Dental caries and caries-associated microorganisms in Uruguayan preschool children

Marina Angulo, Beatriz Cabanas, Nelly Camporeale and Claes-Göran Emilson
Department of Microbiology, Faculty of Odontology, Montevideo, Uruguay and Department of Cariology, Faculty of Odontology, Göteborg, Sweden

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The prevalence of dental caries was studied in 3–5-year-old Uruguayan children ($n = 76$) living in 2 areas with different socioeconomic and cultural conditions. More children from the low socioeconomic area of Las Acacias had caries (68%) than children from the middle- to high-class neighborhood of Pocitos (19%). They also had poorer oral hygiene and a significantly higher caries prevalence ($P < 0.05$) than those from Pocitos. The occurrence of mutans streptococci and lactobacilli was determined in whole unstimulated saliva and compared with that in debris collected with a loop from the dorsum of the tongue. Mutans streptococci were detected in 42% of the children with significant correlations between the salivary levels of the microorganism and caries experience. Lactobacilli were recovered less frequently (18%). The detection of mutans streptococci in the tongue-loop samples was significantly correlated with that in whole saliva. □ *Cariogenic microorganisms; dental caries; preschool children*

Claes-Göran Emilson, Institute of Odontology, Box 450, SE-405 30 Göteborg, Sweden. Tel. +46 31 7733203, fax. +46 31 7733220, e-mail. emilson@odontologi.gu.se

In recent studies on dental caries in Uruguay it was demonstrated that 12–13-year-old children had moderate caries levels with a mean DMFT of 4.2; 51% of the children had high levels of mutans streptococci, the microorganisms most often associated with dental caries (1). The cariogenic microflora leading to dental caries is acquired early in life (2, 3) and the earlier these microorganisms are established the sooner dental caries may be diagnosed (4–6). No national information about caries-associated microorganisms and dental caries in Uruguayan preschool children is available.

A saliva sample is commonly used when evaluating cariogenic bacteria. Since mutans streptococci primarily colonize the teeth, their contribution to the salivary pool may reflect their presence in the dentition, and indications of a positive relationship have been reported in different studies (7, 8). However, it is not always easy to take a saliva sample, especially in the case of young children. A simplified procedure for estimating the prevalence of mutans streptococci in adult volunteers has been described and tested, i.e., by collecting a sample from the tongue with a loop (9). Since the method has shown a high correlation with the number of mutans streptococci in the saliva, and is simple to use, it is useful for collecting saliva also from young children who don't know how to spit.

The purpose of the present study was to determine the prevalence of dental caries in 3–5-year-old Uruguayan children and to study the relationship between caries-associated micro-organisms and dental caries. In order to evaluate a suitable method for determining the load of mutans streptococci and lactobacilli in young children we compared the tongue-loop method with standard microbiological plating of unstimulated saliva.

Materials and methods

Subjects

A total of 76 children from two preschool kindergartens in Montevideo, Uruguay were examined. One school was situated in Las Acacias, a low socioeconomic and cultural area and the other in Pocitos, a middle to high socioeconomic and cultural area. The children were 3–5 years old, with 55 of them (72%) in the age range 37–48 months. The children from Pocitos were slightly older (mean 3 months) than those from Las Acacias.

Family data

The parents answered questions concerning the family's socioeconomic and cultural conditions and the dietary habits of their children. Socioeconomic status was evaluated by questions about the educational level of the parents, their occupation and type of housing conditions. The answers were recorded on a scale of 1–4 with 1 denoting low level and 4 high level. The result was a mean value for the family.

Clinical data

The clinical examination was carried out using plane mirrors and new probes under artificial light. Oral hygiene was expressed as the gingival status and scored 03 if there was general gingivitis with bleeding on probing, 02 if there was gingivitis but without bleeding on probing, and 01 if the gingiva was practically free from clinical signs of inflammation (10). Dental caries was recorded using the

Table 1. Oral hygiene and prevalence of caries in 3–5-year-old children

		Gingival status score			Caries-free		Children with caries		
Area	No. of children	01	02	03	No.	(%)	No.	(%)	Mean dmft
Las Acacias	22	0	12	10	7	(32)	15	(68)	6.7 ± 3.6
Pocitos	54	47	7	0	44	(81)	10	(19)	4.1 ± 3.1*
Total	76	47	19	10	51	(67)	25	(33)	5.7 ± 3.6

* $P < 0.05$ (*t*-test).

criteria issued by the World Health Organization (11). Radiographs were not taken.

Sampling

Samples of unstimulated saliva were collected by expectoration into a sterile bottle after the child had been instructed by the dentist how to perform the sampling. A second sample was then immediately taken from 62 of the children from the dorsum of the tongue using a 10-μL Difco loop. Debris was collected by drawing the loop down the center of the anterior two-thirds of the tongue as described by Beighton (9). The filled loop was dislodged into 1 ml 0.05 M phosphate buffer. All samples were transported in a cooler to the Department of Microbiology, University of Montevideo and processed within 3 h.

Salivary processing

Since it is difficult for some children to expectorate enough unstimulated saliva to process both microorganisms, mutans streptococci were preferred because of their importance in the early establishment in the mouth. The lactobacilli were thus enumerated in only 66 children. The samples were dispersed on a Whirlimixer for 1 min and diluted in 10-fold steps in 0.05 M phosphate buffer (pH 7.3). Portions of 0.1 ml of appropriate dilutions were plated onto Mitis Salivarius Bacitracin (MSB) agar (12) for enumeration of mutans streptococci and Rogosa agar for lactobacilli. The plates were incubated for 48 h at 37°C in 10% CO_2 and left for 24 h at room atmosphere and temperature. Counts of colonies with a morphology characteristic of mutans streptococci were made on MSB agar (13) and of lactobacilli on Rogosa agar and the number of colony-forming units per milliliter (CFU/ml) saliva calculated. Representative colonies of mutans streptococci were subcultured onto Trypticase Soy Agar (Difco) supplemented with 5% (v/v) horse blood and grown at 37°C in 95% N_2 and 5% CO_2 for 24 h at 37°C and identified as *Streptococcus mutans* and *Streptococcus sobrinus* using a set of biochemical and physiological tests (14, 15).

Statistical analysis

Levels of mutans streptococci and lactobacilli were treated as a categorical variable (presence or absence) and also as a continuous variable. When treated as continuous, the levels were transformed to $\log_{10}$ CFU/ml of saliva. Comparisons of dmft scores were done using the Kruskal-Wallis test. Regression analysis was performed on comparing dmft in relation to mutans streptococci levels. Also Pearson's correlation test was used to measure the association between the plating method versus the loop technique in enumeration of mutans streptococci.

Results

The answers to the questions concerning family socioeconomic and cultural conditions indicated that in Las Acacias 16 families had low conditions and 6 low to middle; in Pocitos, 9 families had low to middle conditions, 9 middle to high, and 36 high conditions. In both areas the dietary sugar intake could not be studied because of difficulties obtaining true data from the parents. Sometimes the parents did not answer the questions or said that they did not remember or know what the children ate.

Oral hygiene and prevalence of caries in Las Acacias and Pocitos are given in Table 1. The number of children with a gingiva practically free from clinical signs of inflammation was 0 in Las Acacias and 47 (87%) in Pocitos, while the number of children with gingivitis was 22 (100%) in Las Acacias and 7 (13%) in Pocitos. The number of caries-free subjects was 51 (67%), with 7 (32%) belonging to the low-class neighborhood of Las Acacias and 44 (81%) to the middle-high class neighborhood of Pocitos. In the 25 children with caries the mean dmft was 5.7 (range 1–16) with a significantly higher prevalence in Las Acacias (6.7) than in Pocitos (4.1) ($P < 0.05$).

Relationship between bacterial salivary counts and caries prevalence

Table 2 gives the prevalence of mutans streptococci and lactobacilli in whole unstimulated saliva and their relationship to dental caries. Mutans streptococci were detected in 32 children (42%). Six children (8%) had 10^5 CFU of mutans streptococci or more. All carriers harbored *S. mutans* and one of them also *S. sobrinus*. Children with low levels of mutans streptococci had a lesser mean dmft score than children with high levels of the microorganism. The

Table 2. Relationship between whole salivary counts of mutans streptococci and lactobacilli and dental caries (dmft)

No. of CFU/ml	*n* (%)	dmft mean ± SD	No. (%) of children with caries
Mutans streptococci			
ND	44 (58)	0.5 ± 1.3[a]	8 (18)
$<10^5$	26 (34)	1.6 ± 4.6	12 (46)
$>10^5$	6 (8)	4.8 ± 3.1	5 (83)
	76		
Lactobacilli			
ND	54 (82)	1.6 ± 3.3	15 (27)
$<10^4$	6 (9)	2.2 ± 2.2	4 (66)
$>10^4$	6 (9)	5.0 ± 4.4	4 (66)
	66		

ND = not detected.
[a] Differences between the three ms groups are statistically significant, $P < 0.01$ (Kruskal-Wallis test).

difference in caries prevalence between children with not detectable levels and low and high levels of mutans streptococci was statistically significant ($P < 0.01$). Also for lactobacilli the children with no lactobacilli showed lower dmft values than the 12 children harboring the organism. However, because of the low number of children with lactobacilli no significant differences were found between different levels of the organism and the caries prevalence.

Comparison of mutans streptococci in whole saliva and in tongue-loop samples

Samples of whole saliva collected by expectoration yielded higher levels of mutans streptococci than the tongue-loop samples ($P < 0.01$, Table 3). There was a significant correlation between the counts of mutans streptococci in whole saliva and in salivary samples from the tongue ($r = 0.715$, $P < 0.003$). There was also a highly significant correlation in detection frequencies between the 2 methods ($P < 0.001$) with an agreement of 76% (Table 4). Although the tongue-loop method identified mutans streptococci in 5 samples where whole saliva was not positive and missed 10 of 23 samples where positive results were found in whole saliva, these differences were not significant.

Discussion

The present study demonstrates striking differences in the prevalence of caries among preschool children from two areas with different socioeconomic and cultural backgrounds. Since the caries process is multifactorial it was important to determine the impact of some caries-promoting factors in explaining this difference.

Table 3. Comparison of the number of mutans streptococci ($\log_{10}$ CFU/ml) in whole saliva and tongue-loop samples

Sample	Mean ± SD
Whole saliva	4.1* ± 1.0
Tongue-loop	3.5 ± 0.7

* Significantly different (paired *t*-test, $P < 0.01$).

Table 4. Comparison of detection frequency of mutans streptococci in 62 pairs of whole saliva and tongue-loop samples

	Tongue-loop technique		
Whole saliva	Yes	No	Total
Yes	13	10	23
No	5	34	39
Total	18	44	62

$\chi^2 = 13.41$, $P < 0.001$, Pearson's correlation test.
Agreement = $(13 + 34)/62 \times 100 = 76\%$.

The level of oral hygiene was much poorer among the low socioeconomic children in Las Acacias than in Pocitos, as reflected in a greater frequency of gingival inflammation. As poor oral hygiene has been shown to be an important causal factor for caries in very young children (16, 17) this may be one factor explaining the differences in the prevalence of caries between the two areas. Furthermore, there is some indication that children in low socioeconomic status groups have a lower toothbrushing frequency than children of higher status groups (18). This may be of importance since 4-year-old children who had their teeth brushed regularly showed significantly lower mean values for dmfs than children who did not have a regular toothbrushing routine (19).

It was not possible to get reliable information about the dietary sugar intake of the children from the two areas. However, it is widely believed that consumption of sweets is one of the most important caries-related factors (20) and this in combination with poor oral hygiene is particularly harmful (21).

Of the 3–5-year-old children, 67% were caries-free. This proportion is of the same magnitude as in some recent studies in which 59–66% of 3–4-year-old children were without caries (22–24). The distribution of caries-free children was distinctly different between the two studied areas, however, as a larger proportion of caries-free children was found in Pocitos with its higher socioeconomic status than in Las Acacias. This difference was also apparent in the children with caries, with a significantly higher prevalence of caries being noted in Las Acacias than in Pocitos. The observed relationship between caries and socioeconomic status is in line with earlier observations (1, 18, 25) and indicates that the socioeconomic status of the family plays an important role in the development of caries. The higher prevalence of caries in Las Acacias may also be a useful predictor of future caries, as recent studies have shown that children who develop caries at an early age are likely to have more caries both in the primary dentition (22, 26) and in their permanent teeth (27). Past caries experience, which in

most caries models has been the strongest predictor of future caries (28), can thus be used in identifying young children with the greatest need of preventive care.

Mutans streptococci were detected in 42% of the children. Similar prevalences have been presented in this age group where unstimulated saliva has been collected (24, 29). In other studies a much higher prevalence of 3–4-year-old children with mutans streptococci has been observed (23, 30, 31), which may be explained by differences not only in caries prevalence but also in various sampling techniques.

There was a significant positive association between levels of mutans streptococci and prevalence of caries. This is in accordance with many studies showing an association between the colonization level of mutans streptococci and caries experience in the primary dentition (23, 24, 29–32). It is interesting to note that in very young children without a long dental history, mutans streptococci have been shown in many studies to be, in addition to socio-demographic factors and past caries experience, the most successful variable in predicting caries development (28). Knowledge of the levels of caries-associated bacteria can thus be used clinically in identified caries risk children for selective measures such as checking the patient's compliance in following dietary recommendations.

The low frequency of isolation of lactobacilli in the present study is in accord with the low frequency reported previously for preschool children (24, 29, 31). Although no significant differences were noted between children with and without lactobacilli, those from whom no lactobacilli were detected showed the lowest caries score.

The yield of mutans streptococci in the tongue-loop samples was significantly correlated with that in unstimulated whole saliva, an observation which is in line with what has been shown by Beighton (9). In contrast to that study the salivary level of mutans streptococci was higher than that obtained by the tongue-loop method. The reason could be the fact that the loop available in Uruguay was a traditional bacteriological loop and not the one chosen by Beighton (9), whose use of a loop with an angled rather than a curved edge may have facilitated the collection of debris from the tongue. However, this study has clearly shown that the simple tongue-loop method was suitable for use in preschool children in estimating the prevalence of mutans streptococci.

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