

Determination of the acquisition of *Mutans Streptococci* in a group of Uruguayan children up to 36 months of age. Pilot study

Determinación de la adquisición del *Streptococcus grupo mutans* en un grupo de niños uruguayos de hasta 36 meses de edad. Estudio piloto.

Determinação da aquisição do *grupo Streptococcus Mutans* em um grupo de crianças uruguaias até 36 meses de idade. Estudo

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Resumen

Introducción: La boca del recién nacido se contamina inmediatamente con microorganismos maternos. Caufield determinó la colonización con *SGM* para niños americanos a los 26 meses de edad. Estudios latinoamericanos la ubican entre los 14,9-18 meses.

Objetivos: Identificar el momento de adquisición y colonización del *SGM* en una población de niños uruguayos y relacionarlo con factores del niño y maternos. Establecer el tiempo necesario para la recolección de la muestra y estudio de su pérdida.

Método: Estudio observacional, longitudinal prospectivo, de 83 pares madre-hijo de un policlínico de Montevideo. De 83 pares examinados, 20 completaron el estudio. Se incluyeron niños de 0-9 meses, con/sin dientes y primera toma salival negativa para *SGM*.

Resultados: Edad promedio de adquisición del *SGM* 16,4 meses ($\pm 4,13$) y colonización 18,6 meses ($\pm 3,80$) de edad. La pérdida de la muestra fue 71,42%.

Conclusiones: La colonización resultó similar a la encontrada por Florio (Brasil), observándose diferencias significativas con Caufield (EEUU).

Palabras clave: *Streptococcus mutans*, ventana de infectividad, flora bucal infantil, microbiología, recién nacido.

Abstract

Introduction: The newborn's mouth is immediately contaminated with maternal microorganisms at birth. Caufield determined that the average age of colonization with *Mutants streptococci (MS)* for American children was 26 months old. Latin American studies indicate that it occurs at an age between 14.9 and 18 months old.

Objectives: To identify the time of *MS* acquisition and colonization in a population of Uruguayan children and to relate it with various child

and maternal factors. To establish the time needed to collect the sample and its loss.

Methods: Observational, longitudinal prospective study, of 83 mother-child pairs from a medical center in Montevideo. From the 83 pairs examined, 20 completed the study. Children between 0-9 months old, with/without teeth, whose first saliva sample was negative for *MS*, were included.

Results: The mean age of *MS* acquisition was 16.4 months (± 4.13), and colonization occurred at 18.6 months (± 3.80) of age. The sample loss was 71.42%.

Conclusions: The colonization found was similar to that found by Florio (Brazil) but showed significant differences with Caufield's results (USA).

Keywords: *Streptococcus mutans*; *mutans streptococci*; *MS*; window of infectivity; infant oral flora; microbiology; newborn

Resumo

Introdução: A boca do recém-nascido é imediatamente contaminada por microrganismos maternos. Caufield determinou a colonização com *SGM* para crianças americanas aos 26 meses de idade. Estudos latino-americanos o situam entre 14,9-18 meses.

Objetivos: -Identificar o momento de aquisição e colonização do *SGM* em uma população de crianças uruguaias e relacioná-lo com fatores da criança e da mãe. Estabeleça o tempo necessário para coletar a amostra e estudar sua perda.

Método: Estudo observacional, longitudinal prospectivo, de 83 pares mãe-filho da uma policlínica de Montevideu. Dos 83 pares examinados, 20 completaram o estudo. Foram incluídas crianças de 0-9 meses com / sem dentes e primeira amostra salivar negativa para *SGM*.

Resultados: Idade média de aquisição do *SGM* 16,4 meses ($\pm 4,13$) e colonização 18,6 meses ($\pm 3,80$) de idade. A perda estabelecida da amostra foi 71,42%.

Conclusões: A colonização foi semelhante aos de Florio (Brasil), observando diferenças significativas com Caufield (EUA)

Palavras-chave: *Streptococcus Mutans*, janela de infectividade, flora oral infantil, microbiologia, recém-nascido.

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Introduction and background

The newborn's mouth is contaminated with microorganisms immediately after birth; these come mostly from the baby's mother. *Mutans streptococci* (*MS*) are known as initiators of the dental caries process, and evidence indicates that infant transmission is mainly vertical, from mother to child⁽¹⁾. Diet and lifestyle are a fundamental factor in the development⁽²⁾ of the disease, as they can modify oral flora levels⁽³⁾. Total sugar exposure during childhood was associated with initial *MS* acquisition in an Australian cohort of young children⁽⁴⁾, and it was found that its adhesion properties may be sensitive to sucrose concentration in the oral cavity⁽⁵⁾. Early consumption of sugary foods and beverages can have a significant dental impact on the establishment of future cariogenic dietary patterns or on the formation of cariogenic bacteria in the oral cavity⁽⁶⁻⁷⁾.

Early childhood caries (ECC), of multifactorial etiology, occurs in a specific patient, and the presence of *MS* seems to be key in its pathogenesis and in this age group, as it increases the risk⁽⁷⁻¹⁵⁾. Research shows that *MS* colonization may occur in edentulous children⁽¹⁶⁻¹⁷⁾.

Despite this, a period of greater⁽¹⁸⁾ susceptibility for acquisition is described as "infectivity window", which occurs after tooth eruption, especially after the eruption of the first deciduous molar.

According to reports from the United States Center for Disease Control and Prevention, dental caries is the most prevalent and common chronic disease in children under the age of three. ECC is five times more common than asthma and seven times more common than hay fever. The time of acquisition of cariogenic microorganisms is of particular importance for ECC, particularly *MS*.

Preventing or delaying *MS* colonization is essential to control dental caries^(4,20-21). Less privileged groups have a disease prevalence between two and five times higher⁽²²⁾.

The time of *MS* acquisition remains controversial, as it is the main microorganism associated with caries⁽²³⁾. Microbiological risk indicators may be present in the mouth even before tooth eruption. A predominantly cariogenic environment includes aciduric and acidogenic flora. Some of the caries-related factors are unique to this age group, such as newly acquired bacterial flora, frequent intakes, immaturity of the immune system and of the tooth enamel.

Saliva is the vehicle through which *MS* reach the child's oral cavity. In addition to *MS* in maternal saliva, the mother's caries record (DMF-T index), education and eating habits are strongly associated with *MS* colonization in the baby^(4,17,23-27).

However, the presence of *MS* alone is not sufficient to predict caries in children^(17,28-29). Three events are fundamental for ECC development: early *MS* acquisition, *MS* colonization until pathogenic levels are reached, and reduced enamel mineralization⁽²⁹⁻³⁰⁾.

Preschoolers with high *MS* levels showed a higher prevalence of caries and a higher risk of developing new lesions compared to

children with low *MS* levels^(20,25,31-32). Some studies agree that infants or preschoolers who acquire *MS* before the age of two have a more considerable caries experience in both dentitions compared to those who have later colonization⁽²⁰⁻²¹⁾. Those with high *MS* levels are five times more likely to develop caries than those with low *MS* levels.

Study objectives:

- To identify the time of *MS* acquisition and colonization in a population of Uruguayan children that attend a public health center, relating this to other factors such as:
 - in the child: sex and number of erupted teeth.
 - in the mother: *MS* presence in saliva, age and educational level.
- To determine the time required for sample collection and sample attrition in this longitudinal study.

Methodology

Ethical issues

This study was approved by the Ethics Committee of the School of Dentistry, Universidad de la República.

Design

Prospective, observational, longitudinal study.

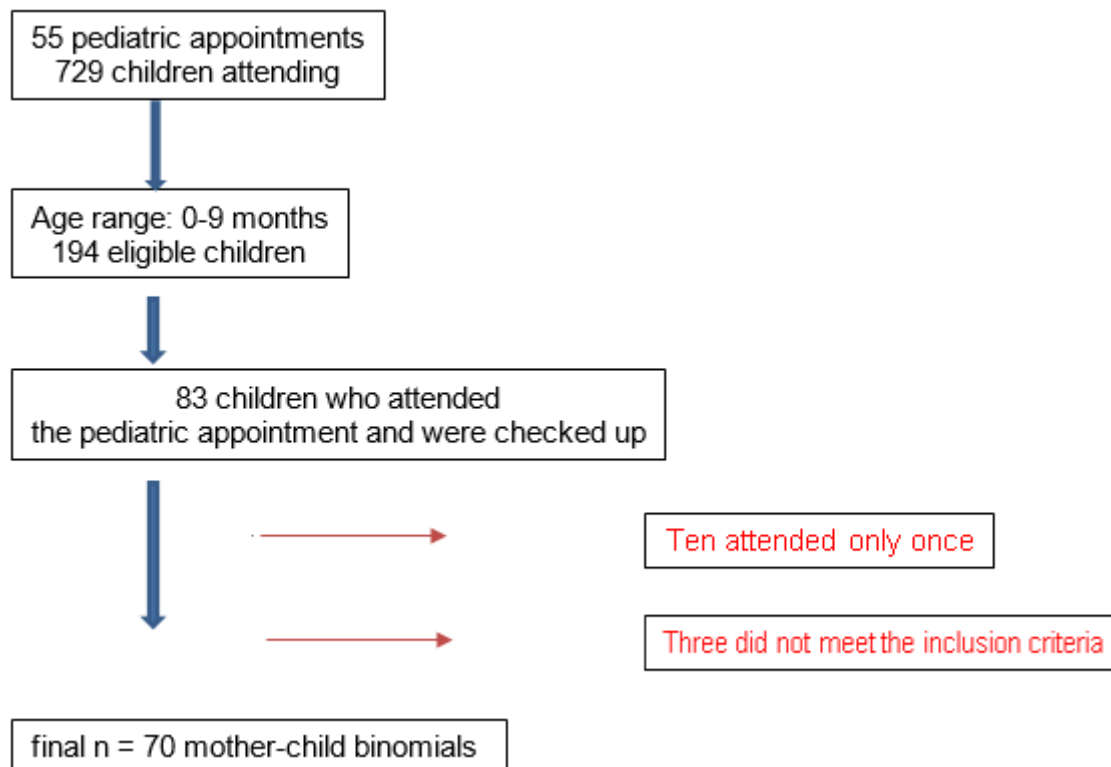
Eighty-three mother-child pairs were examined at the Piedras Blancas clinic Dr. Badano Repetto, while attending the clinic for their pediatric checkups. This clinic is part of the State Health Services Administration (ASSE, for its acronym in Spanish) of the Ministry of Public Health and is located on the outskirts of Montevideo, at 4145 Capitán Tula Street.

Sample selection

The selection of mother-child binomials was made for convenience reasons. Of all the children aged 0-9 months attending pediatric checkups on Wednesday and Friday mornings for 13 months (July 2011 - August 2012). Pediatric care records were reviewed from 29-Jul-2011 to 29-Aug-2012. In this period, 36 survey visits were conducted (twice a week in the morning). At these visits, there were

55 pediatric appointments including 729 children, but only 194 were within the age range for this study. Finally, the total number of patients reviewed was 83 mother-child binomials, who were the ones that actually attended the pediatric appointment in that period. Of these, three binomials did not meet the inclusion criteria and ten only attended once. The final sample included 70 pairs (Fig. 1).

Fig. 1: Sample selection flowchart



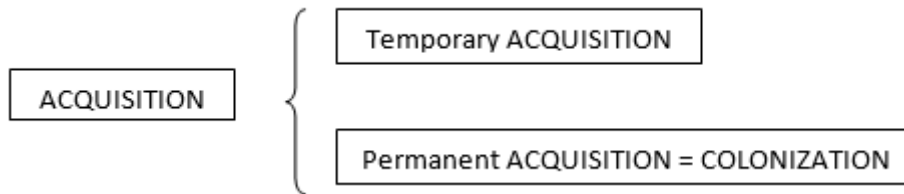
Overall healthy mothers and children took part in the study. The children included had the following characteristics: 0 to 9 months of age with or without teeth, whose first saliva sample tested negative for *MS*. Binomials in which either member had a systemic disease were excluded. In the case of medical treatment with antibiotics, saliva sampling was delayed at least ten days after the treatment was completed.

- Calibration and established criteria

The operators were two dentists specialized in Pediatric Dentistry, calibrated for the World Health Organisation (WHO) DFM-T caries index (decayed, filled, and missing teeth). The Kappa intra-operator test value obtained was 0.95 for operator 1 and 0.96 for operator 2. The resulting inter-operator Kappa value was 0.98. Criteria on the presence or absence of visible plaque, gingivitis, white spots and cavitations were agreed on. According to other studies, two

operational definitions were established: a) "acquisition": time when the presence of the microorganism in saliva is detected, and b) "patient with stable colonization" (permanent microorganism acquisition): after two consecutive *MS*-positive saliva samples⁽²⁶⁾ (Fig. 2).

Fig. 2: Operational definitions on *MS* contamination in salivary flora



Data collection

The following was done on mother-child binomials:

- mothers: medical history, structured survey and one-time collection of approximately 3 ml of unstimulated saliva in sterile bottles at the start of the study.
- children: medical history and saliva sample taken with plastic loop (Beighton technique) at each checkup, until *MS* was detected in two consecutive samples.

The structured survey recorded names, socio-demographic variables, mother and child eating habits, behaviors conducive to transmissibility, oral hygiene habits of the mother and child, among others.

The clinical examination was performed in a medical office with LED frontoluz lighting (located on the operator's forehead). The mother's DMF-T examination was performed visually, with a dental mirror and a sickle probe. Unstimulated saliva was collected once in a sterile bottle.

The child's clinical examination was performed visually, with the infant on the mother's lap. The infant's saliva was sampled with a sterile plastic loop at each appointment.

Saliva samples were identified with the participant's ID number, stored in ice, transferred and processed no later than four hours after collection.

The infant's saliva was processed by placing it in 1 ml of sterile saline solution; 0.1 ml was taken and expanded with a glass rod in Gold medium (culture medium selective for *MS*). Three dilutions were made from the mother's saliva sample, placing 9 ml of buffer solution in each tube and diluting 1 ml in the first tube, from which 1 ml was transferred to the second tube and from there 1 ml to the third tube.

From the last two dilutions, 0.1 ml of each was streaked in Gold medium.

Plates were incubated for 48 hours in the oven at 37°C under anaerobic conditions; they were left at room temperature for better visualization of macroscopic morphology. Colony-forming units (CFU) were read and counted and biochemical tests were performed for identification purposes.

The fieldwork lasted 29 months in total.

Results

Population characteristics

The population studied was at high risk for *MS*, considering their socioeconomic and cultural level, high *MS* count and past experience of maternal caries, mother and child's eating and oral hygiene habits.

Time of *MS* acquisition and colonization

The average age of acquisition in this group was 16.4 months (± 4.13).

Of the 20 children who completed the study, 4 had temporary acquisition, at an average age of 10.6 months (± 1.11).

The average age of colonization was 18.6 months (± 3.80). Seventy-five per cent of colonized children had molars and 25% erupted incisors (Table 1).

Table 1: Type of *MS* contamination (acquisition/colonization) correlated with age and erupted teeth

RESULTS						
Type of <i>MS</i> contamination	Average age (months)	SD	Range (months)	Average number of teeth	SD	Range (number of teeth)
ACQUISITION	16.4	4.13	8.8 - 21.8	9.9	3.95	2 - 16
COLONIZATION	18.6	3.80	11.6 - 25.7	12.05	4.56	2 - 20

The children contaminated with *MS* had a minimum count of 1×10^2 CFU and a maximum count of 2.5×10^4 , with an average of 2×10^3 .

Although on average girls were colonized earlier than boys, this difference was not found to be significant applying the Cox model (p -value = 0.61).

Maternal factors

All the mothers were colonized with *MS*, with an average value of 1.94×10^6 CFU, with a range between 1×10^3 and 3.59×10^6 CFU.

The mother's average age was $23.8 (\pm 6.5)$.

Mothers had completed $8.2 (\pm 1.9)$ years of education (basic cycle incomplete) on average.

Survival data models were used to develop charts 1 and 2, which show no statistically significant differences between age (p-value = 0.65) and maternal education (p-value = 0.21), and the child's colonization age.

Chart 1: Relationship between mother's age and infant's colonization age

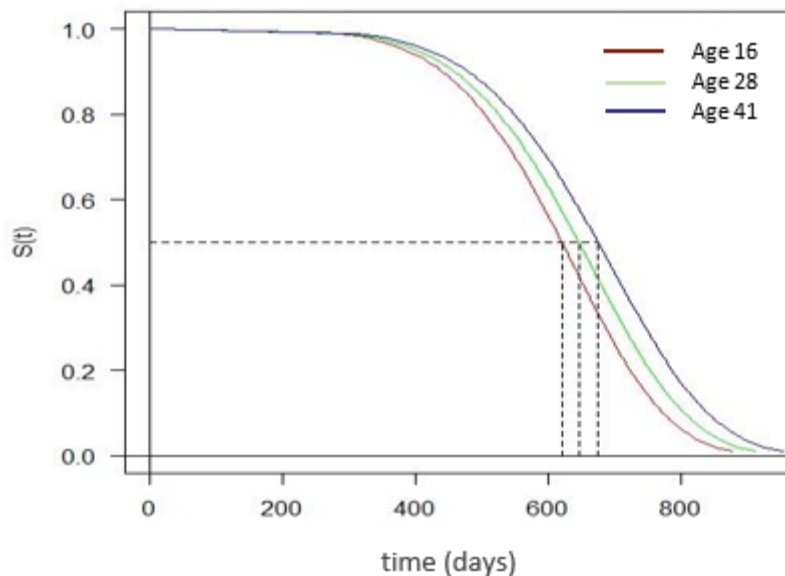
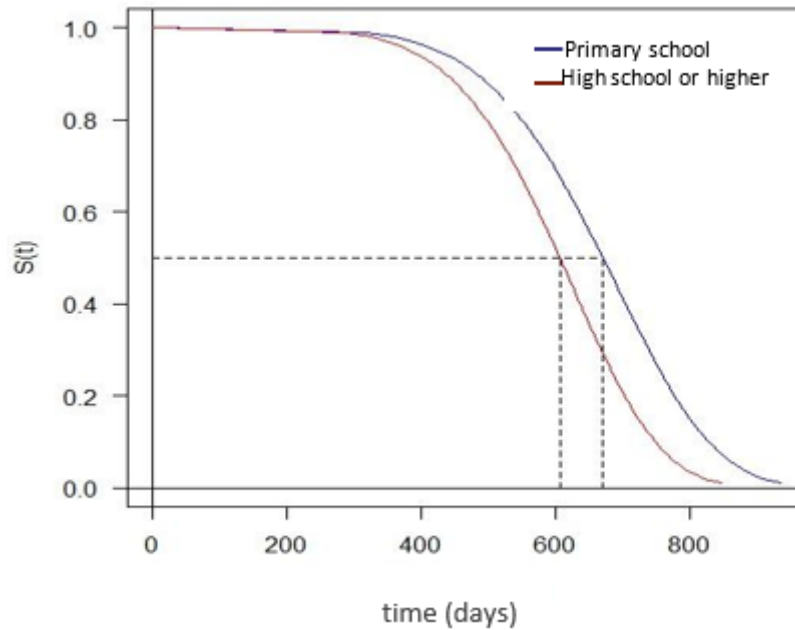


Chart 2: Relationship between mother's education and infant's colonization age



Sample attrition

Eighty-three mother-child binomials were invited to participate in the study; 10 did not agree to participate and 3 were excluded for not meeting the inclusion criteria (Table 2). In the end, 70 mother-child pairs participated in the study. The highest attrition occurred between the 2nd and 5th sample collection, when 64.27% of participants left ($n=45$). Total attrition over the 29 months of the study was 71.42% ($n=50$) (Table 3).

Of the total number of mother-child pairs who withdrew from the study, 88.9% ($n= 48$) did so for undetermined reasons and 11.1% ($n= 6$) because the family had moved or changed health care providers.

Table 2: Binomials not included in the study during the sampling process

Mother-child binomials excluded from the study	
Do not meet the inclusion criteria	4.28 % ($n=3$)
Do not agree to participate	14.29 % ($n=10$)
Total	18.57 % ($n=13$)

Table 3: Sample attrition during the study period, classified according to gender

Description of sample attrition during study							
	Attrition in 1st to 2nd collection	Attrition in 2nd to 3rd collection	Attrition in 3rd to 4th collection	Attrition in 4th to 5th collection	Attrition in 5th to 6th collection	Attrition in 6th to 7th collection	Total attrition
Females	18.57 % (n=13)	12.86 % (n=9)	4.28 % (n=3)	5.71 % (n=4)	1.43 % (n=1)	2.86 % (n=2)	45.71 % (n=32)
Males	4.28 % (n=3)	11.43 % (n=8)	4.28 % (n=3)	2.86 % (n=2)	2.86 % (n=2)	0 % (n=0)	25.71 % (n=18)
Total	22.85 % (n=16)	24.29 % (n=17)	8.56 % (n=6)	8.57 % (n=6)	4.29 % (n=3)	2.86 % (n=2)	71.42 % (n=50)

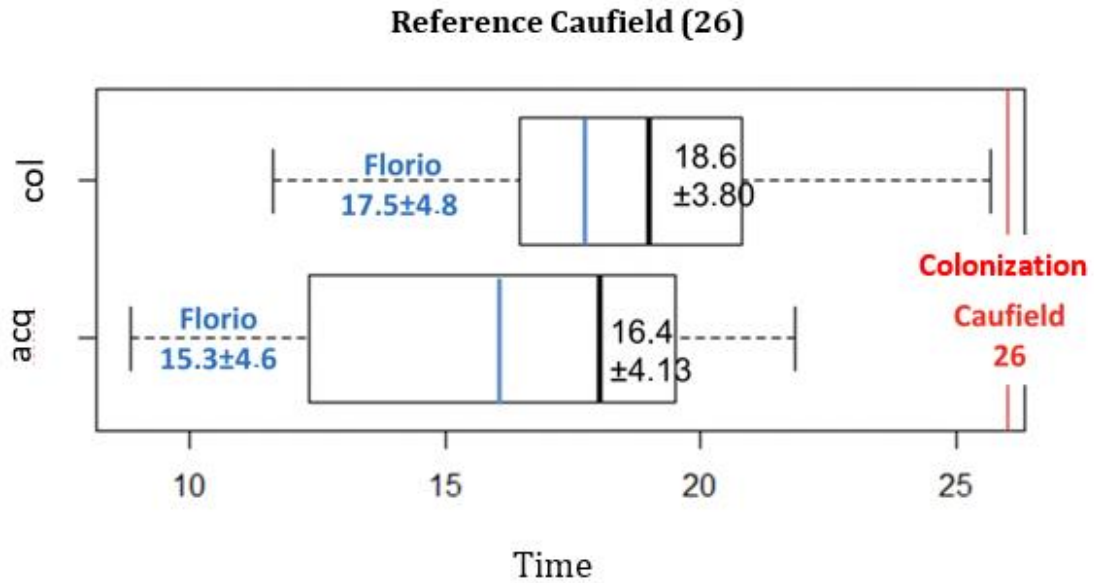
Discussion

In young children, the early colonization of dental plaque by *MS* is important, as well as its relationship with the increased risk of developing early childhood⁽³³⁾ caries. Several countries have studies that relate this early colonization to different factors such as socioeconomic and cultural level, eating habits, sex and age, among others.

In Uruguay, there are no epidemiological data in this age group, possibly because of how difficult it is to access this population, its mobility patterns, the length of the study and the high costs. Additionally, research in these groups requires specialized operators. Caufield et al. (USA)⁽¹⁸⁾ set the "window of infectivity" for *MS* at an average age of 26 months, and Florio et al.⁽²⁶⁾ (Brazil) at 17.5 months (± 4.8). This study finds that colonization occurs at 18.6 (± 3.80) months of age, results that are closer to the values found by Florio et al. Likewise, Florio et al. found a mean acquisition age of 15.3 (± 4.6), and the value in this study was 16.4 (± 4.13).

In brief, the values found in this study in terms of age of acquisition and colonization were similar to those of Florio et al. from Brazil (p -value = 0.221 and 0.209 respectively), while a statistically significant difference was observed regarding the findings of Caufield et al. in the United States (p -value < 0.001) according to the Wilcoxon rank test (Chart 3).

Chart 3: Comparison of the distribution of time of *MS* colonization and acquisition - Caufield et al., Florio et al. and Caviglia-García



Caufield et al.	N= 46
Florio et al.	N= 26
Caviglia – García	N= 20

The results according to sex observed in this study are consistent with what Caufield et al. reported, since girls became infected with *MS* earlier than boys, but the difference found was not significant (p -value = 0.61).

It is difficult to compare studies from different countries as there are differences between different populations (eating habits, sociocultural factors, oral hygiene practices, microbiological differences and *MS* levels) in addition to variations in the methodology used in sample collection and processing techniques.

Although several authors^(4,17), including Florio et al., describe the presence of the microorganism in edentulous children, this could not be confirmed in this study⁽²⁶⁾.

Regarding dental caries, this study found that 15% of colonized children developed caries. However, Florio et al. (Brazil, 2004) reported that 23% of colonized children had caries, while Wan et al. (Australia 2003) found that only 9% developed dental caries. These differences can surely be explained through socioeconomic and cultural factors that should be studied^(4,26).

The sample attrition recorded in this study (71.42% over 29 months) is much higher than that reported by Florio et al. (21.2% in 24 months) and Wan et al. (32.4% in 24 months). Caufield et al.'s five-year study does not report any attrition. These results are probably related to the local health system, the safety of health controls in place and other sociocultural factors of the population studied^(4,18,26).

Conclusions

The presence of *MS* is one of the risk factors described in ECC and Severe Early Childhood caries along with dietary and behavioral factors. This pilot study was useful to obtain national data that will make it possible to design more comprehensive studies taking into account local difficulties.

The colonization values found were similar to those of Florio et al. in Brazil, with significant differences observed with those found by Caufield et al. in the United States, perhaps due to cultural similarities or the populations selected.

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ACKNOWLEDGEMENTS

We would like to thank:

- Our tutors, Prof. Dr. Ma del Carmen López Jordi MSc, Statistician Ramón Álvarez MSc, Prof. Dr. Virginia Papone.
- The following professionals, who helped us process samples: Dr. Josua Álamo, Dr. Gabriela Morteo and Dr. Carolina Verolo.
- Librarians Claudia Silvera, Carina Patrón and Verónica Pérez.
- The RAP authorities.

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- The authorities of the Piedras Blancas Clinic, especially Dr. Giselle Benítez, and to the nursing staff.