EARLY ACQUISITION OF *STREPTOCOCCUS MUTANS* FOR CHILDREN

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**ABSTRACT**
Existing evidence reveals that in Early Oral Infection the main route of transmission of *Streptococcus mutans* is mother-child saliva contacts and that initial acquisition takes place during a specific period of time denominated “Window of Infectivity” that lapses between 6 and 30 months of the child’s life, with a higher risk between 18 and 30 months of age.

The aim of the present study was to analyze Early Oral Infection. The levels of *Streptococcus* mutants in saliva and bacterial plaque in the binomial mother-child 6 to 18 months after childbirth were evaluated.

Twenty-four mother-child binomials that attended the University Hospital of Maternity and Neonatology of Córdoba participated in the study. Samples of saliva and dental plaque were taken from mother and child and seeded in selective Agar Mitis Salivarius Bacitracin medium (0.28 mg/ml) and cultured for 48 hours at 37°C and in 5% CO₂ to allow for the growth of *Streptococcus* mutants. The colonies were identified morphologically and biochemically.

At 6 months after childbirth: 58.33% of the samples of the mother’s saliva were positive, while the presence of the microorganism was not detected in 100% of the samples of the infants’ saliva.

At 18 months of after childbirth: 79.16% of the samples of saliva and 100% of the samples of the mothers’ dental plaque were positive, whereas the samples of saliva and dental plaque of the infants were positive for *Streptococcus* mutants in 20.83% and 70.83% of the cases respectively.

The initial acquisition of *Streptococcus* mutants would have occurred in 20.8% of the children at the age of 18 months. Eruption of primary molars occurs at this age.

**Key words:** *Streptococcus* mutants, binomial mother-child, transmission, acquisition.

**INTRODUCTION**
Birth is the first environmental event that conditions the allogeneic succession in the oral cavity. In the uterus, the fetus is free from microorganisms and up to 8 hours after birth the mouth remains sterile. Oral colonization begins when the baby passes through the birth canal and by interaction with the outside world. During the first stages of colonization it is possible to detect staphylococci, enterobacteria, neisseria, yeast, and *Streptococcus*
in particular of the viridans group, and more specifically *Streptococcus mitis* and *salivarius* that mainly colonize oral mucosa. The growth and development of the oral microbiota of infants follows the pattern of ecological succession described for other ecosystems. Tooth eruption triggers significant ecological changes in the oral cavity. This physiological event creates appropriate conditions for the development of new species of microorganisms that must adhere to hard surfaces to colonize. Among these microorganisms, the presence of Streptococci of the mutans group, i.e. *Streptococcus mutans* (SM) and *Streptococcus sobrinus* (SS), is necessary but not sufficient for the development of dental caries (1, 2).

In recent years several studies have been performed to determine “from whom”, “when” and “how” initial acquisition of *Streptococcus mutans* occurs. Regarding early childhood caries, Berkowitz (3) supports the notion that scientific evidence has demonstrated that it is an infectious and transmissible disease, characterized by a “three phase model”. The first phase involves infection by mutans streptococci, the second consists of an increase in the population in the biofilm as a result of prolonged exposure of the oral cavity to a cariogenic substrate, and the third corresponds to rapid demineralization and cavitation of tooth enamel.

Slavkin (4) describes the source of infection and mode of transmission as an interesting paradox. The mother can transmit infectious microorganisms to the infant by intimate contact and can also transmit the antibodies that protect the infant from different microorganisms by breast-feeding. This author also states that the mother’s oral health status might be a health risk factor for the fetus or the newborn.

Similarly, Caufield, Cutter and Dasanayake (5) suggested a direct association between the levels of mutans streptococci in mothers and their children and pointed out that the prevalence of SM acquisition increases with the child’s age and with the number of erupted teeth.

The existing evidence suggests that the main SM transmission route in Early Oral Infection is the saliva contacts between mother and child and that initial acquisition occurs during a specific period of time termed “Window of Infectivity” that coincides with tooth eruption and spans the 6-30 month old age period, with a higher risk between 18 and 30 months of age (5, 6, 7). Mohan et al. (8) found that 20% of children under the age of 14 months were infected with SM.

The present study involved the evaluation of the levels of SM and SS in the binomial mother-child at 6 and 18 months after childbirth.

**MATERIALS AND METHODS**

**Population and study design**

The population under study was a group of pregnant women in their last trimester of gestation that attended the University Hospital of Maternity and Neonatology of Córdoba (9, 10). The concentration of fluoride in the drinking water in the city of Córdoba ranges, according to the neighborhood, from 0.6 to 1.0 ppm.

The study group was comprised of 24 mother-child binomials. Saliva samples were taken at 6 months after childbirth and saliva and dental plaque samples were taken at 18 months after childbirth. Informed consent was obtained from all the participating mothers (11).

**Clinical odontological examination**

The infants were examined clinically at 6 and 18 months of age in their homes under good natural light. The number of erupted teeth was recorded and the mother was asked to describe the child’s history of tooth eruption (12).

**Study of saliva and dental plaque samples of the mother-child binomial**

**Sample obtention**

Samples of non-stimulated saliva of the mother-child binomial 6 and 18 months after childbirth were taken in the morning, 30 minutes after the last meal. The mother’s sample was collected by direct salivation for 5 min in a sterile, calibrated container. The infant’s sample was collected from the floor of the mouth with a pipette.

The saliva samples were stored at -20ºC until processing.

The dental plaque samples of mother and child were collected 18 months after childbirth from the vestibular, occlusal, lingual or palatine dental surfaces with sterile cotton swabs.

The dental plaque samples were placed in Brain Heart Broth and stored in the fridge until processing.

**Isolation and Typification of *Streptococcus mutans* from the mother-child binomial**

The saliva and dental plaque samples of the mother-child binomials were seeded in Agar Mitis.
Salivarius (DIFCO) medium additioned with 0.281 mg/mL of Bacitracin (ICN Biomedicals, 71% activity) and incubated at 37ºC under microaerophilic conditions. The morphological analysis of the colonies was performed by Gram staining and light microscopy analysis. Typification was performed by biochemical assays employing the commercial kit Api 20 Strep (bioMérieux) in keeping with the instructions of the manufacturer. The kit is ideally suited to distinguish between most of the Streptococci relevant to medical bacteriology and distinguishes between SM and SS (13, 14).

The levels of CFU/mL in saliva and plaque were classified as high and low risk according to the criteria of Jensen and Bratthall (15) and Bordoni (16).

RESULTS
Clinical odontological examination
The families were visited at their homes 6 and 18 months after childbirth and the mother was asked to provide information on the child’s history of tooth eruption. The mean age of eruption of the lower incisors was 8 months (±2 months).

Analysis of saliva and dental plaque in the mother-child binomials
Six months after childbirth 58.33% of the mothers’ saliva samples were positive for SM. Of these samples, 45.83% had ≥10⁵ CFU/mL SM (high risk level) and 12.5% had <10⁵ CFU/mL SM (low risk level). The presence of the microorganism was not detected in 100% of the saliva samples of the infants (Figure 1). At 18 months after childbirth 79.16% of the saliva samples of the mothers were positive for SM. 54.16% of the samples had high risk counts and 25% had low risk counts.

100% of the dental plaque samples of the mothers tested positive. 37.5% of the samples had -20,000 CFU/mL (low risk level) and 62.5% had +20,000 CFU/mL (high risk level); 54.16% were positive for SM and 8.33% were positive for SS.

The saliva samples of the infants at 18 months of age were positive for SM in 20.83% of the cases. 12.5% of the samples had ≥10⁵ CFU/mL. The dental plaque samples tested positive with +20,000 CFU/mL in 70.83% of the cases. 50% were positive for SM and 8.33% were positive for SS (Figure 1).

Figure 2 shows that at 6 months after childbirth the saliva samples of all the infants corresponding to SM positive mothers tested negative.

DISCUSSION
The acquisition of microorganisms by the body can occur by direct transmission from a host or indirectly via another living organism. Inanimate objects, food and water can also transmit pathogens (17).

Saliva is considered the major vehicle of transmission of SM by physical contact (18, 19). The mother is considered the main source of infection for the child. An association between the time at which most children acquire SM in their oral flora and primary dental eruption at 8 months to 3 years of age has been described (5).
Fig. 2. Streptococcus mutans in saliva of the binomials at 6 months after childbirth.

Fig. 3. Streptococcus mutans in saliva of the binomials at 18 months after childbirth.

Fig. 4: Streptococcus mutans in dental plaque of the binomials at 18 months after childbirth.
The present study revealed that eruption of the first tooth took place approximately at 8 months (± 2 months) of age in keeping with Kreiborg (20). Initial colonization by SM occurred at 18 months of age in 20.8% of the infants.

The primary habitat of SM and SS is human dentition. Once these microorganisms have colonized dentition they are found in saliva, the tongue, oral mucous membranes, etc. (21).

According to Costerton and Lewandowski (22), the biofilm establishes a dynamic link between the dental surfaces and the different components in the saliva and the microorganisms in the oral flora that live in symbiosis and antibiosis.

The prevalence of SM infection in the binomials was markedly higher than SS prevalence (97.66% and 8.33% respectively). A noteworthy finding was the absence of SS in the saliva of the binomials at 6 and 18 months after childbirth whereas SS was isolated from dental plaque of the binomials at 18 months after childbirth. Lindquist (23) reported differences in the dentition colonization pattern between SM and SS, i.e. SS is more frequently isolated from the posterior surface of teeth. When both SM and SS are present they colonize oral surfaces equally but SM is prevalent on all other surfaces.

Within the context of initial acquisition, Masuda and Berkowitz (24, 25) detected SM colonies in 40% of the children at 24 months of age and in 29% of the children at 8 to 18 months of age.

The present study of 24 mother-child binomials from 6 to 18 months after childbirth revealed that initial acquisition would have occurred in 20.8% of the infants at 18 months of age. These data are in keeping with Caufield (5) who studied 46 mother-child binomials from birth to 5 years after childbirth and reported that initial acquisition of SM occurred at 19 months of age in 25% of the infants and at 31 months in 75% of the infants.

Similarly, Karn et al. (26) also demonstrated the presence of SM at a very early age, i.e. in 25% of the infants before one year of age and in 60% of the infants at 15 months of age.

Milgrom et al. (27) evaluated 199 children from 6 to 36 months of age and detected the presence of SM in 25% of the infants before dental eruption and in 53% of the infants from 6 to 12 months of age. Early initial acquisition has become particularly relevant as a predictive marker of future caries activity in primary and permanent dentition.

In the 80’s, Köhler et al. (28) among others had already demonstrated that early detection of SM was associated to caries experience. Caufield, Cutter and Dasanayake (5) provided evidence that supports the association between the presence of mutans streptococci in mother and child’s saliva.

Our results suggest that the risk of infection would be more closely related to the CFU/mL of mutans streptococci present in the dental plaque of the mother than in her saliva.

In terms of the source of infection, our data showed that at 18 months after childbirth, 38.46% of the infants of mothers with high risk values of CFU/mL of SM exhibited SM positive saliva. The dental plaque of 66.66% of the infants of mothers with high risk dental plaque (+20,000 UFC/mL) and of 77.77% of the infants of mothers with low risk dental plaque (-20,000 UFC/mL) were positive for mutans streptococci. The high percentage of colonization by SM in infants of mothers with low risk plaque suggests the existence of other sources of infection within the family and/or the contribution of other factors involved in colonization such as family eating and oral hygiene habits.

Klemonskis et al. (29) analyzed biological risk factors, life style and social environment of mother-child binomials in two communities. Their results showed that 15 months after childbirth there were no statistically significant differences in the level of mutans streptococci infection between the mothers in both groups. However, statistically significant differences were found in the level of infection between the infants in both groups. The Window of Infectivity could be associated to family eating habits. The eating habits of the infants are associated to those of their mothers. Furthermore, early eating habits condition future patterns of eating behavior. According to Alaluusua (30), another requirement for early colonization is that the child should frequently eat refined carbohydrates.

Mohan et al. (8) reported that 20% of the children under 14 months of age exhibited SM infection and suggested that the risk of SM colonization is lower in infants who feed on milk rather than on sweetened beverages.

Although the influence of the mother’s level of infection in early colonization is important, other factors that would participate in initial colonization should also be considered.
CONCLUSION
Based on our results we may conclude that:
Initial colonization by mutans streptococci in the binomials under study occurred in 20.8% of the infants at 18 months of age, concomitantly with the age of temporary molar eruption.
The high incidence of infection by mutans streptococci in children of mothers with high and low risk dental plaque suggests the influence of other factors that favor early colonization.

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