolation of SMS in children. A model was constructed that examined the effect of bottle content

(dichotomized as milk and water groups together vs. sweetened beverage group - "BOTDI" in Tables 3 to 6) on the dependent variable SMS - (dichotomized as presence or absence). Bottle content was found to be a significant factor in SMS isolation ( $p < .01$ ), and children drinking sweetened beverages in this model were three times more likely to have SMS than children in either milk or water groups (Table 3). Other independent variables, including number of teeth ("TEETH") and age ("AGE"), both entered as continuous variables, were included with the bottle content term to examine their effects, separately and combined, on SMS isolation. The teeth term significantly affected SMS isolation frequency when examined with bottle content alone ( $p = .001$ ) (Table 4), and when examined in the presence of age and bottle content ( $p < .05$ ) (Table 6). Age was significant when examined with bottle content being the only other term in the model ( $p < .05$ ) (Table 5); however, age did not approach significance in the presence of the teeth term and the bottle content term (Table 6).

Another model was constructed that examined the effect of bottle contents on the dependent variable (SMS - dichotomized) since bottle content was noted to be a significant variable in the isolation of SMS. In this model, the bottle content term was categorized as water, milk and sweetened beverages, with the water group used as the referent category. The bottle variable was found to be a significant factor in whether children had SMS ( $p < .01$ ) (Table 7). In subsequent models, teeth and age were included, separately and together with the bottle content term (Tables 8 to 10). Although teeth continued to be a significant term when examined with bottle content ( $p < .01$ ) (Table 8) and when

examined in the presence of age and bottle content ( $p < .05$ ) (Table 10), age only approached significance when examined with bottle content alone ( $p = .05$ ) (Table 9) and was not a significant additive to the model containing teeth and bottle content (Table 10). SMS levels in mothers were dichotomized (presence or absence) and included in a model. This model was, however, based on a reduced number of subjects, since data was not available from all mothers. There was no effect of mother's SMS and therefore, mothers' SMS levels were excluded from other analyses (Table 11).

A final exploratory model was constructed that included the independent variables age, teeth and bottle content (restricted to the milk and sweetened beverage groups), with the presence of SMS as the dependent variable. Multiple logistic regression showed that bottle content was a significant predictor in the isolation of SMS ( $p < .01$ ) (Table 12). Age did not add to the model when examined separately or in the presence of the teeth variable (Table 13; Table 14). The most parsimonious model suggested that teeth and bottle content were good predictors of SMS isolation ( $p < .05$ ) (Table 15).

Preliminary findings suggested that salivary flow in the younger age group (6 to 15-month-olds) may not have been adequate for SMS recovery. Therefore, it was investigated whether a new method of sampling for saliva, using the back of a mouth mirror, might be better than the traditional technique of sampling with a tongue blade. The back surface of the mouth mirror is non-absorbable, unlike the tongue blade, and therefore might have allowed higher SMS recovery. Sampling of saliva was performed with both a mouth mirror and

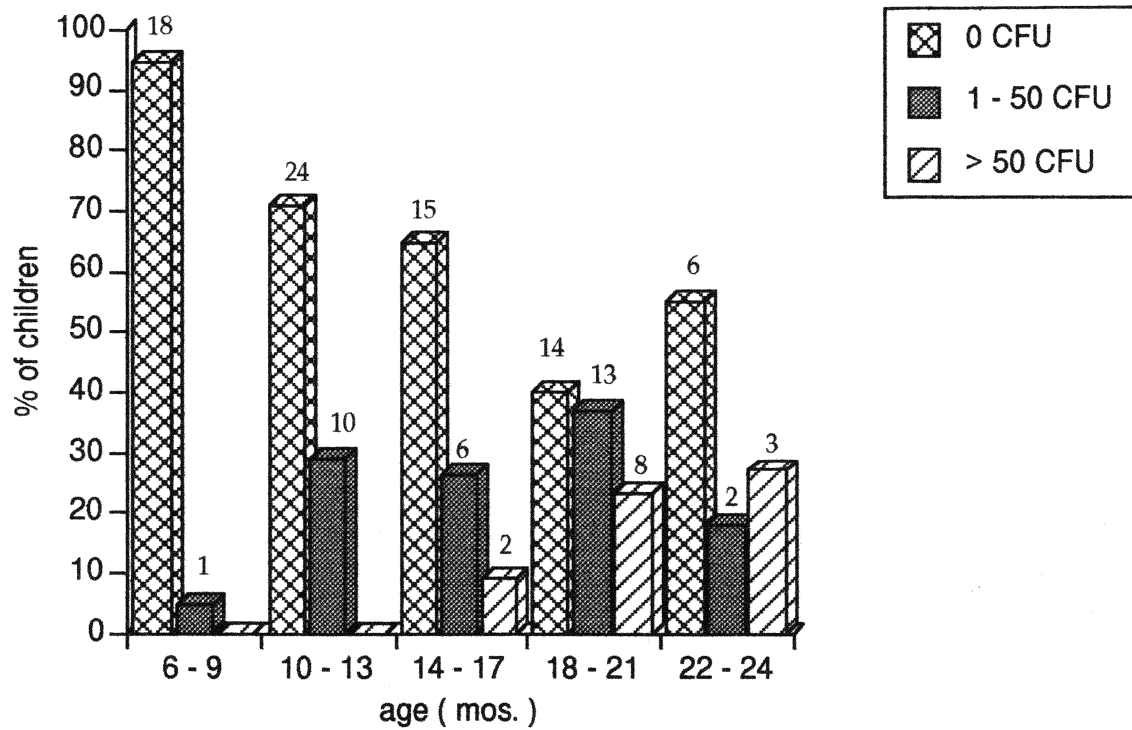
a tongue blade on 86.2% of children in the 16 to 24-month-olds. A strong correlation was found between the two methods ( $r_s = 0.82$ ,  $p < .05$ ), using the Spearman's correlation coefficient (Figure 8).

Although, in general, SMS levels in saliva samples collected with a mouth mirror were correlated with those collected with a tongue blade, the mouth mirror yielded higher numbers of colony forming units of SMS in many cases (Figure 8). Greater numbers of SMS were found in all three categories of bottle content when the mouth mirror was used for saliva sampling (Table 16; Figure 9, Figure 10).

To analyze the relationship between age and SMS levels in the older group of children (16- to 24 months), age was categorized into three groups of 3-month intervals. Isolation frequency of SMS using a mouth mirror suggested a trend of increasing frequency of SMS isolation with increasing age. Levels of SMS infection increased from 38% in the 16- to 18-month age group to 69% in the 22- to 24 month age group (Table 17; Figure 11). However, there was no clear trend observed from data collected by the tongue blade (Table 17; Figure 12).

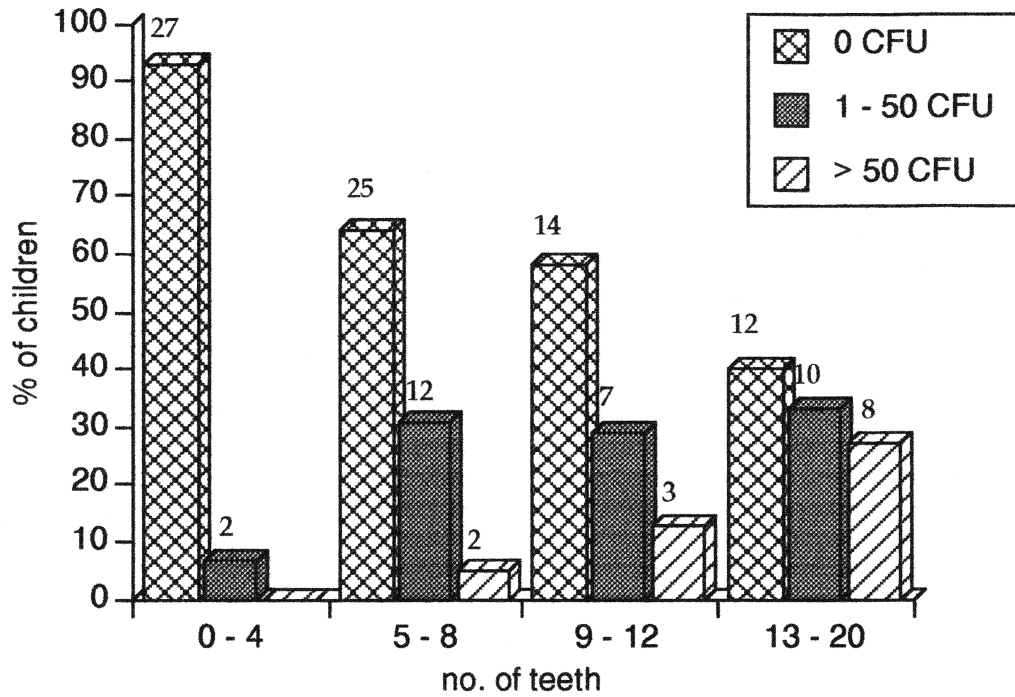
Among the 131 children in this study, only three had developed dental caries at the time of examination. A profile of these children revealed that they all were between 20 and 21 months old and used a baby bottle. Two of the three children were in the sweetened beverage group, and were in the <50 CFU SMS group. The other child was in the milk group, and in the 1 to 50 CFU SMS group (Table 18). Carious lesions were located on the maxillary incisors and first primary molars in these children (Table 19).

Figure 1. Percentage and number of children in five age ranges by levels of salivary mutans streptococci (tongue blade sampling technique)



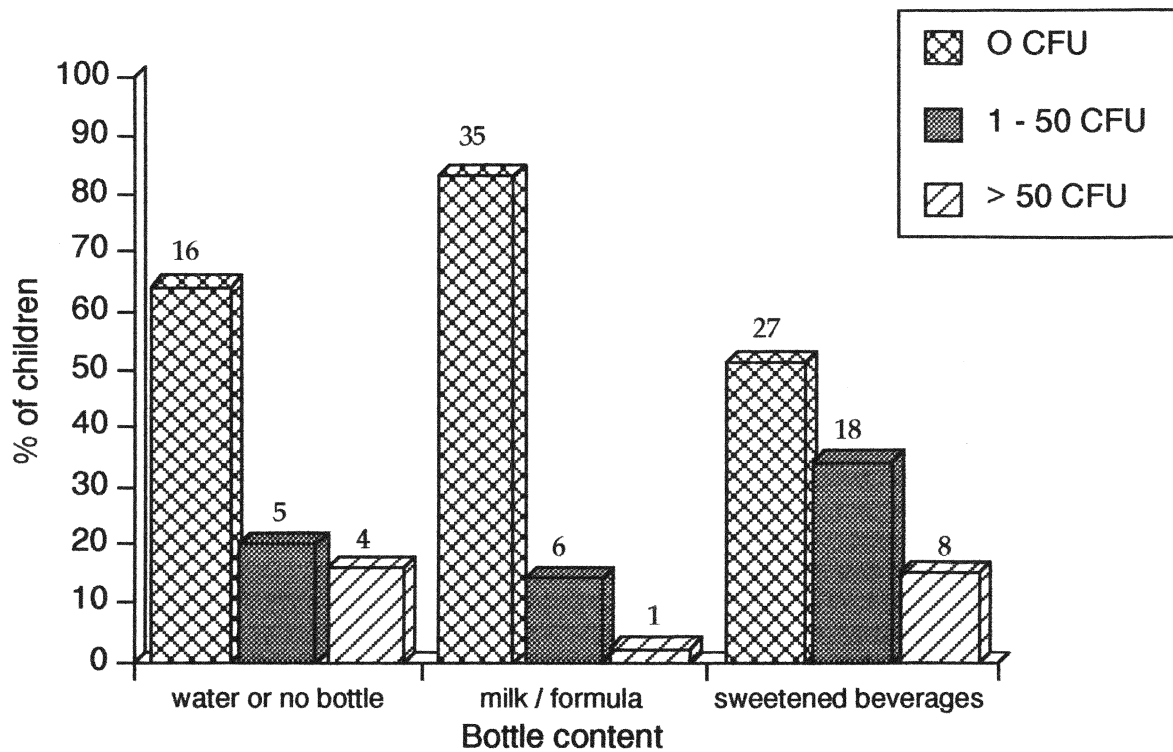
Note : Numbers above bars represent numbers of children

Figure 2. Percentage and number of children in four categories of tooth eruption by levels of salivary mutans streptococci (tongue blade sampling technique)



Note : Numbers above bars represent numbers of children

**Figure 3. Percentage and number of children in three categories of bottle content by levels of salivary mutans streptococci (tongue blade sampling technique)**

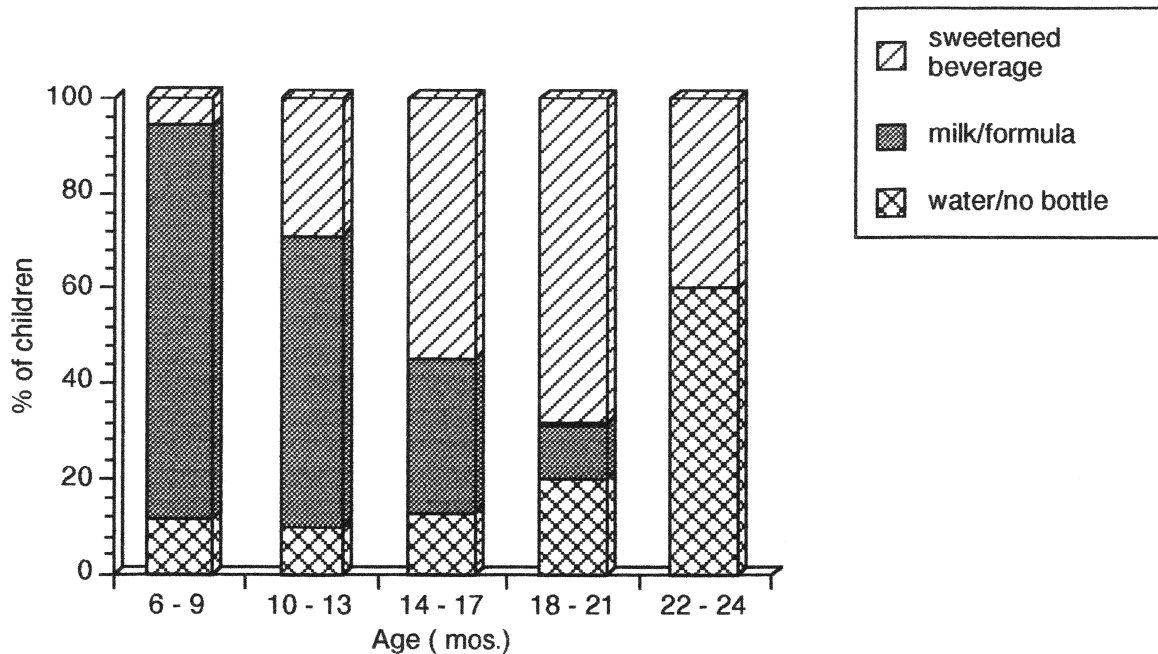


**Note :** Numbers above bars represent numbers of children

Table 1: Percentage of children in the three categories of bottle content, by age

Bottle content	Age (mos.)					Total
	6 - 9 (%)	10 - 13 (%)	14 - 17 (%)	18 - 21 (%)	22 - 24 (%)	
water/ no bottle	2 (11.8)	3 (9.7)	4 (12.9)	7 (20)	9 (60)	25 (19.4)
milk/ formula	14 (82.4)	19 (61.3)	10 (32.3)	4 (11.4)	0 (0.0)	47 (36.4)
sweetened beverage	1 (5.9)	9 (29)	17 (54.8)	24 (68.6)	6 (40)	57 (44.2)
Total	17 (13.2)	31 (24)	31 (24)	35 (27.1)	15 (11.6)	129 (100)

Figure 4. Percentage of children in three categories of bottle content, by 5 different age categories





**Table 2 : Percentage of children in the three categories of bottle content, by number of teeth.**

Bottle content	Number of teeth				Total
	0 - 4 (%)	5 - 8 (%)	9 - 12 (%)	13 - 20 (%)	
water/ no bottle	3 (9.7)	4 (9.8)	7 (29.2)	11 (33.3)	25 (19.4)
milk/ formula	21 (67.7)	20 (48.8)	2 (8.3)	4 (12.1)	47 (36.4)
sweetened beverage	7 (22.6)	17 (41.5)	15 (62.5)	18 (54.5)	57 (44.2)
Total	31 (24)	41 (31.8)	24 (18.6)	33 (25.6)	129 (100)

**Figure 5. Percentage of children in three categories of bottle content, by 4 different teeth categories.**

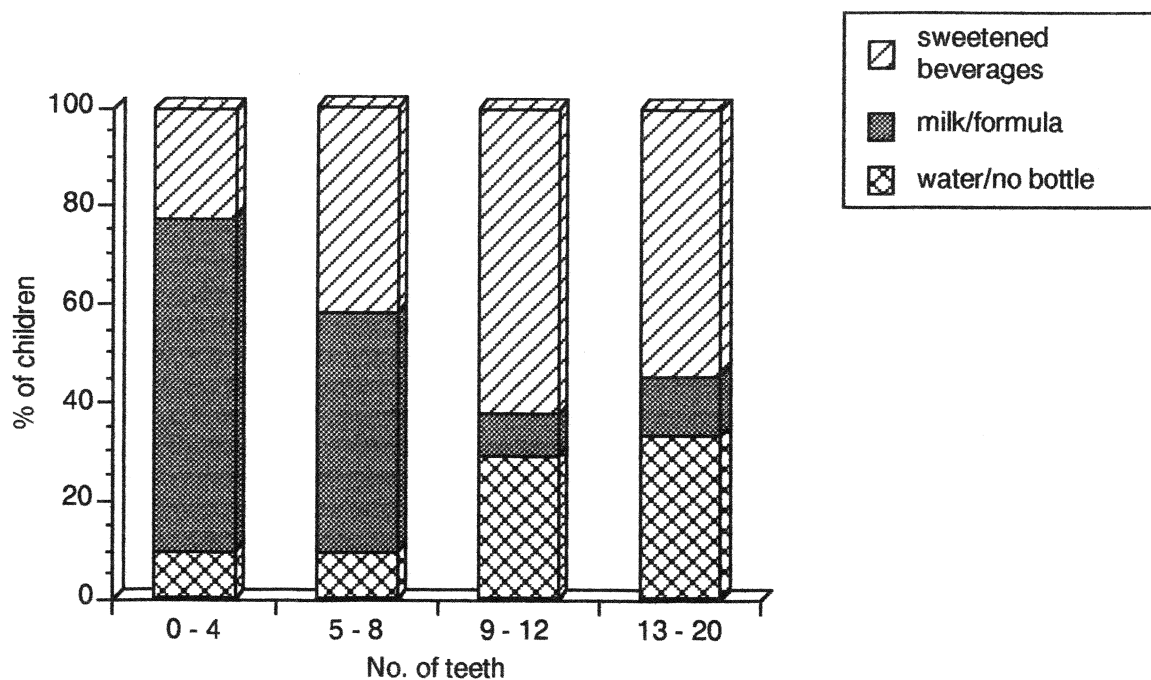
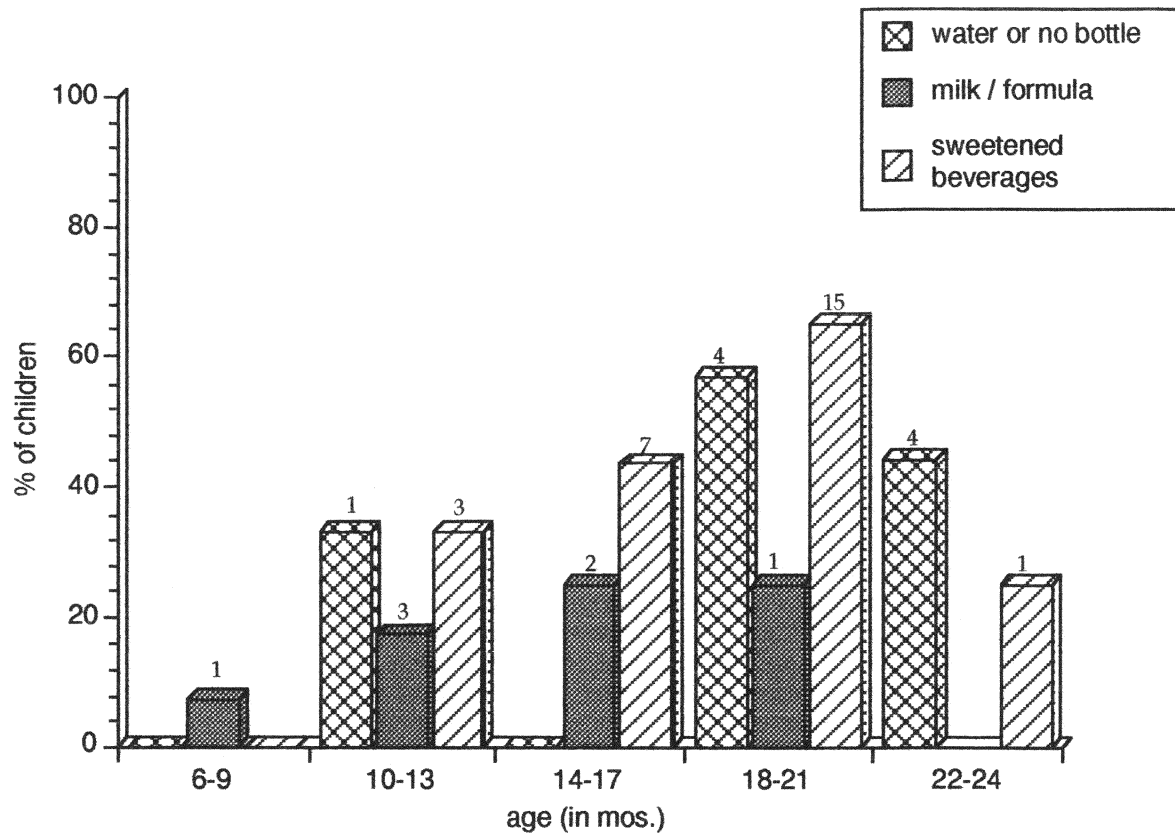
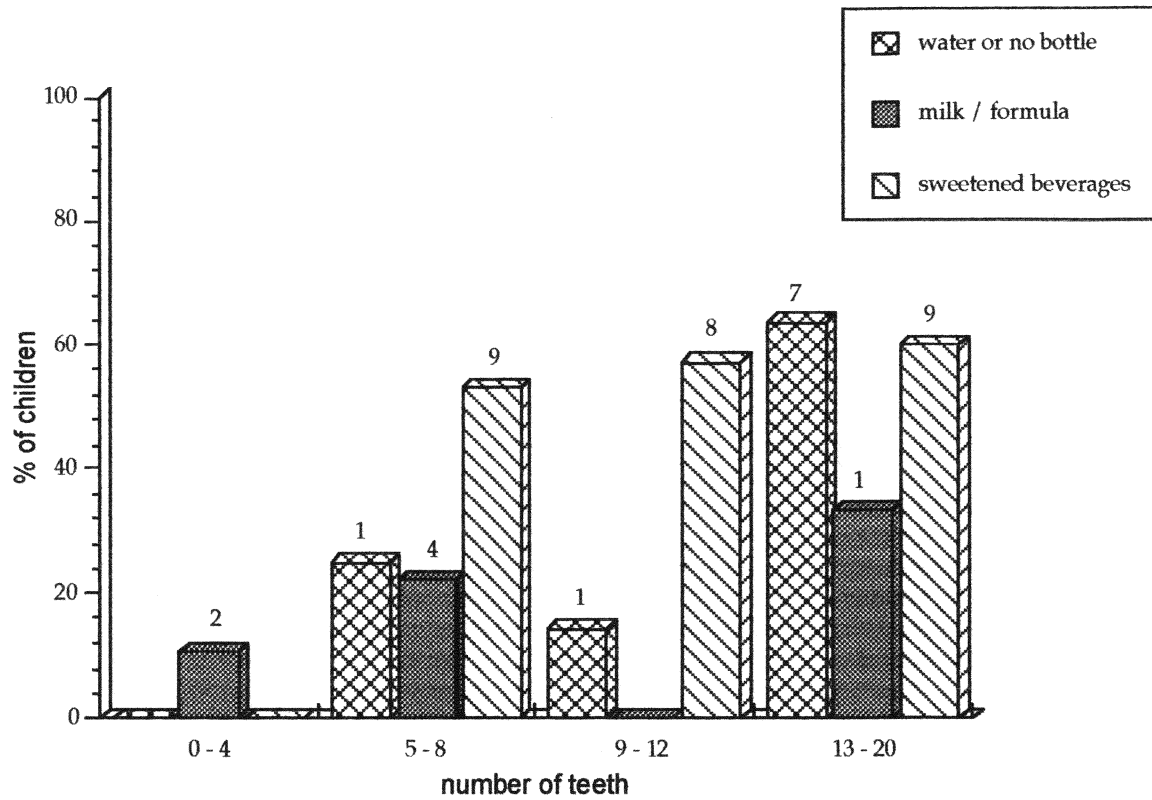


Figure 6. Percentage of children harboring salivary mutans streptococci (SMS >0 CFU), by age and bottle content



Note : Numbers above bars represent numbers of children

**Figure 7. Percentage of children harboring salivary mutans streptococci (SMS > 0 CFU), by number of teeth and bottle content**



**Note :** Numbers above bars represent numbers of children

## LOGISTIC REGRESSION MODELS

Table 3

Independent variable	$\beta$	Significance* (p value)	Prevalence Odds ratio	95% Confidence interval
BOTDI**	1.122	.005	3.07	(1.41, 6.68 )

Table 4

Independent variable	$\beta$	Significance (p value)	Prevalence Odds ratio	95% Confidence interval
BOTDI	.893	.033	2.44	(1.07, 5.56 )
TEETH	.144	.001	1.15	(1.06, 1.26 )

Table 5

Independent variable	$\beta$	Significance (p value)	Prevalence Odds ratio	95% Confidence interval
BOTDI	.916	.026	2.5	(1.12, 5.59 )
AGE	.118	.012	1.13	(1.03, 1.23 )

Table 6

Independent variable	$\beta$	Significance (p value)	Prevalence Odds ratio	95% Confidence interval
BOTDI	.932	.028	2.54	(1.10, 5.84 )
AGE	-.10	.324	.905	(.74, 1.1 )
TEETH	.224	.017	1.25	(1.04, 1.5 )

\* based on Wald statistic

\*\* milk and water groups together vs. sweetened beverage group

## LOGISTIC REGRESSION MODELS (CONTD.)

Table 7

Independent variable	$\beta$	Significance* (p value)	Prevalence Odds ratio	95% Confidence interval
BOTTLE				
WATER	0	.007	1	
MILK	-.1.03	.078	.356	(.11, 1.12 )
SWEETENED BEVERAGES	.538	.281	1.71	(.64, 4.55 )

Table 8

Independent variable	$\beta$	Significance (p value)	Prevalence Odds ratio	95% Confidence interval
BOTTLE				
WATER	0	.098	1	
MILK	-.2.51	.706	.778	(.21, 2.87 )
SWEETENED BEVERAGES	.768	.148	2.15	(.76, 6.1 )
TEETH	.136	.004	1.15	(1.04, 1.26 )

Table 9

Independent variable	$\beta$	Significance (p value)	Prevalence Odds ratio	95% Confidence interval
BOTTLE				
WATER	0	.072	1	
MILK	-.424	.529	.655	(.18, 2.45 )
SWEETENED BEVERAGES	.707	.175	2.03	(.73, 5.64 )
AGE	.102	.051	1.11	(1.0, 1.23 )

\* based on Wald statistic

Table 10

Independent variable	$\beta$	Significance* (p value)	Prevalence Odds ratio	95% Confidence interval
BOTTLE				
WATER	0	.076	1	
MILK	-.446	.516	.639	(.17, 2.46 )
SWEETENED BEVERAGES	.716	.179	2.05	(.72, 5.81 )
AGE	-.117	.265	.889	(.72, 1.09 )
TEETH	.225	.017	1.25	(1.04, 1.50 )

Table 11

Independent variable	$\beta$	Significance (p value)	Prevalence Odds ratio	95% Confidence interval
BOTTLE				
WATER	0	.145	1	
MILK	-.106	.881	.899	(.23, 3.57 )
SWEETENED BEVERAGES	.808	.151	2.24	(.74, 6.76 )
TEETH	.143	.005	1.15	(1.04, 1.28 )
MSMOM	.005	.431	1.00	(1.00, 1.02 )

\* based on Wald statistic

## LOGISTIC REGRESSION MODELS (CONTD.)

Table 12

Independent variable	$\beta$	Significance* (p value)	Prevalence Odds ratio	95% Confidence interval
BOTMILK**	1.571	.002	4.81	(1.82, 12.74 )

Table 13

Independent variable	$\beta$	Significance (p value)	Prevalence Odds ratio	95% Confidence interval
BOTMILK	.1.26	.025	3.52	(1.17, 10.6 )
AGE	.071	.262	1.07	(.95, 1.21 )

Table 14

Independent variable	$\beta$	Significance (p value)	Prevalence Odds ratio	95% Confidence interval
BOTMILK	.1.37	.019	3.96	(1.24, 12.61 )
AGE	-.184	.162	.832	(.64, 1.08 )
TEETH	.243	.028	1.27	(1.03, 1.58 )

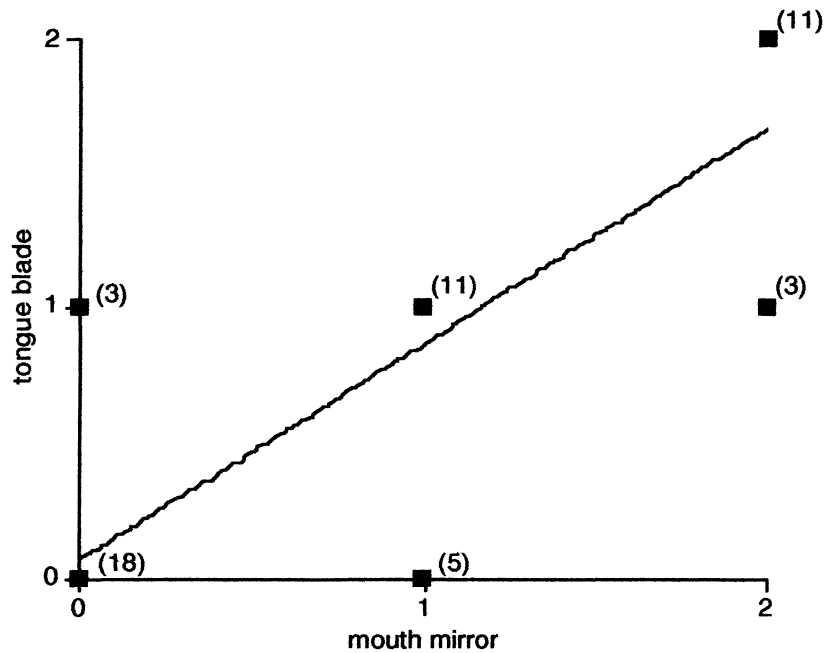
Table 15

Independent variable	$\beta$	Significance (p value)	Prevalence Odds ratio	95% Confidence interval
BOTMILK	1.11	.041	3.04	(1.05, 8.8 )
TEETH	.109	.037	1.12	(1.01, 1.24 )

\* based on Wald statistic

\*\* milk group vs. sweetened beverage group

Figure 8. Correlation between mutans streptococci counts obtained with the mouth mirror and the tongue blade



$r_s = 0.82$  (with 56 pairs)

(-)- number of data values at each point

0 - 0 CFU of salivary mutans streptococci

1 - 1 to 50 CFU of salivary mutans streptococci

2 - > 50 CFU of salivary mutans streptococci



**Table 16 : Comparison of salivary mutans streptococci in children by bottle content using two sampling techniques**

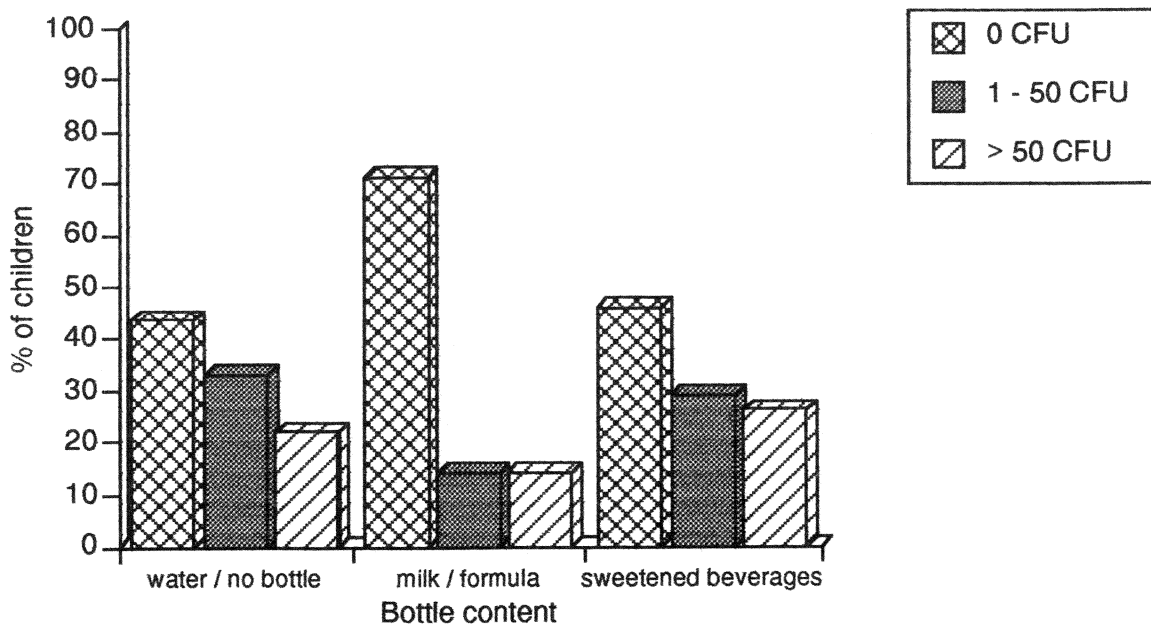
**MS levels in children**

Bottle content	0 CFU		1 to 50 CFU		> 50 CFU	
	MM*	TB**	MM	TB	MM	TB
water / no bottle	8	11	5	4	4	4
milk / formula	5	5	1	1	1	0
sweetened beverages	16	15	10	10	9	7

\* mouth mirror

\*\* tongue blade

**Figure 9. Percentage of children in the three categories of salivary mutans streptococci in three different categories of bottle content (mouth mirror sampling technique)**



**Figure 10. Percentage of children in the three categories of salivary mutans streptococci in three different categories of bottle content (tongue blade sampling technique)**

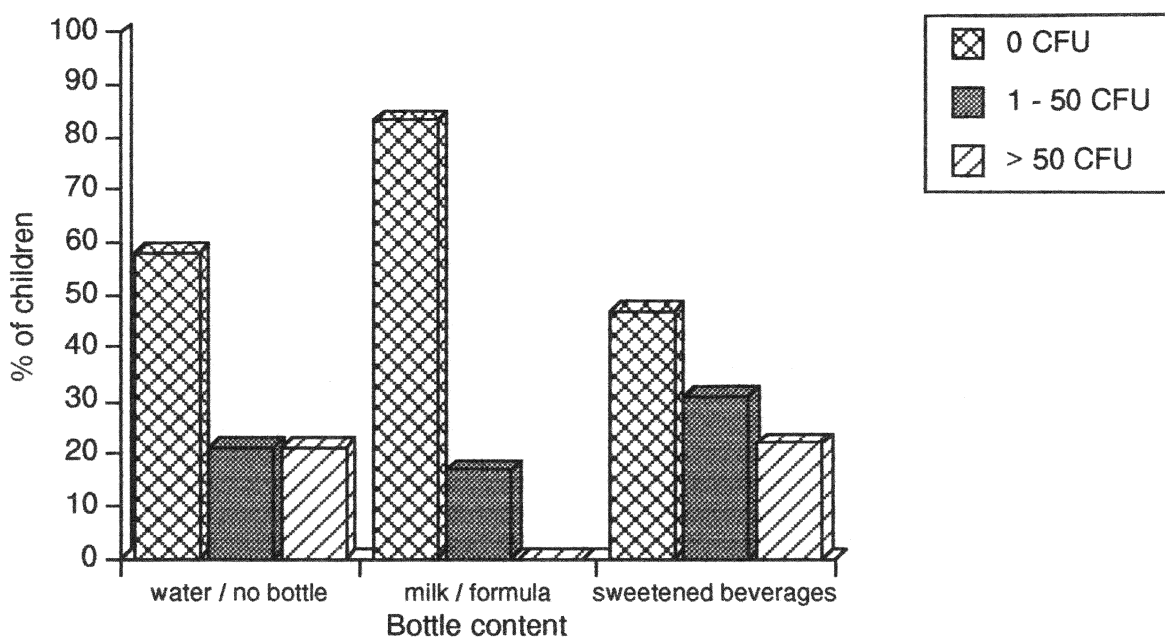


Table 17 : Comparison of salivary mutans streptococci in children by age using two sampling techniques

AGE (in mos.)	MS levels in children					
	0 CFU		1 to 50 CFU		> 50 CFU	
	MM*	TB**	MM	TB	MM	TB
16- to 18	16	15	6	6	4	4
19- to 21	9	10	7	9	7	4
22- to 24	4	6	6	2	3	3

\* mouth mirror

\*\* tongue blade

Figure 11. Percentage of children in the three categories of salivary mutans streptococci in three different age categories (mouth mirror sampling technique)

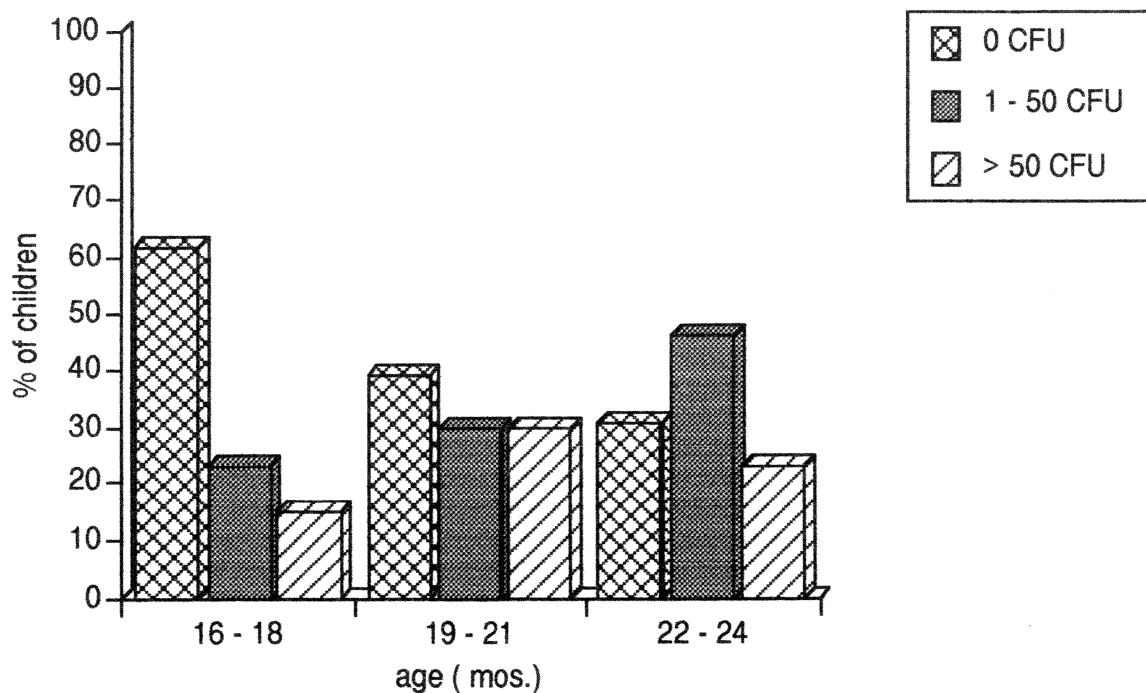


Figure 12. Percentage of children in the three categories of salivary mutans streptococci in three different age categories (tongue blade sampling technique)

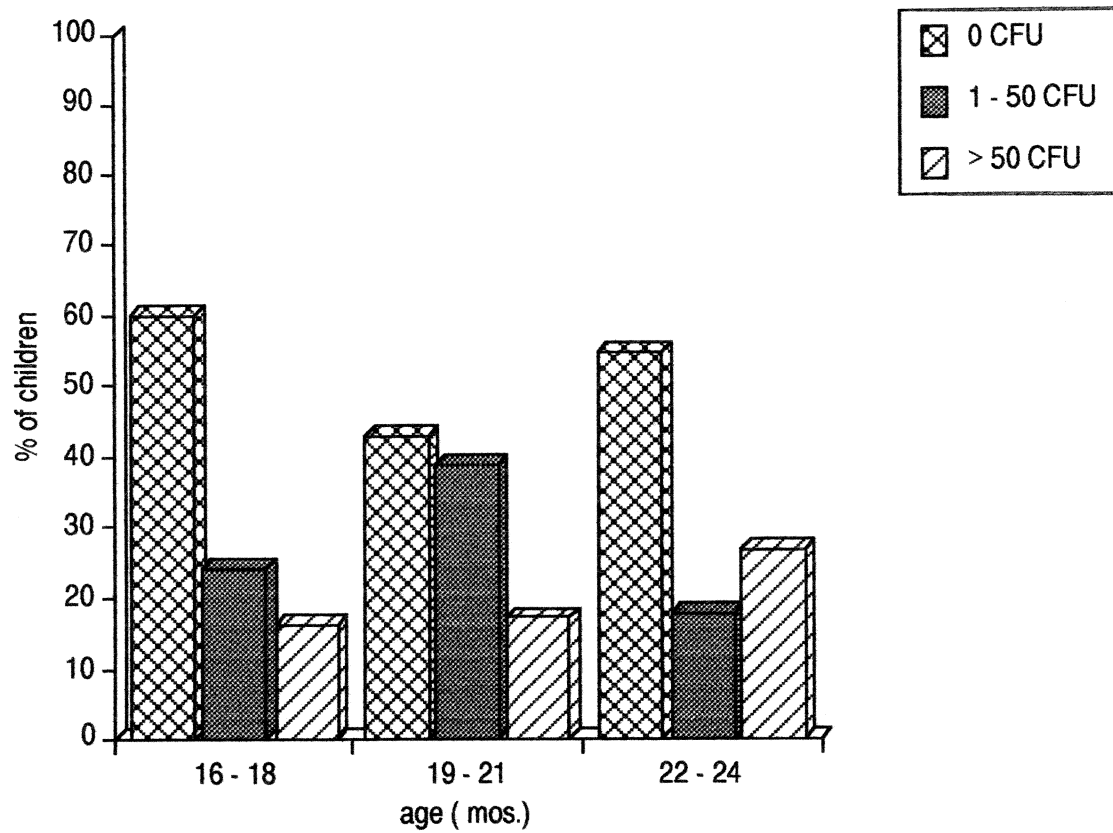


Table 18 : Profile of children with dental caries at time of examination

Children	Age (mos.)	No. of teeth	Bottle content	ms level	ms level (mother)
Subject 44	20	12	sweetened beverage	> 50 CFU	1 to 50 CFU
Subject 33	21	20	milk	1 to 50 CFU	> 50 CFU
Subject 13	21	16	sweetened beverage	> 50 CFU	1 to 50 CFU

Table 19 : Location and number of carious lesions in the three children who had dental caries

Children	Number of carious lesions	Location of carious lesions
Subject 44	4	Maxillary incisors
Subject 33	3	2 maxillary incisors & 1 mandibular first primary molar
Subject 13	7	4 maxillary incisors, 2 maxillary first primary molars & 1 mandibular primary molar

## DISCUSSION

Although a few studies have explored prevalence, background characteristics and patterns of dental caries in 3- to 5-year-old children, there is little information on initiation and mechanisms of this disease in children under age two. For example, mutans streptococci (MS) are known to be important factors in the caries process (van Houte et al., 1982; Milnes and Bowden, 1985; Loesche, 1986). However, controversy exists about the age at which these microorganisms are acquired in the oral cavity and the number of teeth required for their colonization. Furthermore, since high-frequency consumption of carbohydrates is known to play a key role in the caries process (James et al., 1957; Winter et al., 1966; Picton and Wiltshire, 1970), and bottle feeding represents the primary source of carbohydrates to infants and toddlers, it is important to consider the varied types of carbohydrates (e.g., milk, formula, juice) in a baby bottle. Although it is generally believed that anything other than water in the baby bottle will result in caries, there are few data to support these beliefs. More specifically, there is general agreement among dental practitioners that milk in a baby bottle is cariogenic; yet, there is evidence to the contrary (Bowen and Pearson, 1993).

The present cross-sectional study examined caries risk factors related to the acquisition of SMS in infants and children aged 6 to 24 months. The majority of these children were African-American, of low socio-economic status and resided in Hartford, Connecticut. Therefore, the results may be limited to this

population and may not be applicable to all children. Additionally, small sample sizes may not have allowed sufficient power for statistical analyses when the population was subcategorized.

One of the chief findings in this study was that salivary mutans streptococci (SMS) was isolated from the mouths of infants under 12 months of age. Although some studies report that isolation of mutans streptococci (MS) increases with age and increased number of teeth (Catalanotto et al., 1975; Matsuda et al., 1979), the time of acquisition of MS is controversial. One study examining 27 one-year-old infants for streptococcal flora found no MS (Carlsson et al., 1970). A recent study reported that the initial acquisition of MS in infants occurred during a well-delineated age range of 19 to 30 months, designated as a “window of infectivity” (Caufield et al., 1993).

Contrary to Caufield et al.’s hypothesis, earlier studies have found colonization of MS in the oral cavity at younger ages. One study showed that children as young as 11 months were infected with MS (Berkowitz et al., 1975); another reported MS colonization of 13-month -old infants (Matsuda et al., 1979). In the present study, SMS was isolated from a child as young as 7 months and there was a progressive increase in the frequency of isolation in the older age groups. Twenty five percent of children under 18 months harbored SMS, with 2.6% of these children having the highest category of SMS, i.e., >50 CFU.

Studies have reported dental caries in children as young as 11 months old (Suher et al., 1953), and another found caries in 0.5% 1-year-old children (Wendt et al., 1991). Three children in the present study had developed early childhood

caries at the time of examination. They were all between 20 and 21 months, with varying levels of SMS. Since MS are necessary for caries initiation and progression, these findings refute Caufield et al.'s hypothesis that the "window of infectivity" begins at 19 months. It is unlikely that these three children could have developed cavitations in as short a period of time as one to two months.

MS are believed to require a non-shedding surface for colonization (Zinner and Jablon, 1969; Edwardsson and Mejare, 1994). The present study isolated SMS from only one edentulous 7-month-old infant. Perhaps this finding was due to transient SMS in the mouth at the time. Since there was greater recovery of SMS with increasing age and number of teeth, it supports the hypothesis that MS requires the presence of non-shedding surfaces (i.e., tooth structure) for colonization, with increased number of tooth surfaces allowing for increased colonization. An earlier report on 92 children, ranging from newborns to 5-year-old children, reported a gradual increase in the isolation frequency of MS with an increase in age and number of teeth (Catalanotto et al., 1975). Two other studies reported isolation of MS in children with only erupted incisors (Berkowitz et al., 1975; Berkowitz et al., 1980). The present study isolated SMS in 36% of children with 5 to 8 incisors, comparable to 28.6% of children with 6 to 8 teeth who harbored MS in a previous report (Berkowitz et al., 1980).

Besides early colonization of MS in the mouths of infants, the role of the baby bottle is believed to be important in the development of early childhood caries (Robinson and Naylor, 1963; Powell, 1976; Johnsen et al., 1980; Derkson and Ponti, 1982). Most clinical studies that have studied the baby bottle



evaluated the duration of use, rather the actual contents of the bottle. Those that examined bottle content involved children who had early childhood caries, well after the disease process had begun. In studying the characteristics of children with early childhood caries, milk was reported to be most frequently used in the bottle and was thus implicated by many authors in the development of early childhood caries (Fass, 1962; Dilley et al., 1980). In fact, the American Academy of Pediatric Dentistry recommends that children should not sleep with a bottle containing “juice, milk, formula, or any other liquid sweetened with fermentable carbohydrate” (American Academy of Pediatric Dentistry Reference Manual, 1995). Other reports on bottle content and snack foods containing sucrose found none of these carbohydrate sources to be a significant factor in children with caries (Johnsen et al., 1980; Richardson et al., 1981).

Recent studies have more carefully explored the potential cariogenicity of milk in a baby bottle and its importance in the development of early childhood caries. One study reported that children whose bottles contained milk or milk/sugar mixtures did not have any more caries than those who did not use the baby bottle (Goose, 1967). Milk also has been shown to be non-cariogenic when fed to rats. In a study that examined 60 desalivated rats that were given milk, water, lactose-reduced milk and a 10% sucrose solution, it was found that only the 10% sucrose solution, promoted the development of dental caries (Bowen et al., 1991). Another study conducted on 121 Sprague-Dawley rats, fed milk containing different concentrations of sucrose, found that milk may be cariogenic only in the presence of added sugar (Bowen and Pearson, 1993). Milk

also has been reported to have protective effects against caries, reported due either to its content of calcium and phosphorus (Jenkins and Ferguson, 1966), casein (Bibby et al., 1980), phosphoproteins that inhibit enamel dissolution (Weiss and Bibby, 1966), or antibacterial factors that interfere with oral microorganisms (Kosikowski, 1970). Additionally, the potential of cariogenic bacteria to utilize lactose, the sugar in milk, as well as they do sucrose, remains unclear (Rugg-Gunn et al., 1985).

In contrast to milk being potentially protective against dental caries or cariogenic microorganisms, high frequency consumption of sucrose has been shown to increase the colonization of MS. In a study that examined *S. mutans* free Osborne-Mendel rats that consumed sucrose-containing diets, it was found that the percentage of *S. mutans* became higher in sucrose-consuming rats, for all serotypes tested (Tanzer, 1979). A similar finding was reported in a study on 60 human subjects, given sucrose-rich or low-sucrose diets. The sucrose-rich diet was found to increase *S. mutans* populations in plaque by 2.1%, as compared with the low-sucrose diet where the percentage of *S. mutans* recovered was 0.7% at the end of the study period (Folke et al., 1972).

The present study evaluated the effect of milk and other bottle contents based on self-reports from the parents of the children. Self-reported questionnaires have their limitations because of both over- and under-estimation of certain foods (Grindejord et al., 1991). An epidemiological study measuring dietary habits in infancy and childhood showed that sucrose-rich foods were underestimated in answering short questions of particular food frequencies.

Researchers suggest that if more valid and reliable feeding data are to be obtained, continuous monitoring or repeated interviews with parents are needed (Persson and Carlgren, 1984).

The present study examined the relationship of SMS to three categories of bottle content: water in the bottle or no bottle usage, milk or formula in the bottle, and sweetened beverages in the bottle. Milk and infant formula were grouped together since they had similar concentrations of lactose (4% and 7% respectively). None of the children in the study had received infant formula that contained sucrose. Sixteen percent of children in the milk group had SMS, compared with 36% in the water group and 49% in the sweetened beverage group. Since there are no studies that have evaluated bottle content in relation to isolation frequencies of SMS, it is not possible to compare the findings of the present investigation with others.

The relationship of SMS to bottle content and age of the children was evaluated, since age alone affects MS colonization and infants may consume various foods in the bottle at different ages. It was noted that milk consumption decreased with age, from 82.4% of children in the 6- to 9-month-old age group to 11.4% in the 18- to 21-month-olds. In contrast, the consumption of sweetened beverages in the bottle was generally higher with increased age. In the 6- to 9-month age group, 5.9% of children consumed sweetened beverages in the baby bottle, compared with 68.6% of children in the 18- to 21-month age group. A similar trend was noticed when bottle content was assessed by number of teeth.

Since the association between bottle content and SMS may have been confounded by age and the number of teeth present, the percentage of children with SMS in the three bottle groups was assessed by different age and teeth groups. When the bottle contained milk, isolation of SMS was generally low in all age groups. The percentage of children who harbored SMS increased from 7.7% in the 6 to 9-month age interval to 25% in the 18 to 21-month age interval. A steep increase in the percentage of children harboring SMS was, however, noted in the sweetened beverage group. No children in the 6 to 9-month-olds who consumed sweetened beverages in the bottle harbored SMS, while 65.2% of the 18 to 21-month-olds harbored SMS in the sweetened beverage group. Similarly, when SMS in children was assessed by number of teeth and bottle content, there was a sharp increase in the percentage of children harboring SMS in the sweetened beverage group, from no children in the 0 to 4 teeth category to 60% of children in the 13 to 20 teeth category. In contrast, percentage of children who were colonized with SMS increased from 10.5% in the 0 to 4 teeth category to 33.3% in the 13 to 20 teeth category. Such observations allow for the speculation that milk may have protective effects or at least, may not be harmful.

Logistic regression was used to investigate the association between the prevalence of SMS and bottle content, while controlling for the potential confounding effects of age and number of teeth. In these analyses, bottle content was shown to be significantly associated with the isolation frequency of SMS. The results of this investigation suggest that a bottle containing sweetened beverages could increase the likelihood of SMS isolation three-fold, compared

with a bottle that contains milk. A recent study reported that consumption of sugar-containing beverages was significantly associated with the development of dental caries at 3.5 years (Grindejord et al., 1995). Other studies have reported that higher caries prevalence in the primary dentition is associated with early establishment of SMS (Alaluusua and Renkonen, 1983; Köhler et al., 1988). Thus, the results of this investigation suggest that bottles containing sweetened beverages might increase the likelihood of dental caries in the population. However, one cannot dismiss the possibility that bottle content might be a marker for other unidentified variables that are important in the caries process.

The high percentage of children found in the water group with high levels of SMS is surprising. This finding may be attributed to the fact that that these children were on other solid and liquid foods that probably included substances with high sucrose levels. Unfortunately, diet analyses of non-bottle foods was not obtained from the parents. The small number of children in the water group also may have contributed to the unexpected results.

Only three children in the present study had dental caries at the time of examination and therefore, the hypothesis that suggested the possibility of a correlation between dental caries and SMS levels by bottle content could not be examined.

Transmission of MS from mothers to their children is well documented, with the species formerly known as serotype 'c' *Streptococcus mutans*, being the most common isolated (Berkowitz and Jordan, 1975; Berkowitz et al., 1981). However, there is less information on the correlation of MS levels in mothers and

their children. Early studies report an association between maternal and infant levels of SMS, with one suggesting that when maternal salivary levels exceed  $10^5$  CFU / ml saliva, higher levels of SMS isolation are likely to be observed in their children (Berkowitz et al., 1981). Other studies have reported similar findings, with one reporting a quantitative correlation between numbers of SMS in children and their mothers (Köhler and Bratthall, 1978) and another finding a significant relation between SMS scores of mothers and their infants (Brown et al., 1985). However, in the present study SMS levels of the mothers were not significantly associated with SMS levels in their children. The wide age range in this study and the important effects of the number of teeth and age may account for differences between the present findings and other reports. For instance, age may have confounded these results since children who were very young may not have had detectable levels of SMS while their mother was highly infected.

Other factors that may have affected the relationship between mother's and child's SMS levels were the small number of mother-child pairs, due to refusal of some of the mothers to participate in the study or the procedural differences in the collection of saliva samples from the mothers in this study, compared with other studies. The present study used the tongue blade sampling procedure (Köhler and Bratthall, 1979), whereas plaque and paraffin-stimulated saliva samples were used in the other studies.

The present study utilized the tongue blade method (Köhler and Bratthall, 1979) to sample saliva, as well as the back of a dental mouth mirror. There are no data on the reliability or validity of the tongue blade in obtaining saliva samples

from children as young as those used in this study. One study that examined 1,189 1-year-old children (Grindejord et al., 1991) used a cotton swab to sample bacteria from the dorsal surface of the tongue, since it was reported that that this method had a high correlation with the number of mutans streptococci in saliva (Beighton, 1986). It was observed during the course of the present study that infants and young children seemed to have inadequate salivary flow, making collection of saliva samples with the traditional tongue blade method difficult. Moreover, many children did not like the feel or the taste of a wooden tongue blade. Therefore, in the older age group (16 to 24 months), it was decided to compare the back surface of a mouth mirror, with its non-absorbable surface, to sample saliva, to sampling obtained with the tongue blade. Numbers of SMS obtained with the mouth mirror sample were numerically higher than those obtained from tongue blade samples. Although a strong correlation existed between the two methods, the ease of sampling saliva in infants and young children and the numerically higher levels with the mirror suggest that the mouth mirror technique should be further evaluated for SMS collection in young children.

In summary, the notable findings of the present study are that SMS can be isolated in children under the age of 12 months and that increases in SMS levels are best associated with number of teeth, age and high levels of sucrose in baby bottles. Children consuming sweetened beverages were three times more likely to have SMS compared with children consuming milk. Thus, the variables used in the present study to predict SMS isolation may be of assistance in

understanding the factors involved in the colonization of mutans streptococci in the mouths of infants.

Longitudinal studies, involving larger populations, examined at more frequent intervals, are required to determine the effects of early MS acquisition on the development of early childhood caries. Although the present study shows that bottle content is an important predictor of SMS isolation in the oral cavity of infants, it has not addressed the effect of carbohydrates in solid foods on SMS isolation. Future efforts should explore the relationship of dietary practices on the acquisition of SMS in larger and more heterogeneous populations.



**APPENDIX A** (Page 1 of 2)

**Section I**

A.	Putting a child to bed with a bottle containing milk can cause cavities in the front teeth.	(1)YES (2)NO
B.	Putting a child to bed with a bottle containing juice can cause cavities in the front teeth.	(1)YES (2)NO
C.	Putting a child to bed with a bottle containing plain water can cause cavities in the front teeth.	(1)YES (2)NO
D.	Children should stop using the bottle by their first birthday.	(1)YES (2)NO
E.	Children who are allowed to have a bottle all the time might get cavities.	(1)YES (2)NO
F.	Cavities in children's front teeth are a health problem.	(1)YES (2)NO

## APPENDIX A (Page 2 of 2)

### Section II

A.	What is your child's normal/regular schedule? (INTERVIEWER: You are looking to elicit information on when child wakes, eats meals and snacks, naps, how parent gets child to go to sleep, what the child drinks and whether from bottle or cup etc.)
B.	How many hours a day does your child use a bottle? (i.e. how long does it take for the child to finish the bottle or how long do they have access to the bottle) (1)0      (2)1-2      (3)3-5      (4)6-8      (5)>8
C.	How often do you put your child to bed with a bottle, either at night or for a nap? (1)almost every time    (2)1-2 times each week    (3)less than once each week (4)never
D.	Does your child ever fall asleep with the bottle? (1)almost always      (2)sometimes      (3)never (4)N/A
E.	Does the bottle remain with the child while he/she is asleep? (1)yes      (2)no      (3)N/A
F.	Does your child wake up in the middle of the night? (1)almost every night (2)1-2 times a week (3)less than once a week (4)just when sick (5)never
G.	If your child wakes up, do you put your child back to bed with a bottle? (1)almost always      (2)sometimes    (3)never      (4)N/A
H.	What have you put in the bottles in the last week (day or night use)? (INTERVIEWER: read all options & circle all that apply) (1)formula (2)milk (3)sweetened milk (4)juice (5)cereal (6)water (7)soda (8)other_____
I.	How often does your child drink from a cup (tippy)? (1)at most meals and snacks    (2)once a day      (3)less than once a day    (4)never
J.	Does your child use a pacifier? (1)yes      (2)no
K.	Do you ever dip it in something sweet such as honey, syrup or molasses? (1)yes      (2)no      (3)N/A

## APPENDIX B

Calculation of sample size:

$$N = \frac{sd^2(z_{\alpha}+z_{\beta})^2}{(x_s-x_p)^2} \quad (\text{Lemeshow et al.,1990})$$

where

N = number of subjects needed in each group to determine statistical significance

sd = population standard deviation

$\alpha$  = probability of type I error

$\beta$  = probability of type II error

$z_{\alpha}$  = tabular Z score for  $\alpha$

$z_{\beta}$  = tabular Z score for  $\beta$

$x_s$  = sample mean

$x_p$  = population mean

In phase 1, mutans streptococci counts were used for mean and standard deviation and

sd = 7.53 (for all counts)

$\alpha$  = 0.05

$\beta$  = 0.20

$z_{\alpha}$  = 2.013

$z_{\beta}$  = 0.850

$x_s$  = 4.95 (all counts taken in 10/95)

$x_p$  = 1.84 (all counts)

therefore N = 48 in each group.

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