Lactobacilli, Mutans streptococci and Dental Caries: A Longitudinal Study in 2-Year-Old Children up to the Age of 5 Years

Key Words
Caries risk
Cohort study
Microbiology
Pre-school children
Primary dentition

Abstract
A 3-year cohort study was carried out in 252 pre-school children for early identification of caries-active individuals. During this period information was collected about the acquisition of mutans streptococci and lactobacilli from the age of 2 till 5 years old. At baseline mutans streptococci were detected in 43% of the children while the detection frequency of lactobacilli was low (11.5%). On an individual level, numbers of colony-forming units of mutans streptococci and lactobacilli in plaque and saliva varied largely during the study period. The correlations between the numbers of lactobacilli and mutans streptococci in the saliva of the mother and the saliva and plaque of the child were low and never exceeded $r = 0.22$. Very low correlations ($< r = 0.22$) were also found between the numbers of mutans streptococci or lactobacilli and the diet in terms of the number of sugar intakes. Nevertheless, in children older than 2.5 years correlations between the clinical caries score and lactobacilli in saliva (range 0.31–0.62) and mutans streptococci in plaque or saliva (range 0.24–0.46) were highly significant ($p < 0.01$).

There is evidence that acidogenic species, such as mutans streptococci (Streptococcus mutans and Streptococcus sobrinus) and lactobacilli, are statistically associated with the presence and the onset of dental decay [Loesche, 1986]. The acquisition of these organisms in young children is only partly described and is ill understood ecologically. Colonization of the mouth by mutans streptococci seems to depend on the presence of hard surfaces, and in young children occurs after tooth eruption [Carlsson et al., 1969; Catalanotto et al., 1975]. Before the age of 2 years, lactobacilli are recovered in low numbers and very often they seem to be present transiently [Carlsson et al., 1975]. A gradual increase in the detection of mutans streptococci is observed, the isolation frequency being highest when the deciduous dentition is completed and approximal contacts between deciduous molars are present with an increase in the number of teeth [Catalanotto et al., 1975]. Similar biotypes of mutans streptococci have been found in the parents (in most cases the mother) and the child [Rogers, 1981]. This focuses attention on the supporter of the child as a source of mutans streptococci. Transfer might occur directly via saliva or by the use of domestic items such as toothbrushes and spoons contaminated with saliva [Köhler and Bratthall, 1978; Svanberg, 1978]. The salivary concentrations of microorganisms such as mutans streptococci and Streptococcus sanguis were suggested to be crucial in their establishment on the tooth surface [van Houte and Green, 1974]. A delay in colonization of the child by mutans streptococci is likely to be bene-
ficial as their early detection was found to be coupled with a higher caries experience of the child [Alaluusua and Renkonen, 1983; Köhler et al., 1988].

Longitudinal data on the development of the oral microflora in young children are still scarce. In this study, the acquisition of mutans streptococci and lactobacilli is described on a group level and on an individual level for a group of children from the age of 2 until 5 years of age. The detection of mutans streptococci and lactobacilli in children was correlated with the occurrence of these microorganisms in the mothers and with the frequency of sugar intake. Furthermore, mutans streptococci and lactobacilli were also related to the development of caries.

Materials and Methods

Two hundred and fifty-two children with an average age of 2.3 years (range 1.9–2.8 years), who visited the Child Dental Health centre in Nijmegen, The Netherlands, participated in a study on caries prediction; 49% of the children were boys and 51% were girls. The social background of the child was assessed at the first examination by interviewing the accompanying adult on the level of education of both parents. The level of education was divided into three categories (low level: primary school or elementary vocational training; intermediate level: secondary or high school education; high level: university training).

Over a 3-year observation period the children were examined clinically at 6-month intervals until the age of 5 years. Due to the young age of the children and their limited cooperation, data were recorded by one and the same dentist on every occasion. Plaque and saliva samples were collected as described below, and further data were obtained by interviewing the accompanying adult, and by intra-oral examinations of the child.

Caries was scored by visual examination of the teeth. After drying the teeth with air, each tooth surface was recorded according to the following criteria: sound = no signs of caries lesions; white spot intact enamel; dark (yellow/brown) discolouration of intact enamel; carious = discolouration accompanied by loss of surface continuity; cavity progressing into dentine; restoration.

Dietary habits of the child were recorded with the help of a 24-hour recall method [van Schaik, 1971]. The average daily numbers of food intakes, in particular those containing sugar, were calculated. When the interval between two food intakes was less than 20 min, they were counted as one.

At every check-up, children and parents were advised on diet, the use of fluoride and on oral hygiene procedures. Initial carious lesions were treated with a fluoride varnish.

Bacteriological Sampling

Saliva samples were taken by moving cotton pellets, held by tweezers, through the oral cavity; care was taken to avoid contact with the teeth. The saliva-soaked pellets (3–5 per child) were collected in a glass bottle until approximately 1 ml of saliva could be extracted. After the collection of saliva, the front teeth were dried with air, and a pooled plaque sample was collected from the approximal surfaces of the upper incisors and the mesial surfaces of the canines using dental floss.

At the first examination a saliva sample was also taken from the parent taking care of the child (generally the mother). Due to non-cooperation of the child or failures in the laboratory procedures, some samples were missing. This was the case for 10% (mutans streptococci in saliva), 11% (mutans streptococci in plaque), and 8% (lactobacilli in saliva) of the children.

The pieces of dental floss were placed in 1 ml of saline and transported to the laboratory. Bacteriological samples were processed within 5 h after collection. Plaque samples were homogenized and cotton pellets were treated by ultrasonic dispersion for 20 s at 0 °C using a Kontes cell disrupter (model 881440; Kontes, N.J., USA) and serially diluted in 10-fold steps. Suitable dilutions were plated onto TSY20B agar [Schaeken et al., 1986]; saliva samples were additionally plated onto Rogosa SL agar (Difco Detroit, Mich., USA). All plates were incubated for 5 days at 37 °C in an atmosphere of 91% N2, 5% CO2 and 4% H2. The plaque samples were used to assess the numbers of mutans streptococci. Numbers of colony-forming units (cfu) of mutans streptococci were counted on TSY20B agar and lactobacilli were counted on Rogosa agar. The level of detection with these methods was 10 cfu/ml.

In order to study possible age effects, the data were related to the age at which they were collected. To this end 7 age groups were constructed (table 1). None of the children entered an age group twice. The group of children younger than 2 years was small and therefore all the children younger than 2.5 years were placed in the same group. As a result of age distribution at baseline, the youngest age group was not representative for the whole baseline group because some of the older children started in group 2.

### Table 1. The size of the age groups during the study

<table>
<thead>
<tr>
<th>Age group</th>
<th>Age range years</th>
<th>Average age, years</th>
<th>Children n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.92–2.49</td>
<td>2.2</td>
<td>182</td>
</tr>
<tr>
<td>2</td>
<td>2.50–2.99</td>
<td>2.7</td>
<td>183</td>
</tr>
<tr>
<td>3</td>
<td>3.00–3.49</td>
<td>3.2</td>
<td>197</td>
</tr>
<tr>
<td>4</td>
<td>3.50–3.99</td>
<td>3.8</td>
<td>195</td>
</tr>
<tr>
<td>5</td>
<td>4.00–4.49</td>
<td>4.3</td>
<td>197</td>
</tr>
<tr>
<td>6</td>
<td>4.50–4.99</td>
<td>4.7</td>
<td>195</td>
</tr>
<tr>
<td>7</td>
<td>5.00–5.52</td>
<td>5.2</td>
<td>198</td>
</tr>
</tbody>
</table>

In this part of the study it was a prerequisite that the children were younger than 3 years at the first bacteriological examination, older than 5 years at the final examination, and that at least 3 examinations had been successful.
time that they reached the age of 5 years, only 193 children fulfilled these demands. At baseline, plaque samples could be collected from 235 children (93.2%) and saliva samples from 225 children (89.3%). Plaque and saliva samples were missing because some children refused to cooperate while others failed to produce an adequate amount of saliva. Both plaque and saliva samples were obtained from 225 children. Mutans streptococci could be detected in 31% of the plaque samples and in 43% of the saliva samples.

The numbers of mutans streptococci and lactobacilli were divided into three categories: not detectable–low, moderate, and high levels. Mutans streptococci in the saliva samples were present in low numbers ($\geq 0$ and $< 10^4$ cfu) in 74% of the children, in moderate numbers ($\geq 10^4$ and $< 10^6$ cfu) in 13%, and in high numbers ($\geq 10^6$ cfu) in 13% of the children. In the plaque samples, mutans streptococci were detected in low numbers ($\geq 0$ and $< 10^4$ cfu) in 77% of the children, in moderate numbers ($\geq 10^4$ and $< 10^6$ cfu) in 9%, and in high numbers ($\geq 10^6$ cfu) in 14% of the children. The Spearman correlation between the numbers of mutans streptococci in plaque and saliva was 0.60 ($p < 0.01$).

Lactobacilli were detected in the saliva samples of 11% of the children. Lactobacilli in saliva samples of the children were present in low numbers ($\geq 0$ and $< 10^3$ cfu) in 94% of the children, in moderate numbers ($\geq 10^3$ and $< 10^5$ cfu) in 5%, and in high numbers ($\geq 10^5$ cfu) in 1% of the children.

**Longitudinal Data**

Since bacteriological samples were only taken once a year and the children entered the study at different ages, individual data were not available in every age group. However, for each age group bacteriological information was obtained from the sample taken at that age or by taking the average of the scores of the previous and following examination. After classification of the numbers of microorganisms in four categories, the occurrence of mutans streptococci and lactobacilli is presented in figures 1–3. Only small variations between successive age intervals were observed.

The variations in levels of mutans streptococci and lactobacilli in plaque and saliva at the sampling occasions are presented in figure 4. Microorganisms could be absent on each occasion, show increasing or decreasing numbers in time, or fluctuate in the long term. Mutans streptococci were detected more frequently in saliva (72% of all samples) than in plaque (56% of all samples). Lactobacilli were present in low numbers ($\geq 0$ and $< 10^3$ cfu) in 94% of the children, in moderate numbers ($\geq 10^3$ and $< 10^5$ cfu) in 5%, and in high numbers ($\geq 10^5$ cfu) in 1% of the children.
Fig. 2. Percentage distribution of the children in the various age groups according to the levels of mutans streptococci (MS) in plaque.

Fig. 3. Percentage distribution of the children in the various age groups according to the levels of lactobacilli in saliva.
detected in 40% of all saliva samples. Children with initial high levels tended to keep high levels, whereas in children with low levels more fluctuations in bacterial counts were observed.

High correlations (0.55–0.61, p<0.01) were found between the numbers of mutans streptococci in plaque and saliva samples. These correlations were little influenced by age of sampling. The correlation between mutans streptococci and lactobacilli in the child fluctuated between 0.22 and 0.49 (p<0.01).

Mutans streptococci were detected in 97% of the saliva samples of the parents, and in 50% of these samples high numbers (>10⁶ cfu) were found. Lactobacilli were observed in the saliva samples of 81% of the parents, and high numbers (>10⁵ cfu) were found in 20% of them. The correlations of mutans streptococci and lactobacilli between parents and children are presented in figure 5. Compared with the mutans streptococci, the correlations between the numbers of lactobacilli in the saliva of the mother and the child were slightly higher, but still low. No significant age effects seemed to be present.

The Spearman correlations between the numbers of mutans streptococci in plaque or saliva and the numbers of sugar intakes were very low (range 0.00–0.22) and were not statistically significant at p<0.05 for most of the age categories. The correlations between sugar intakes and lactobacilli were not higher than those for mutans streptococci.

The dmfs score and the percentage of children with caries related to the various age groups are presented in table 2. Looking at dentinal lesions alone, 0.6% of the children in the youngest age group and 28.1% in the oldest age group had caries.

Spearman correlations between visual caries scores (including dark discolourations of the enamel with loss of surface continuity and cavities progressing into the dentin) and the numbers of mutans streptococci and lactobacilli are presented in figure 6. For the youngest age group, no positive correlation was found between the mutans streptococci or lactobacilli and the caries score. Above the age of 3.5 years the correlations with the caries score were higher for lactobacillus counts than for mutans streptococci in saliva.

Significant negative correlations were found between the level of education of the mother and the levels of mutans streptococci or lactobacilli in the saliva of their children (fig. 7).
Discussion

The number of children with detectable mutans streptococci entering this investigation was higher than in similar previous studies [Catalanotto et al., 1975; Alaluusua and Renkonen, 1983]: The strong correlation found between the presence of mutans streptococci in plaque and saliva samples was in accordance with earlier findings [Keene and Horton, 1982; van Houte et al., 1978; Köhler et al., 1981; Schaeeken et al., 1987; Mundorff et al., 1990]. In saliva samples, mutans streptococci were detected more frequently than in plaque samples. This is explained by the localized establishment of mutans streptococci in the mouth. Plaque samples collected from the upper incisors are not supposed to reflect the whole dentition, in contrast to a saliva sample. The low detection frequency of lactobacilli in the children was in accordance with the findings of Carlsson et al. [1975].

This study did not confirm previous findings that mutans streptococci are isolated more frequently as the child grows older [Alaluusua and Renkonen, 1983; Catalanotto et al., 1975; Fujiwara et al., 1991]. Perhaps this is because the dental plaque flora has established already by the age of 2. At that age in most of the children only the second deciduous molars still have to erupt and this change and subsequent developments may not have major effects on the oral microbiota. At the age of 5 years the numbers of mutans streptococci were comparable with those in other 5-year-old children [Alaluusua et al., 1989].

The fluctuations of the numbers of mutans streptococci and lactobacilli in the samples may be due partly to problems with the sampling technique. However, these fluctuations may well reflect normal shifts in population size of these organisms. A study using the tongue depressor for saliva sampling in 2- to 6-year-old children demonstrated variations in the numbers of mutans streptococci isolated during the day [Weinberger and Wright, 1990]. In that study, significant differences (p < 0.01) were found between mid-morning and after lunch, and between before and after lunch. A false negative result was found in 5% of the samples. In the present study the effect of fluctuations during the day will have been limited as all the samples were collected in the morning.

The larger fluctuations of mutans streptococci in plaque than in saliva samples may partially have been the result of differences in the amount of plaque collected. To reduce the negative effects of storage, the samples were always kept in a refrigerator and processed within 5 h. It should be realized that large variations in the size of populations of plaque bacteria are often found in longitudinal analyses of dental plaque [Bowden et al., 1975; Köhler et al., 1981]. The present results are in agreement with findings by Marsh et al. [1978, quoted by König, 1982], who collected 10 approximal plaque samples during a 3.5-year period in a group of...
64 schoolchildren. They found stable increases in the viable counts of mutans streptococci in only 22% of the children. Evidently, shifts in the numbers of mutans streptococci and lactobacilli occur, resulting in numbers below the detection level. In this study the variation in the bacterial counts cannot be explained by differences in the frequency of sugar intake. Small differences in the diet will not affect the counts of mutans streptococci and lactobacilli in children [Carlsson, 1989].

With increasing age more carious tooth surfaces were found. The caries experience of the children was low in comparison to other studies performed in The Netherlands during the same period [Frencken et al., 1990; Truin et al., 1991]. In the study of Truin et al. [1991] 40% of the 5-year-old children showed lesions progressing into the dentine.

High correlations between mutans streptococci in parents and children have been reported in various studies [Berkowitz and Jordan, 1975; Bergowizt et al., 1980; Rogers, 1981]. In our study, the correlations between mutans streptococci in the parent and the child were very low and often not statistically significant at \( p < 0.05 \). Our results are more in accordance with those of van Houte et al. [1981], who studied the relationship between levels of colonization by mutans streptococci in the saliva of 5- to 8-year-old children and their parents. In that study no positive association was found between the levels of mutans streptococci in parents and children have been reported in various studies [Berkowitz and Jordan, 1975; Bergowizt et al., 1980; Rogers, 1981].

The positive correlations between the numbers of lactobacilli and mutans streptococci in saliva, and the caries development in this study are even higher than those reported in several other studies [Klock and Krasse, 1977; Crossner, 1981; Zickert et al., 1983; Newbrun et al., 1984]. The correlations between the dietary scores (according to the 24-hour recall method) and the numbers of mutans streptococci in plaque and saliva were low and not statistically significant in most of the age groups. Although the levels of lactobacilli are thought to be strongly associated with the number of sugar-containing food intakes [Karjalainen et al., 1987] this could not be demonstrated in the present study. The results were similar to the correlations between the numbers of mutans streptococci and the number of dietary intakes. In a study by Carlsson [1989] of adults, no differences were found in the composition of the oral microflora between groups with a cariogenic and a non-cariogenic diet. It is suggested here that although the numbers of mutans streptococci and lactobacilli increase with the frequency of sugar intake, this only applies to low sugar intake diets. In other words, saturation levels of these organisms may be attained at relatively low frequencies of sugar intake.

While the composition of the oral microflora in the mother had a limited effect on the oral microflora of the child, the level of education of the mother showed a significant correlation with the numbers of mutans streptococci and lactobacilli in the child. This may result from a lower dental awareness associated with a lower level of education, thus subjecting the child to more cariogenic conditions [de Vries and Ruiken, 1987].

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