Universidade Estadual de Campinas

Faculdade de Odontologia de Piracicaba

Thaís Manzano Parisotto

"ASSOCIAÇÃO ENTRE CÁRIE PRECOCE DA INFÂNCIA, COMPOSIÇÃO MICROBIOLÓGICA DO BIOFILME DENTÁRIO, DIETA, HIGIENE BUCAL E FATORES SÓCIO-ECONÔMICOS EM PRÉ-ESCOLARES DE 36 A 48 MESES"

Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como requisito para obtenção do título de Mestre em Odontologia, Área de Odontopediatria.

Orientadora: Profa. Dra. Marinês Nobre dos Santos Uchôa

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Pela iluminação e força...

À minha querida família, em especial meus pais, José Antônio e Flávia

Pela dedicação incondicional, amor, carinho, suporte e compreensão...

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"Valeu a pena? Tudo vale a pena
Se a alma não é pequena.
Quem quer passar além do Bojador
Tem que passar além da dor.
Deus ao mar o perigo e o abismo deu,
Mas nele é que espelhou o céu".

Fernando Pessoa

RESUMO

A prevalência da cárie precoce da infância (CPI) no Brasil é alta e sua severidade aumenta com a idade. Assim, métodos sensíveis para o diagnóstico precoce e a identificação de indicadores de risco são importantes para o controle desta doença. Essa dissertação, constituída por três artigos teve como objetivos: (1) revisar sistematicamente os trabalhos que evidenciaram associação entre os níveis de estreptococos do grupo mutans (SM) e a prevalência e progressão da CPI; (2) investigar a prevalência da CPI em pré-escolares após inclusão das lesões de mancha branca (LMB) no critério de diagnóstico e a influência destas lesões no perfil epidemiológico da população estudada; (3) identificar os principais indicadores de risco da CPI através da avaliação dos fatores microbiológicos, dietéticos, sociais e hábitos de higiene bucal, considerando os estágios de desenvolvimento da doença. No levantamento dos artigos da revisão (1951-2007) foram utilizadas as bases de dados: Pubmed, Scopus e Cochrane. Na realização dos estudos dois e três utilizou-se uma amostra constituída de 351 e 169 crianças, respectivamente. Estes pré-escolares, de 36-48 meses e ambos os gêneros, freqüentavam creches e pré-escolas municipais de Itatiba-SP. Os exames clínicos para determinação do índice de cárie foram realizados com auxílio de gaze, sonda e espelho sob luz artificial. No terceiro estudo as crianças foram divididas em 3 grupos experimentais (livres de cárie, LMB, lesões de cárie cavitadas). Para a avaliação da dieta foi empregado um diário, enquanto higiene bucal, renda familiar, etnia e escolaridade foram avaliados por questionário. A coleta do biofilme de todas as superfícies dentárias vestibulares e palatinas foi realizada com auxílio de alças esterilizadas (1 μl) para padronizar a quantidade removida. Técnicas quantitativas de cultura microbiológica foram empregadas para determinar o número de colônias de SM, microrganismos totais (MT) e lactobacilos (LB). Os dados da revisão foram avaliados qualitativamente, enquanto aqueles inerentes aos estudos dois e três foram analisados pelo teste t-pareado e pela regressão logística múltipla, respectivamente (α=0,05). Dos 119 artigos levantados na revisão, 16 foram avaliados e apenas 1 alcançou alto nível de evidência científica. No estudo dois, o índice de cárie aumentou significativamente ($p<0,05$) com a inclusão das LMB, que predominaram na maioria dos dentes, principalmente nas superfícies lisas livres. No terceiro estudo, dentre os indicadores de risco analisados, os mais significativos para o

desenvolvimento de LMB foram: altos níveis de SM (OR=2,3, CI=1,01-5,14), alta freqüência diária de consumo de açúcar total (OR=5,4, CI=1,42-20,88) e presença de biofilme nos incisivos superiores (OR=2,3, CI=1,01-5,14). Os fatores significativos para a progressão da CPI foram: altos níveis de MT (OR=4,6, CI=1,56-13,74) e presença de LB (OR=20,3, CI=4,03-102,51). Através da revisão foi concluído que os níveis de SM são um forte indicador de risco para a CPI; entretanto, estudos longitudinais com maiores níveis de evidência científica são necessários para que os níveis de SM sejam apontados como fortes fatores de risco. As conclusões dos estudos dois e três revelaram que a inclusão das LMB no diagnóstico da cárie possibilitou a identificação precoce de pré-escolares de risco à cárie e o direcionamento de medidas preventivas.

Palavras-chave: cárie dentária, pré-escolar, epidemiologia, microbiologia, placa dentária.

ABSTRACT

The prevalence of early childhood caries (ECC) in Brazil is high and its severity increases with age. This way, sensitive methods for the early caries diagnosis and risk indicators identification are important for the disease control. This thesis, comprised by three manuscripts, aimed: (1) to undertake a systematic review of studies which have evidenced the association between mutans streptococci (MS) levels and the prevalence and progression of the ECC; (2) to investigate the increase of caries prevalence in young children after the inclusion of early caries lesions (ECL) to WHO thresholds caries detection and the influence of these lesions in the epidemiological profile of the studied population; (3) to identify the main risk indicators of the ECC, with regards to the microbiological, dietary and social factors, as well as oral hygiene habits, considering the development stages of dental caries. In the review, Pubmed, Scopus and Cochrane Library databases were searched for papers (1951-2007). In studies two and three the sample comprised 351 and 169 children, respectively. These preschoolers, aging 36 to 48 months, from both genders, attended public nurseries and preschools in the city of Itatiba-SP. The clinical examinations for caries index determination were performed using gauze, probe and mirror under artificial light. In the third study, the children were divided in three experimental groups (caries free, ECL and cavitated lesions). A chart was employed for the diet evaluation whereas oral hygiene, family income, ethnicity and education level were assessed by a questionnaire. Dental biofilm was collected from all buccal and lingual surfaces with a sterilized handle (1 μl) in order to standardize the amount removed. Quantitative microbiological culture techniques were performed to determine the number of mutans streptococci (MS) colonies and total microorganisms (TM) and lactobacilli (LB) counts. The review data were appraised trough qualitative analyses; the data from studies two and three were statistically analyzed by paired t-test and multiple logistic regression, respectively (α=0.05). Out of the 119 articles yielded in the review, 16 were appraised and only one article has achieved high value as evidence. In study two, the caries index has significantly increased ($p<0.05$) when the ECL were included; these ECL were the predominant caries lesion in the majority of the teeth, particularly on smooth surfaces. In the third study, among all risk indicators studied, the statistically significant indicators

associated with ECL development were: high levels of MS (OR=2.3, CI=1.01-5.14), high daily frequency of total sugar consumption (OR=5.4, CI=1.42-20.88) and biofilm presence on maxillary incisors (OR=2.3, CI=1.01-5.14). The significant factors associated with ECC progression were: high levels of TM (OR=4.6, CI=1.56-13.74) and lactobacilli presence (OR=20.3, CI=4.03-102.51). From the review it was concluded that MS levels are a strong risk indicator for early childhood caries; however, longitudinal studies with high levels of scientific evidence are required to point out MS levels as a remarkable ECC risk factor. From studies two and three it was concluded that the inclusion of ECL in the caries diagnosis allowed the earlier identification of caries risk preschoolers and targeting of preventive measures.

Key-words: dental caries, preschool child, epidemiology, microbiology, dental plaque.

SUMÁRIO

I- INTRODUÇÃO GERAL

A cárie precoce da infância é definida como a presença de uma ou mais superfícies dentárias cariadas (cavitadas ou não), perdidas ou obturadas em crianças com idade inferior a 06 anos. A presença de padrões atípicos, progressivos, agudos ou rampantes desta doença é designada cárie precoce da infância severa (Drury *et al.*, 1999).

Clinicamente, as lesões iniciais apresentam-se na forma de mancha branca opaca no terço cervical da superfície vestibular e lingual dos incisivos decíduos superiores (Ramos-Gomez *et al.*, 2002). Se a doença não for diagnosticada e controlada na fase precoce, essas lesões cavitam e progridem. Em seqüência, outros dentes são acometidos, o que pode culminar na destruição de toda a dentadura decídua. A perda precoce de dentes decíduos pode acarretar em uma série de transtornos no desenvolvimento adequado do sistema estomatognático. Em conseqüência, a função mastigatória, a fonação e a deglutição ficam comprometidas e a instalação de hábitos para-funcionais é favorecida, além de ocorrer a perda do guia de erupção dos dentes permanentes (Moyers, 1988). Ainda, verificam-se piores condições na qualidade de vida considerando-se os aspectos psico-sociais (Thomas e Primosch, 2002, Filstrup *et al.*, 2003, Feitosa *et al.*, 2005), peso e altura reduzidos (Ayhan *et al.*, 1996) e um maior número de faltas escolares (Gift *et al.*, 1992, Feitosa *et al.*, 2005).

Levantamentos epidemiológicos evidenciaram que no Brasil a CPI apresenta-se como um problema de saúde pública. No último relatório de saúde bucal, SB Brasil (Ministério da Saúde, 2004), o país não atingiu a meta estabelecida pela Organização Mundial de Saúde (OMS), a qual preconizava que 50% das crianças com idade de zero a cinco anos deveriam estar livres de cárie. Comparando-se o Brasil com outros países do mundo, verifica-se que a prevalência da CPI é alta e varia de 10,1 a 43,4% de acordo com, Bönecker *et al.* (2002), Rosenblatt e Zarzar (2004), Ribeiro *et al.* (2005), Ferreira *et al.* (2007), Rihs *et al.*, (2007), Oliveira *et al.* (2008). Mais importante, a população infantil que apresenta CPI possui maior risco ao desenvolvimento de cárie no futuro, sendo a experiência passada desta doença considerada um dos preditores de risco mais significativos (Sclavos *et al.*, 1988, Peretz *et al.*, 2003).

Os fatores primários relacionados à etiologia da cárie dentária e da CPI são a presença de bactérias cariogênicas, carboidratos fermentáveis, e hospedeiro/superfície dentária susceptível, que interagem em determinado período de tempo (Harris *et al.*, 2004, Selwitz *et al.*, 2007). Dentre esses fatores, a freqüência de exposição à sacarose tem sido destacada como responsável pelas alterações microbiológicas (Loesche 1986, Nobre dos Santos *et al.*, 2002) no biofilme dentário.

Com relação a microbiota, esta é representada por bactérias capazes de colonizar a superfície dentária e produzir ácido, em velocidade superior à capacidade de neutralização do biofilme, quando o pH encontra-se abaixo do crítico. Os estreptococos do grupo mutans apresentam tais características e vários estudos mostram que o mesmo está intimamente relacionado ao desenvolvimento da cárie na infância (Mattos-Graner *et al.*, 1998, Nobre dos Santos *et al.*, 2002, Vachirarojpisan *et al.*, 2004). Considerando a progressão desta doença, a presença dos lactobacilos desempenha um papel importante, visto que contribuem para a produção de ácidos que desmineralizam os tecidos dentários.

Ainda, visto que a CPI é multifatorial, os fatores comportamentais e sócio-econômicos também exercem influência no desenvolvimento desta doença. Neste contexto, hábitos de higiene bucal (Tsai *et al*., 2006) que estão intimamente relacionados à presença do biofilme dentário, etnia (Hallet O'Rourke, 2006), renda familiar e grau de escolaridade (Oliveira *et al.*, 2008) também devem ser considerados.

A análise da literatura evidencia que a despeito de existir um extenso número de trabalhos que demonstraram a associação entre os estreptococos do grupo mutans e a cárie precoce da infância, uma avaliação qualitativa crítica dos mesmos possibilita conclusões mais sólidas. Apesar da presença destes microrganismos ser necessária para o desenvolvimento da doença ela não é suficiente, o que torna importante a identificação de outros indicadores de risco. Ainda, a inclusão das lesões iniciais de mancha branca no critério de diagnóstico da CPI fornece informações adicionais que favorecerão o entendimento da doença.

II – PROPOSIÇÃO

Os objetivos da presente dissertação foram:

1. Revisar de forma sistemática os trabalhos que evidenciaram a associação entre os estreptococos do grupo mutans e a prevalência e progressão da cárie precoce da infância, considerando a qualidade dos mesmos.

2. Investigar a prevalência da CPI em pré-escolares após a inclusão das lesões de mancha branca (LMB) no critério de diagnóstico de cárie, bem como a influência destas lesões no perfil epidemiológico da população estudada.

3. Identificar os principais indicadores de risco da CPI através da avaliação dos fatores microbiológicos, dietéticos, sociais e hábitos de higiene bucal, considerando os estágios de desenvolvimento da doença (LMB e cavitação).

III – CAPÍTULOS

Essa tese está baseada na Resolução CCPG/002/06/UNICAMP que regulamenta o formato alternativo para teses de Mestrado e Doutorado e permite a inserção de artigos científicos de autoria ou co-autoria do candidato (Anexo 1). Por se tratar pesquisas envolvendo seres humanos, o projeto de pesquisa destes trabalhos foi submetido à apreciação do Comitê de Ética em Pesquisa da Faculdade de Odontologia de Piracicaba, tendo sido aprovado (Anexo 2). Assim sendo, essa tese é composta de três capítulos, conforme descrito abaixo:

✓ Capítulo 1

"Early childhood caries and mutans streptococci: a systematic review". Parisotto TM, Steiner-Oliveira C, Souza e Silva CM, Rodrigues LKA, Nobre-dos-Santos M. Este artigo foi submetido para publicação no periódico *Oral Health and Preventive Dentistry*.

✓ Capítulo 2

"Assessment of noncavitated and cavitated caries lesions in 3-4 years old children: A comparative study". Parisotto TM, Steiner-Oliveira C, Souza e Silva CM, Rodrigues LKA, Peres RCR, Nobre-dos-Santos M. Este artigo será submetido para publicação no periódico *Caries Research.*

✓ Capítulo 3

"Identification of risk indicators for different stages of early childhood caries". Parisotto TM, Steiner-Oliveira C, Rodrigues LKA, Peres RCR, Duque C, Nobre-dos-Santos M. Este artigo será submetido para publicação no periódico *Journal of Dental Research.*

CAPÍTULO 1

Early childhood caries and mutans streptococci: a systematic review

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Key words: dental caries, review literature, mutans streptococci, primary dentition, preschool child

Abstract

Purpose: The aim of this article was to undertake a systematic review of the relationship between mutans streptococci levels in the biofilm/saliva/tongue samples from children younger than 6 years-old and early childhood caries (ECC). **Methods:** The authors searched Pubmed, Scopus and Cochrane Library databases for papers from 1951 to 2007. The minimal inclusion requirements were assessment of preschool children reporting mutans streptococci counts, mainly in saliva and biofilm samples, and caries assessment. Since the heterogeneity of the studies did not allow a meta-analysis (X^2 test), a qualitative analysis was conducted. **Results:** The electronic search yielded 120 abstracts, but only 16 scientific articles were critically appraised. Of these 16 scientific papers included in the review, only one cross-sectional study achieved high value as evidence. **Conclusion:** It was concluded that mutans streptococci levels are a strong risk indicator for early childhood caries. However, further well designed longitudinal studies with high evidence values are required to point out mutans streptococci levels as a remarkable ECC risk factor.

Introduction

Dental caries is an infective-contagious disease that affects a large number of preschool children. Although caries prevalence decreased over the last few decades, especially because of water supply fluoridation and fluoridated dentifrice use, this multi-factorial health care problem is still present. It is, however, not uniformly distributed in the population, and continues to be concentrated in high-caries-risk groups (Bankel et al, 2006; Petti et al, 2000).

According to the Workshop sponsored by the National Institute of Dental and Craniofacial Research, the Health Resources and Services Administration and the Health Care Financing Administration (Drury et al, 1999) the presence of any decayed, missing or filled surface in primary teeth in children younger than 6 years old is designated early childhood caries (ECC). Early childhood caries lesions might become clinically evident as early as 12 to 16 months of age, usually appearing first on the labial, gingival and lingual surfaces of the maxillary incisors (Ramos-Gomez et al, 2002). Subsequently, the lesions rapidly spread to other primary teeth, resulting in the eventual destruction of primary dentition. An intact primary arch is of extreme importance for the child continued well-being and adequate development of the stomatognatic system.

The ECC prevalence achieves high values, particularly in developing countries (Carino et al, 2003), and it is related to physical, biological, environmental, behavioral and lifestyle-related factors. In addition, in young infants, this health care problem is also associated with the frequent use of a baby bottle containing sweetened fluids with fermentable carbohydrates over extended periods, poor oral hygiene as well as high level of mutans streptococci infection (Selwitz et al, 2007). Frequent sugar intake by liquids or solids leads to low pH conditions in the oral environment and in dental biofilm, favoring the growth of acidogenic and aciduric species, such as mutans streptococci. Moreover, sweetened liquids usually contain sucrose, which is a specific substrate for glucan production leading, to mutans streptococci adherence to oral biofilm (Loesche, 1986).

Several clinical studies demonstrated a positive correlation between the number of mutans streptococci and caries prevalence (Bankel et al, 2006; Ersin et al, 2006; Vachirarojpisan et al, 2004; Olmez et al, 2003; Nobre dos Santos et al, 2002; Ramos-

Gomez et al, 2002; Milgrom et al, 2000; Petti et al, 2000; Mattos-Graner et al, 1998, Douglass et al 1996, Hallonsten et al 1995, O'Sullivan and Tinanoff et al 1993, Matee et al, 1992; Fujiwara et al, 1991) as well as caries increment in young children (Mattos-Graner et al, 2001; Thibodeau and O'Sullivan, 1996).

However, the quality of studies has to be appraised in order to reach reliable conclusions. Thus, the aim of this article was to undertake a systematic review of the relationship between mutans streptococci levels in the biofilm/saliva/tongue samples from children younger than 6 years of age and ECC.

Material and methods

Question Addressed by this Review

Based on the current quality of the literature regarding the relationship between early childhood caries and mutans streptococci, are these microorganisms levels a strong risk indicator/factor for early childhood caries?

Literature searching

The electronic search was conducted in Pubmed, Scopus and Cochrane Library databases, and studies dated between December 1951 and November 2007 were selected. No manual search was used. Based on the aim of this systematic review, the following search descriptors were used together with "mutans streptococci": "early childhood caries", "nursing caries", "baby-bottle tooth decay", "maxillary anterior caries", "labial caries", "rampant caries" and "nursing bottle caries".

Inclusion and exclusion criteria

The literature search enabled a total of 120 non-duplicate articles to be identified. The minimal inclusion requirements were assessment of preschool children reporting mutans streptococci counts, mainly in saliva and biofilm samples, and caries assessment. Interim case reports, reviews, protocols, brief/short communications, and articles in other language than English were dismissed. Excluded studies and the main reason for the exclusion are detailed in Table 1. When the abstract did not provide the necessary information to meet all the inclusion criteria, the full text was obtained and after detailed

screening, 16 scientific articles (Table 2) and one systematic review (Harris et al, 2004) were selected. The systematic review was only considered in the discussion session.

Evaluation of Scientific articles

The articles that met all the inclusion criteria were submitted to critical appraisal by five project group members. Even after the evaluation criteria standardization, any disagreement between the reviewers was solved by discussion among them until consensus was reached. Based on predetermined methodology quality and performance criteria (Egger et al, 2001; Clarke and Oxman, 2002), as defined in Table 2, each report was given scores, from 0 to 2, and only the total score was retained. Thus, the final level of evidence was judged according to the total score, which ranged from 0 to 18. Scores between 0 and 8 were considered as poor value as evidence, whereas scores from 9 to 15 and 16 to 18 were rated as moderate and high level as evidence, respectively.

Data synthesis

Heterogeneity among the studies, particularly with respect to the varying quality, methodology and presentation of results, precluded use of statistical data pooling methods such as meta-analysis. Nevertheless, even the articles that provided information that could be grouped and tested through chi-square analysis were not considered homogenous ($p<.001$), therefore definitely dismissing meta-analysis.

Results

Out of the 120 articles from the original literature search, 16 (14 cross-sectional and 2 longitudinal) met all the inclusion criteria and were therefore included and critically appraised (Table 2). According to Table 2, only one cross-sectional study (Vachirarojpisan et al, 2004) presented high level as evidence, with score 18, whereas 10 articles achieved scores raging from 9 to 15 (Bankel et al, 2006; Ersin et al 2006; Nobre dos Santos et al, 2002; Milgrom et al, 2000; Petti et al, 2000; Mattos-Graner et al, 1998; Douglass et al, 1996; Hallonsten et al, 1995; O'Sullivan and Tinanoff, 1993; Mattos-Graner et al, 2001) with moderate value as evidence. The remaining articles, with scores ranging from 5 to 8 (Olmez et al, 2003; Ramos-Gomez et al, 2002; Thibodeau and O'Sullivan, 1996; Matee et al, 1992; Fujiwara et al, 1991) were considered limited or of poor value as evidence.

All the 16 articles included for evaluating scientific evidence were used as a basis for conclusions.

Discussion

The present review systematically estimated the substantial literature in order to achieve solid conclusions about the relationship between mutans streptococci and ECC. Therefore, with regard to Dentistry based on scientific evidences, systematic reviews play a very important role. Moreover, this article will probably contribute to emphasizing the need for developing articles with high level as evidence in the study design to provide data applicable to the whole population.

Studies appraisal

Since the heterogeneity of the studies did not allow a meta-analysis, they were qualitatively analyzed to obtain evidences that would clarify the question addressed. The study from Olmez et al (2003) scored 5, and was the only one that did not verify a significant association between mutans streptococci counts and ECC (Table 3), because all age groups presented high caries prevalence and there was no comparison between children with caries and caries-free children. All the others 15 selected articles showed significant association between early childhood caries and mutans streptococci levels in the dental biofilm or saliva samples (Table 3). However, only the cross-sectional study by Vachirarojpisan et al (2004) provided high level as evidence.

This article, along with the 8 other cross-sectional studies that reached scores 11 and 15, such as Bankel et al (2006), Petti et al (2000), Milgrom et al (2000), Douglass et al (1996), Hallonsten et al (1995), O'Sullivan and Tiannoff (1993), Nobre dos Santos et al (2002) and Mattos-Graner et al (1998), presented a well designed and representative sample, except for the latter two studies that only randomized the children, without mentioning how. These 8 cross-sectional studies did not achieve the maximal score, because the authors did not mention kappa intra-examiner values (Petti et al, 2000; Nobre dos Santos et al, 2002; Milgrom et al, 2000; O'Sullivan and Tinanoff, 1993), kappa inter and intra- examiner values (Bankel et al, 2006; Hallonsten et al, 1995), did not calibrate them at all (Douglass et al, 1996), did not stratify the sample for gender and age (Nobre dos

Santos et al, 2002; Douglass et al, 1996, Hallonsten et al, 1995; O'Sullivan and Tinanoff, 1993) or did not consider white chalky spot lesions as caries (Petti et al, 2000; Nobre dos Santos et al, 2002; Douglass et al, 1996; O'Sullivan and Tinanoff, 1993). Stratification by gender and age is of great relevance because the number of erupted primary teeth, and consequently the number of mutans streptococci varies among young children (Vachirarojpisan et al, 2004; Erickson et al, 1998; Fujiwara et al, 1991). Moreover, the fact of not calibrating inter and/or intraexaminer and not considering white chalky spot lesions as caries have led to results that did not match the true reality.,

The other five cross-sectional studies conducted by Ramos-Gomez et al (2002), Ersin et al (2006), Matee et al (1992), Olmez et al (2003) and Fujiwara et al (1991) that received scores of 9 or lower, did not obtain higher values as evidence because they did not consider a representative number of children, did not calculate the sample size based on the caries prevalence already established in previous or pilot studies, or did not include all the children from a determined area in a pre-established age group. Moreover, these studies rated as moderate or of poor values as evidence, did not perform adequate allocation concealment, because they did not randomize the sample or did not specify how this procedure was done. Still, the work from Olmez et al (2003) did not include a control group in their study.

It is important to emphasize that this systematic review considered the following as bias: lack of intra and/or inter examiners calibration (not showing kappa values) and studies that did not consider white chalky spot lesions as caries, leading to doubtful results.

With respect to the longitudinal studies, the fact that mutans streptococci levels are a strong risk factor for early childhood caries remained unclear. This happened because all these studies reached poor or moderate values as evidence, with scores ranging from 6 to 14 (Table 2). The main reason was that these studies (Mattos-Graner et al, 2001; Thibodeau and O'Sullivan, 1996) worked with convenience samples, without the description of sample size calculation, leading to results that could not be generalized. The other reasons were lack of a homogeneous group of children, including stratification by gender and age, inclusion and exclusion criteria not clearly defined, as well as lack of defined and valid

methods for caries diagnosis, including inter and intraexaminer calibration mentioning kappa values (Thibodeau and O'Sullivan, 1996).

Based on the cross-sectional articles that reached the highest scores, especially Vachirarojpisan's et al (2004) study, this systematic review confirmed that mutans streptococci levels is a strong risk indicator for early childhood caries. However, it is important to emphasize that findings from cross-sectional studies have some limitations, such as the assumption that a certain factor preceded caries development, and not considering the child's response to this factor during the disease process.

Furthermore, it should be highlighted that mutans streptococci levels are not *sine qua non* for caries manifestation. Their ability to synthesize alkali-soluble polysaccharide (Nobre dos Santos et al, 2002; Mattos-Graner et al, 2000) and its diversity of genotypes (Marchant et al, 2001; Alaluusua et al, 1996) in the same child are also relevant factors. The systematic review by Harris et al (2004), the only one identified in the electronic search strategy, related to the question addressed, pointed out that early acquisition of mutans streptococci also favors caries development. Nevertheless, ECC is a multi-factorial disease and other factors/variables, such as dietary habits, oral hygiene and socio-economical status should be considered.

Biofilm/saliva/tongue samples

The biofilm samples collected to enable mutans streptococci counts were not homogeneous due to the great variability in the collection area. Whereas Matee et al (1992) and Milgrom et al (2000) used the primary maxillary incisor area, Bankel et al (2006) chose the primary maxillary and mandibular molar and incisor areas, Hallosten et al (1995), worked with all occlusal and smooth surfaces and Nobre dos Santos et al (2002) used the primary maxillary incisors, canines and maxillary and mandibular molar areas. Although all these studies found a significant association between ECC and mutans streptococci counts in the biofilm samples from these different areas (Table 3), it was already demonstrated that mutans streptococci decrease in prevalence from the molars to the anterior teeth, (Lindquist et al, 1989) except for the anterior caries pattern (Nobre dos Santos et al, 2002).

Saliva samples were also used for microorganism detection (Table 3), leading to positive association between ECC and mutans streptococci levels in the great majority of

the studies. The articles by Bankel et al (2006), Petti et al (2000), Ramos-Gomez et al (2002), Ersin et al (2006), Vachirarojpisan et al (2004), Olmez et al (2003), Mattos-Graner et al (1998), Douglas et al (1996), O'Sullivan and Tinanoff (1993), Matee et al (1992), Mattos-Graner et al (2001) and Thibodeau and O'Sullivan (1996) all took saliva samples into account. The reason for the no significant association verified by Olmez et al (2003) only, has already been discussed above.

It was also noticeable that the studies by Milgrom et al (2000), Matee et al (1992) and Bankel et al (2006) considered more than one sample type (Table 3). Bankel et al (2006) and Matte et al (1992) considered biofilm and saliva samples, both leading to positive association between mutans streptococci and ECC. In this context, Lindquist et al (1989) showed that mutans streptococci levels in saliva reflect dental biofilm conditions. Nevertheless, biofilm and tongue samples were reported by Milgrom et al (2000). While in the biofilm samples, mutans streptococci counts were significantly associated with dental caries, the opposite occurred with regard to the tongue samples. The reasons for this finding could be the adherence characteristics of mutans streptococci, because the tongue provides a nonshedding surface (Berkowitz, 1996). Furthermore, it was recently demonstrated that in children aged from 9-24 and 25-36 months, the values for mutans streptococci in dental biofilm were significantly higher than those found in tongue samples (Barsamian-Wunsch et al, 2004).

Caries diagnosis criteria

The criteria used to diagnose caries lesions were described in the Table 3. While Bankel et al (2006), Ramoz-Gomez et al (2002), Vachirarojpisan et al (2004), Milgrom et al (2000), Mattos-Graner et al (1998;2001) and Hallonsten et al (1995) considered white chalky spot lesions as initial caries, the majority of studies did not (Ersin et al, 2006; Olmez et al, 2003; Nobre dos Santos et al, 2002; Petti et al, 2000; Douglass et al, 1996; Thibodeau and O'Sullivan, 1996; O'Sullivan and Tinanoff, 1993; Matee et al, 1992; Fujiwara et al, 1991). Therefore, the first clinical manifestation of dental caries can easily be underestimated, leading to less accurate results. In this respect, Ersin et al (2006) were the only authors to report that in spite of presenting white spot lesions with no cavitations, some children may have been classified as caries-free.

Examiner calibration

Another confounding factor considered in the present systematic review was the lack of kappa value description for intra and/or inter examiner calibration in many studies (Bankel et al, 2006; Petti et al, 2000; Nobre dos Santos et al, 2002; Milgrom et al, 2000, Thibodeau and O'Sullivan, 1996; O'Sullivan and Tinanoff, 1993). Moreover, in the articles by Ramos-Gomez et al (2002), Olmez et al (2003), Douglass et al (1996), Matee et al (1992) and Fujiwara et al (1991) the examiners were not calibrated at all. The fact that no calibration was done became worse when there were several examiners in the study, which happened in the research by Hallonsten et al (1995).

Good or excellent calibration, demonstrated by kappa values ranging from 0.61 to 1.00 (Landis and Koch, 1977), is important to assure that there was intra and/or inter examiner agreement with regard to caries diagnosis, providing reliable data.

From this systematic review it was, therefore, concluded that mutans streptococci levels are a strong risk indicator for early childhood caries. However, further well designed longitudinal studies with high evidence values are required to point out mutans streptococci levels as a remarkable ECC risk factor.

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Table 1. Excluded studies and the main reasons for exclusion.

Reason for exclusion	First author
Case reports	Tinanoff, 1999; Walsh, 1990
Reviews	Ly, 2006, Berkowitz, 2003; Douglass, 2004; Ramalingam, 2004; Lynch, 2003; Davies, 1998; Bowen, 1998; Horowitz, 1998; Seow, 1998; Berkowitz, 1996, Tinanoff, 1995; Krasse, 1989
Protocols	Hildebrandt, 2004; Yengopal, 2003
Other language than English	Jokicc, 2006; Tong, 2004; Behrendt, 2002; Liu, 2001_a; Liu, 2001_b; Qian, 2001; Karn, 1998; Buttner, 1996; Lacatusu, 1996; Liu, 1996; Buttner, 1995; Wetzel, 1993; Berkowitz, 1984
Children six years old or older	Chambers, 2006; Hata, 2006, Law, 2006; Bedi, 2005; Corby, 2005; Chase, 2004; Koga-Ito, 2004; Becker, 2002; Dasanayake, 2002; Krishnakumar, 2002; Mojon, 1998; Kreulen, 1997; Budtz-Jlrgensen, 1996, Dasanayake, 1995; Aaltonen, 1990; MacEntee, 1990; Smith, 1990
Children with any type of syndrome	de Soet, 1997
Subjects submitted to antimicrobial therapy	Zhan, 2006; Plotzitza, 2005; Amin, 2004; Gripp, 2002; Soderling, 2001; Ogaard, 2001; Isokangas, 2000; van Lunsen, 2000; Lopez, 1999; Clark, 1994; Epstein, 1991; Boue, 1987
Children already treated for ECC	Peretz, 2003
Caries-free group only	Lamas, 2003; Habibian, 2002; Lopez, 2000
Predental children only	Wan, 2001a; Wan, 2001b
Rat subjects	Zhang, 1999; Ooshima, 1994; van Raamsdonk, 1993; O'Connell, 1991
Not available in Brazil	Ali, 1998
Did not count mutans streptococci	de Carvalho, 2006; Marchant, 2001; Milnes, 1985
Not related to the question addressed	Persson et al., 2007, Park, 2006; Tanabe, 2006; Saxena, 2005; Barsamian-Wunsch, 2004; Benson, 2004; Glenny, 2004; Marinho, 2003; Marinho, 2002; Smith, 2002; Wan, 2002; Emanuelsson, 2001; Primosch, 2001; Li, 2000; Mattos-Graner, 2000; Erickson, 1999; Naspitz, 1999; Emanuelsson, 1998; Erickson, 1998; Mohan, 1998; Redmo Emanuelsson, 1998; Alaluusua, 1997; Alaluusua, 1996; Wright, 1996; Alaluusua, 1994; Li, 1994; Matee, 1993; Grindefjord, 1991; Alaluusua, 1990; Masuda, 1979

Table 2. Criteria for scoring assessed papers that met the inclusion criteria.

Scoring criteria		First author: Bankel [8 CS]	Ersin [42 CS]	Vachirarojpisan [116 CS]	Olmez [93 CS]	Nobre dos Santos [90 CS]	Ramos- Gómez [103 CS]	Milgrom [85 CS]	Petti [98 CS]	Mattos-Ganer [84 CS]	Douglass [34 CS]	Hallonsten [48 CS]	O'Sullivan [95 CS]	Matee [81 cs]	Fujiwara [43 cs]	Mattos-Graner [82 L]	Thibodeau [112 L]
High values as evidence (score 2)	Adequate allocation concealment	X	-	X	-	X	-	X	X	-	X	X	X	-	-	-	-
	Method of sample size calculation mentioned	X	-	X	-	-	-	X	X	-	X	X	X	-	-	-	-
	Representative sample-results are able be generalized	X	-	X	-	-	-	X	X	-	X	X	X	-	-	-	-
	Inclusion and exclusion criteria clearly defined	X	X	X	-	X	X	X	X	-	X	X	X	-	X	X	-
	Control group	X	X	X	-	X	X	X	X	X	X	X	X	X	X	X	X
	Homogeneous sample- taking into account sex, age and social group	X	-	X	X	-	-	X	X	X	-	X	-	X	-	X	-
	Defined and valid methods for caries diagnosis	-	X	X	-	-	-	-	-	X	-	-	-	-	-	X	-
	Bias taken in account	-	-	X	-	-	-	-	-	X	-	-	-	-	-	X	-
	Statistical analysis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Moderate value as evidence (score 1)	Random allocation but method used to conceal unknown	-	-	-	-	X	-	-	-	X	-	-	-	-	-	X	-
	Sample defined- but results could not be generalized	-	X	-	X	X	X	-	-	X	-	-	-	X	X	X	X
	Inclusion and exclusion criteria poorly described	-	-	-	-	-	-	-	-	X	-	-	-	X	-	-	-
	Methods for clinical caries diagnosis not completely described or validated	X	-	-	-	X	-	X	X	-	-	X	X	-	-	-	X
Limited or of poor value as evidence (score 0)	Inadequate allocation concealment or controlled clinical trial	-	X	-	X	-	X	-	-	-	-	-	-	X	X	-	X
	No method, or none mentioned for sample size calculation	-	X	-	X	-	X	-	-	X	-	-	-	X	X	X	X
	Inclusion and exclusion criteria not described	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-
	Non calibrated examiner	-	-	-	X	-	X	-	-	-	X	-	-	X	X	-	-
	Scores	**15**	**9**	**18**	**5**	**11**	**7**	**15**	**15**	**13**	**12**	**15**	**13**	**8**	**7**	**14**	**6**

Modified from Egger et al (2001) and Clarke and Oxman (2002). CS: cross-sectional; L: longitudinal. The "X" s indicate papers that addressed the issues above.

Table 3. Results of references appraised.

First author	Study design	Age months	Subjects	Caries index	Considered ICL	Sample for MSC	Association ECC x MSC/*Sm*C
Bankel [8]	CS	24-36	221	Koch 1967	Yes	Saliva Biofilm	Significant
Ersin [42]	CS	15-35	101	NIDCR	No	Saliva	Significant
Vachirarojpisan [116]	CS	6-19	520	WDR	Yes	Saliva	Significant
Olmez [93]	CS	9-57	95	WHO	No	Saliva	No significant
Nobre dos Santos [90]	CS	18-48	60	Radike 1972	No	Biofilm	Significant
Ramos-Gomez [103]	CS	3-55	146	NIDCR	Yes	Saliva	Significant
Milgrom [85]	CS	6-36	163	ICL and MCL	Yes	Biofilm Tongue	Significant
Petti [98]	CS	36-60	1404	WHO	No	Saliva	Significant
Mattos-Graner [84]	CS	12-30	142	ICL and MCL	Yes	Saliva	Significant
Douglass [34]	CS	48	127	Radike 1972	No	Saliva	Significant
Hallonsten [48]	CS	18	200	ICL and MCL	Yes	Biofilm	Significant
O'Sullivan [95]	CS	36-48	369	Radike 1972	No	Saliva	Significant
Matee [81]	CS	12-30	34	WHO	No	Biofilm Saliva	Significant
Fujiwara [43]	CS	0-24	356	WHO	No	Saliva	Significant
Mattos-Graner [82]	L 1 year	24-48	101	ICL and MCL	Yes	Saliva	Significant
Thibodeau [112]	L 2 years	44	146	Radike 1968	No	Saliva	Significant

CS: cross-sectional study; L: longitudinal study; NIDCR: National Institutes of Dental and Craniofacial Research's 1999[3]; WDR: Workshop on diagnosing and reporting ECC for research purposes 1999 [3]; WHO: World Health Organization 1987; ICL: initial caries lesion-white chalky spot; MCL: manifested caries lesion-cavity; MSC: mutans streptococci counts; *Sm*C: *Streptococcus mutans* counts.

CAPÍTULO 2

Assessment of noncavitated and cavitated caries lesions in 3-4 years old children: A comparative study

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Declaration of interests

The authors declare that there is no potential conflict of interest because none of the authors has a personal or financial relationship that might introduce bias or affect their judgment.

ABSTRACT

As the prevalence of early childhood caries (ECC) is high in developing countries, sensitive methods for the early diagnosis of caries lesions are of prime importance for the establishment of preventive measures. Thus, the aim of the present study was to investigate the caries prevalence in young children after including early caries lesions (ECL) to WHO thresholds caries detection as well as its influence in the epidemiological profile of the studied population. A total of 351 3-4 years old preschoolers of both genders and living in an optimally fluoridated Brazilian community took part in the study. Clinical examinations were conducted by one calibrated examiner using the following criteria: World Health Organization (WHO) and WHO + ECL. During the examinations, mirrors, ball-ended probe, gauze, and artificial light were used. The intra-examiner Kappa values at tooth and surface levels were 0.93/0.87 for WHO and 0.75/0.78 for WHO + ECL criteria. The data were statistically analyzed by paired t- test and Mc-Nemar's test ($\alpha = 0.05$). The results have shown that the number of decayed, missing and filled surfaces were significantly higher ($p<0.05$) when WHO + ECL criteria was used. The prevalence of dental caries was 40% and 70% for WHO and WHO + ECL criteria, respectively. Statistical differences between caries-free children according to the two criteria were also found. Additionally, the ECL were the predominant caries lesion in the majority of teeth, particularly on the smooth surfaces. In conclusion, the WHO + ECL criteria used was able to diagnose dental caries earlier in preschool children, providing the establishment of preventive measures to avoid frank cavitations.

INTRODUCTION

Over a number of decades, caries in the primary dentition was diagnosed by criteria that could evidence caries only in advanced stages. The most popular caries index used in the world, so far, had been the number of decayed, missing and filled surfaces [World Health Organization, 1997] due to its versatility. However, changes in the epidemiology of the disease and the understanding of the caries process have progressed far beyond the point of restricting the first clinical evidence for dental caries to cavitation [Pitts, 2004_a], since the early mineral loss, evidenced by the white chalky spot lesion, is an absolute necessity to reach the cavitation at the enamel surface [Biesbrock et al., 2004].

The early diagnosis of caries, especially in young children with high caries activity but without cavity, is of extreme importance because it can provide valuable information for the establishment of preventive measures. These measures should be able to enhance tooth remineralization, and avoid treatment negligence as well as frank cavitations, corroborating with the international trend to move away, wherever possible, from operative interventions towards preventive treatment in the clinical practice [Pitts, 2004_b]. In this context, the wide range of fluoridated products and antimicrobial agents available nowadays enables interventions in the caries process since its first stage [Anusavice, 2005].

It is also important to highlight that the early childhood caries (ECC) progresses very rapidly [Grindefjord et al., 1995], due to lower mineralization [Wilson and Beynon, 1989], higher carbonate content [Clasen and Ruyter, 1997] and higher porosity [Shellis, 1984; Lindén et al., 1986,] of the primary teeth compared to the permanent. In light of this, when the diagnosis is delayed in a young child, many primary teeth may already be destructed or missed, leading to serious consequences such as: problems in speech and mastication, installation of incorrect oral habits, loss of the guidance for the permanent teeth eruption [Moyers, 1988], reduced percentile category for height and weight [Ayhan et al., 1996] and loss of school days [Gift et al., 1992]. Furthermore, the scientific literature presents few studies considering caries diagnosis criteria in the primary dentition that includes early caries lesions (white chalky spot lesions) in developing countries [Mattos-Graner et al., 1998; Mattos-Graner et al., 2001; Gonzaléz et al., 2003, Vachirarojpisan et al., 2004]. Thus, the aim of the present study was to investigate the caries prevalence in

young children after including early caries lesions to WHO thresholds caries detection as well as its influence in the epidemiological profile of the studied population.

MATERIALS AND METHODS

Ethics considerations

This study was approved by the Ethical Committee in Research of Piracicaba Dental School/State University of Campinas (UNICAMP) in agreement with resolution 196/96 of the National Committee of Health Department (Brazil) under 015/06 protocol. The nurseries and preschools granted permission for the study and an informed positive consent term was signed by the children's responsibles.

Sample

All 3 to 4 years old children enrolled in public nurseries and preschools in the urban area of Itatiba-SP/Brazil were invited to participate in the study. This age range was chosen because in this stage of life, all primary teeth are supposed to be erupted and no permanent teeth should be present in the mouth. The city of Itatiba is located in the State of São Paulo, 80 km from the capital, and has a population of about 91 000 habitants. Most of these habitants live in the urban area, where the tape water supply has been optimally fluoridated since 1980 and heterocontrol of this fluoridation process showed that the levels of fluoride were from 0.6-0.8 ppm during this study. The oral health program in the city includes preventive measures and curative treatments. Moreover, children from public nurseries and preschools in Itatiba are from mid socioeconomic backgrounds.

A minimum sample size of 123 children was required to achieve a level of precision with a 0.07 standard error. The 95 percent confidence interval level and caries prevalence (0.72) found in a previous pilot study carried out with part of these children were used for the sample size calculation. It was decided to invite all 3 to 4 years old children in the present study in order to minimize eventual problems that would contribute to a sample size smaller than the minimum calculated. Out of the 546 children invited to take part in the study, only 351 have participated. Thus, the final sample size was 351 preschoolers, comprising 173 males and 178 females. The exclusion criteria were: children whose parents did not attend the scheduled school meetings at entrance/exit time to understand the study's

aims and/or its importance and children whose parents refused to sign the informed positive consent term. Reason for not completing the study was: patients who had not collaborated with the necessary procedures for the clinical examinations.

Diagnostic criteria

The two criteria used for early childhood caries diagnosis in the present study were: WHO (WHO, 1997) and WHO + early caries lesions (ECL) [Nyvad et al., 1999, Assaf et al., 2006, Kassawara et al., 2007], which are described in table 1. According to WHO criteria, caries was recorded if a frank cavitation was present. On the other hand, considering the WHO + ECL criteria, the early caries lesions were also defined as caries. This happened when there was a rough white spot lesion, with chalky appearance and without breakdown of the surface, usually adjacent or close to the soft tissue margin where the biofilm accumulates. For the occlusal surface, ECL were recorded on lesions extending along the walls of the fissure, where increased roughness and opacity were evident. Additionally, according to WHO + ECL criteria, cavities alone or adjacent to fillings were classified as active when softened floor was detected and as inactive when the cavity floor was hard, brownish or black. The tooth structure texture (rough/hard/soft) was tested by gentle probing.

The units of evaluation used in the clinical exams were dmfs (decayed, missing and filled surfaces) and dmft (decayed, missing and filled teeth), according to each criteria described.

Calibration of the examiner

Intra-examiner reliability (Kappa calculation) with regards to all components from the diagnostic criteria (WHO and WHO + ECL) was assessed by reexaminations of approximately 10 percent of the children with a -1week-interval period, to avoid dental examiner memorization. The intra-examiner agreement, measured using Kappa calculation regarding the tooth and surface level, were 0.93/0.87 for WHO criteria and 0.75/0.78 for WHO + ECL criteria, respectively.

Theoretical discussions using clinical photographic slides were held to give instructions to the examiner about the use of the criteria and the examination method,

including explanations about the exams for early caries lesions. The entire time spent on the calibration process (theoretical discussions, training and calibration exercises) was 30 h.

Clinical examination

The clinical exams were conducted with a focusable flashlight at the nurseries and preschools using a mirror and a ball-ended probe to confirm questionable findings. Gauze was employed in order to dry or clean the teeth favoring the early caries lesions identification. A portable flashlight was also used to make noncavitated lesions easier to be recorded. The dental examiner sat behind the child, who was lying on a table, and was assisted by a scribe. All the examinations were carried out by a single dentist (T.M.P.) following rigorously strict cross-infection control measures.

Statistical analysis

For data analysis, the proportions of caries-free children and mean dmfs scores were calculated. Mc-Nemar's test was used to compare the proportion of caries-free children according to the two different criteria. Paired t-test was used to compare dmfs/dmft means according to WHO and WHO + ECL criteria, in order to demonstrate the influence of early caries lesions inclusion in the caries diagnostic criteria. The analyses were carried out using the SPSS 9.0 (SPSS Inc., Chicago, IL, USA) statistical program.

RESULTS

Epidemiological examinations under the WHO diagnostic criteria presented significant differences ($p<0.05$) when compared with the epidemiological examinations under WHO + ECL criteria (Table 2). The statistical significant differences between caries-free children according to the two criteria are also presented in Table 2. The non-uniform distribution of the dmfs in the population, characterized by many children without caries and a smaller group with very high caries prevalence (caries polarization), is shown in Figure 1. The mean and standard deviations (SD) of the components of the dmfs indexes is evidenced in Table 3. In this Table it is also shown that the ECL and the cavitated surfaces corresponded to the major components of the number of dmfs index according to the criteria that included the ECL. Furthermore, the distribution of the decayed, missing or filled surfaces according to the surface type when the WHO + ECL criteria was used is

presented in Figure 2. The early caries lesions were predominant on the smooth surfaces and the cavities were uniformly distributed among the three surface types. Moreover, the restorations without cavity or ECL occurred more frequently on the occlusal surface whereas the restorations with decay were more common on the smooth surface. Figure 3 shows that the ECL are the predominant caries lesion type in the majority of teeth.

DISCUSSION

The increase of caries prevalence in the primary dentition after the inclusion of early caries lesions to WHO thresholds caries detection has influenced significantly the epidemiological profile of the studied population. Despite the fact that Kappa values decreased when the ECL were considered, the study from Assaf et al. [2006] have shown that with enough training and examiners calibration, a good reliability is possible, encouraging future studies with this criteria that includes noncavitated lesions with power to predict future caries.

The mean of the dmfs scores considering WHO + ECL criteria was twice as much as WHO criteria (Table 2), highlighting that the first clinical evidence of dental caries (ECL) has a great prevalence among 3 to 4 years old children, corroborating with results found by Gonzaléz et al. [2003] in a developing country.

When the ECL were included to WHO thresholds caries detection in the present research, the percentage of caries-free children decreased from 59 to 32 % (Table 2). This means that 27% of the preschoolers present white chalky spot lesions only. Therefore, the percentage of children that showed ECL together with other decay component, such as cavities or fillings, is about 40%. In light of this, it could be verified that the majority of the ECL were present in children with past history of caries, which is in accordance with studies from Warren et al. [2002] and Autio-Gold and Tomar [2005] who also worked with young children. Additionally, the fact that nearly 30% of the children presented ECL only is remarkable and could be explained by the children´s early stage of life: when caries active children get older the early caries lesions will certainly have progressed and new ECL will continue to appear, until the disease is controlled. This was demonstrated by the study from Kassawara et al. [2007], which was conducted with 7-10 years old Brazilian

children living in an optimally fluoridated tap water area and has verified that the difference between caries-free children regarding WHO and WHO + ECL criteria was less than 10%. In this context, it is strongly emphasized that the ECL should be included in the early diagnosis of caries in order to minimize the chance of a high caries active young child not to receive the appropriate early intervention. Thus, the younger the child, the higher the necessity of including ECL in the caries diagnosis.

As dental caries is a multi-factorial disease, the high ECL prevalence in the children here evaluated (Table 3 and Figure 3) was not surprising considering that these children usually present inappropriate feeding practice such as consumption of sweetened fluids in a baby bottle with a high frequency and at their age, they are already colonized by mutans streptococci [Mattos-Graner et al., 1998; Mattos-Graner et al. 2001; Hallett and O'Rourke, 2006].

The urban area of Itatiba, where the present study took place, has been optimally fluoridated since 1980 (0.6-0.8 ppm). The widespread of fluoridated tap water and dentifrice use have led to a decrease in caries prevalence, even tough it was still high in the studied population, and to a polarization of this disease in the high caries-risk groups [Narvai et al., 2006]. This polarization is shown in Figure 1, where it can be seen that less than 10% of the children presented a dmfs index higher than 15 according to WHO+ECL and WHO criteria.

As observed in Table 3, WHO + ECL criteria have shown more details about the carious lesions, which enabled children classification regarding caries activity and also the identification of the high caries-risk group. The focus on the early targeting of these groups is of great significance for appropriate preventive measures implementation, such as supervised toothbrushing, parental education about oral hygiene/dietary habits and topical fluoride application. These measures aim at controlling dental caries and avoid cavitations by stopping lesions progression, considering that ECC severity increases with age [Sclavos, 1988; Peretz et al., 2003, Mattila et al., 2005]. Although these preventive measures should be targeted at high caries-risk group, they also should be provided to all children, as the caries free group can also develop caries lesions.

It is noticeable that the majority of the early caries lesions were present on the smooth surfaces (Figure 2), as previously demonstrated by Gonzaléz et al. [2003]. In the present research as well as in the study from Gonzaléz et al. [2003] this may have occurred because caries diagnosis is favored in these areas. In addition, carious lesions were more prevalent on the maxillary central incisors (Figure 3) in agreement with the findings from Wyne et al. [2001]. Since the anterior caries pattern has a more aggressive course [Peretz et al., 2003], early interventions are of prime importance because the lesions might rapidly spread to the other teeth, which could lead to the entire primary dentition destruction. Also, no extractions due to caries process and only a few restorations were found in the present study (Table 3), indicating that the access to dental offices at this age is limited in Itatiba-SP, Brazil. This is in line with the study from Rihs et al., 2005 in a similar Brazilian community, where they found that there is a high necessity of dental services coverage for young children.

In conclusion, the present study strongly supports, in a representative sample of the city population, that the diagnosis method WHO+ECL was able to identify early caries lesions in this age range and to classify caries activity, then providing valuable information for the earlier establishment of preventive measures for controlling dental caries.

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Table 1. Summary of caries diagnosis criteria codes according to WHO and WHO + ECL.

WHO Codes		**WHO + ECL Codes**	
A	Sound	A	Sound, excluding early caries lesions
B	Cavitated	ECL	Early caries lesion (white chalky spot lesion)
C	Filled with cavity	B	Cavitated, without ECL
D	Filled, without cavity	BECL	Cavitated+ECL
E	Missing, as a result of caries	C	Filled+cronic cavity
F	Missing, any other reason	CECL	Filled+cavity +ECL
-		D	Filled, without cavity
-		DECL	Filled+ECL
-		4	Missing, as a result of caries
-		5	Missing, any other reason

Addapted from Assaf et al., 2006 and Kassawara et al., 2007.

Table 2. Means (±SD) of dmfs and dmft of the epidemiological evaluation and number and percentage of caries-free children according to according to WHO and WHO + ECL criteria.

	Caries diagnosis criteria	
	WHO	**WHO+ECL**
Number of caries-free children	206	114*
% of caries-free children	59	32*
Mean (SD) dmfs	3.0 (±6.9)	6.1 (±9.1)*
Mean (SD) dmft	1.9 (±3.9)	3.8 (±4.3)*

*values in the same line differed statistically ($p<0.05$); SD: standard deviation; %: percentage.

Table 3. Components of the dmfs among children according to WHO and WHO + ECL criteria.

Caries diagnosis criteria		Mean (SD)
WHO + ECL	Early caries lesions (ECL)	3.2 (±4.4)
	Cavitated, without ECL	0.2 (±1.4)
	Cavitated+ECL	2.0 (±5.3)
	Filled+cronic cavity	0.0 (±0.2)
	Filled+ECL+active cavity	0.1 (±0.8)
	Filled, no cavity	0.6 (±1.9)
	Filled+ECL	0,0 (±0.3)
	Missing due to caries	0,0 (±0.0)
WHO	Cavity	2.2(±5.7)
	Filled, with cavity	0.2 (±0.9)
	Filled, no cavity	0.6 (±2.1)
	Missing due to caries	0.0(±.0.0)

*SD: standard deviation.

Figure 1. Distribution of the number of decayed, missing or filled surfaces according to WHO and WHO + ECL criteria.

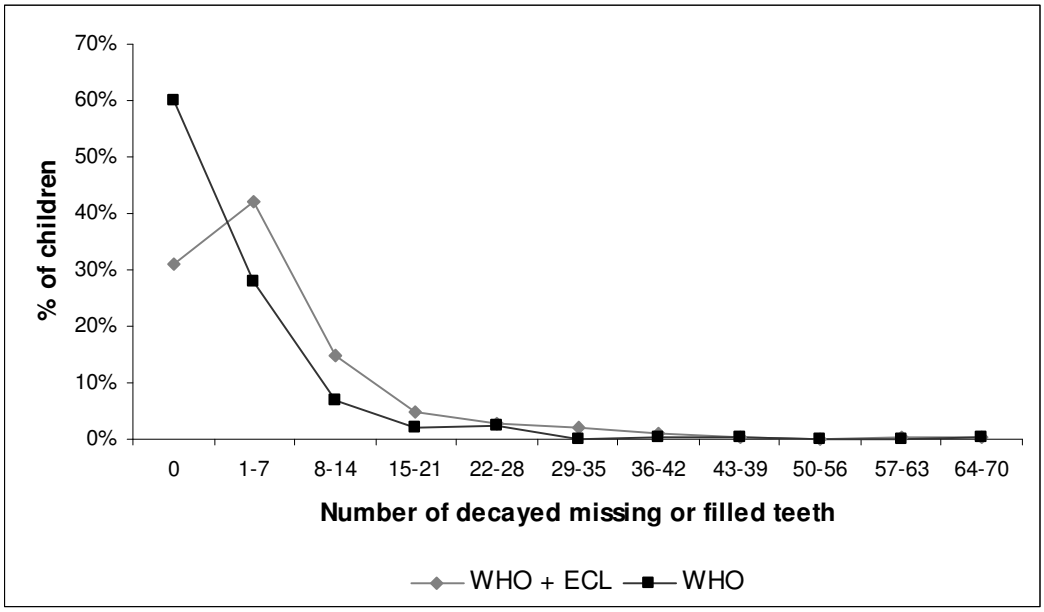


Figure 2. Distribution of the dmfs components by surface type in the children, according to WHO + ECL criteria.

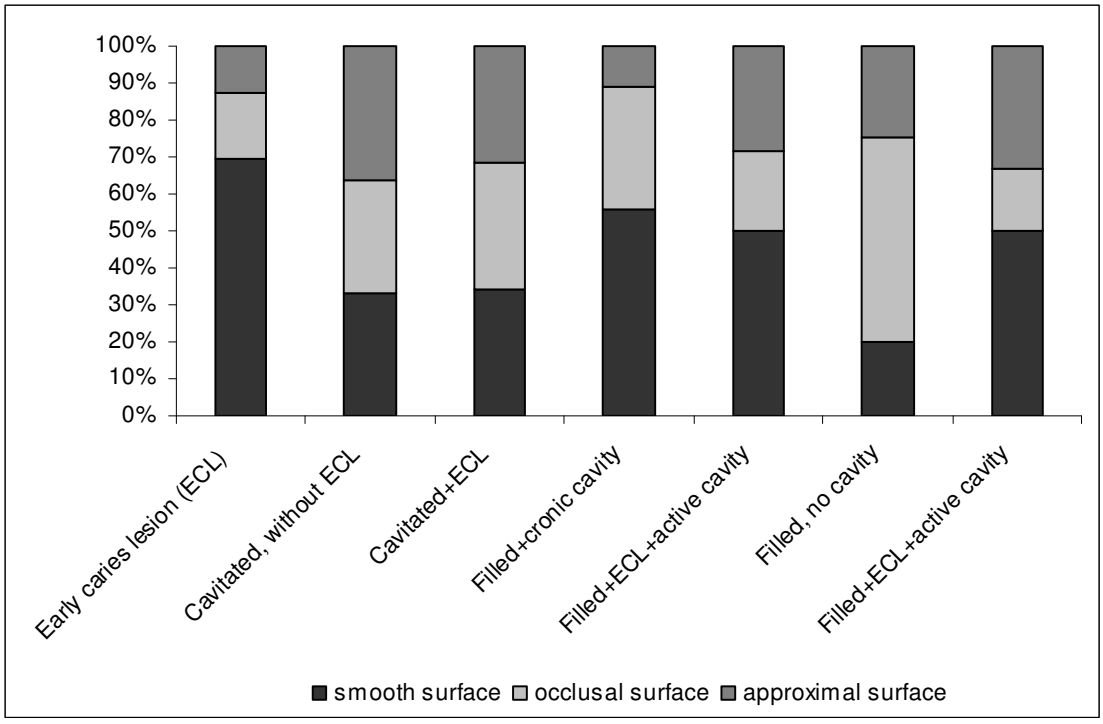
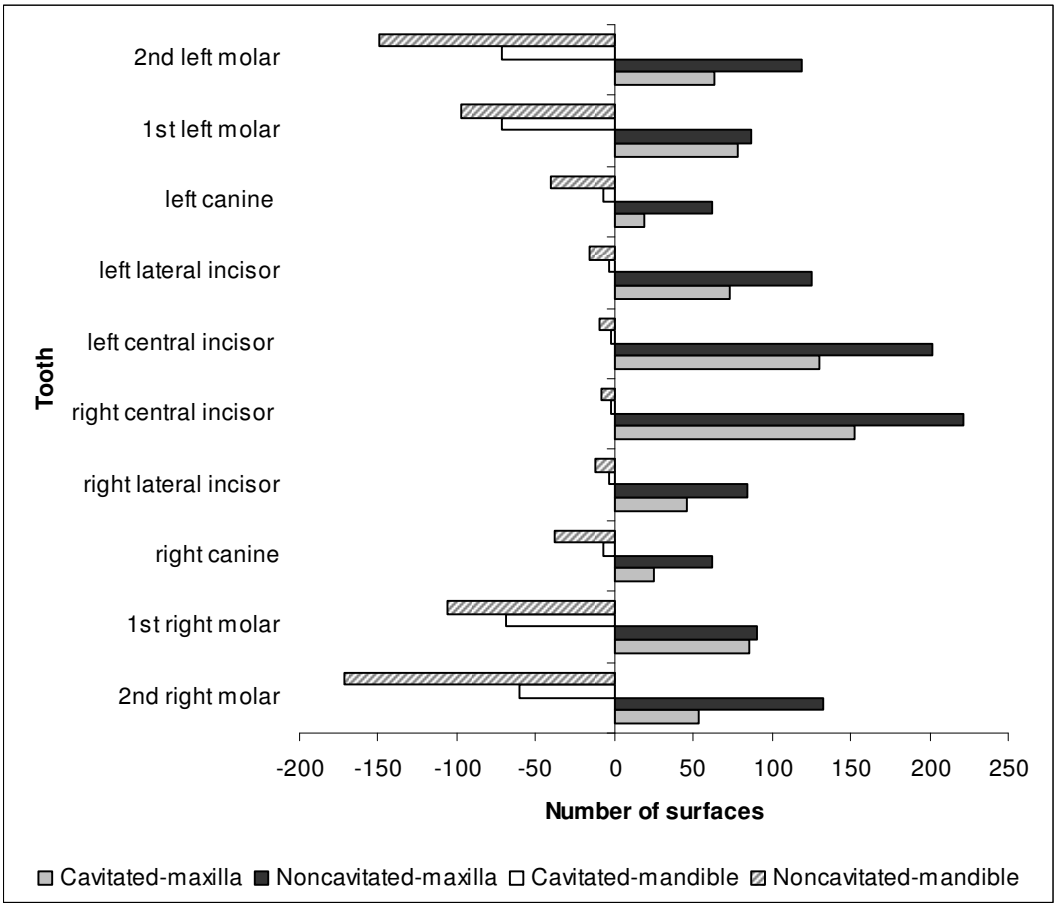


Figure 3. Prevalence of cavitated and noncavitated lesions of maxillary and mandibular teeth according to WHO + ECL criteria.



CAPÍTULO 3

Identification of risk indicators for different stages of early childhood caries

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ABSTRACT

This study aimed to identify risk indictors that may influence early childhood caries, with regard to microbiological composition of dental biofilm, dietary and social factors as well as oral hygiene habits, considering dental caries stages. A total of 169 children were divided in three groups: caries-free (n=53), presenting early caries lesions (n=56) and with cavitated caries lesions (n=60). Dental examinations were conducted using the WHO + early caries lesions (ECL) diagnosis criteria. Before these procedures, the presence of clinically visible dental biofilm on maxillary incisors was recorded. Daily frequency of meals containing sugar was assessed by a diet chart whereas social factors and toothbrushing frequency were evaluated by a questionnaire. The number of colony-forming units of mutans streptococci and total microorganisms as well as presence of lactobacilli was assessed in supragingival biofilm collected from all buccal and lingual smooth surfaces. The data were analyzed by chi-square test, followed by multiple logistic regressions, expressed by odds ratios (OR) with a confidence interval (CI) of 95%. The statistically significant risk indicators associated with ECL were: high levels of mutans streptococci (OR=2.3, CI=1.01-5.14), high daily sugar exposure (OR=5.4, CI=1.42-20.88) and clinically visible dental biofilm presence on the maxillary incisors (OR=2.6, CI=1.07-6.27). The indicators related to cavitated caries lesions were: high total microorganisms counts (OR=4.6, CI=1.56-13.74) and lactobacilli presence (OR=20.31, CI=4.03-102-51; OR=3.4, CI=1.33-8.49). Mutans streptococci counts, daily total sugar exposure and dental biofilm presence may be good predictors for the development of early caries lesions, while total microorganisms counts and presence of lactobacilli may predict caries lesions progression. However, a longitudinal study should be designed to evaluate these possibilities.

INTRODUCTION

The term “early childhood caries” (ECC) includes all dental caries, encompassing non-cavitated lesions that occur in the primary dentition of children younger than 6 years of age. The ECC begins with white chalky spot lesions in the maxillary primary incisors along the gingival margin, where dental biofilm usually accumulates (Selwitz, 2007). Without an early diagnosis allowing effective preventive measures to reverse the caries activity, the lesions can progress very rapidly and, in a period of one year, active early lesions can progress into cavities (Grindefjord *et al.*, 1995). The higher carbonate content (Clasen and Ruyter, 1997), the lower mineralization (Wilson and Beynon, 1989) and the higher porosity (Shellis, 1984; Lindén *et al.*, 1986) of the primary teeth compared to the permanent teeth certainly contribute to this rapid progression.

This disease represents a serious public health problem in disadvantaged communities in both developing and developed countries (Carino *et al.*, 2003; Peressini *et al.*, 2004). In young children, high numbers of cariogenic bacteria, poor oral hygiene and inappropriate feeding practice have been identified as important predisposing caries indicators (Mattos-Granner *et al.*, 1998; Tougher-Decker and van Loveren, 2003; Tsai *et al.*, 2006). Social status, deprivation and number of years of education are also related to caries risk (Oliveira *et al.*, 2008).

The serious consequences of ECC include pain, infection, chewing difficulty, malnutrition, gastrointestinal disorders and low self-esteem (Ramos-Gomez et al., 2002). Furthermore, preschoolers with ECC present a higher risk for new caries lesions development in their permanent teeth (Peretz *et al.*, 2003; Mattila *et al.*, 2005). The information regarding white chalky spot lesions in these children should provide important additional components for the understanding of the early childhood caries process (Drury *et al.*, 1999). Moreover, there is no comparison available in the scientific literature with respect to microbiological composition and presence of dental biofilm, dietary and social factors as well as oral hygiene habits, concerning dental caries stages. In this context, the aim of the present study was to identify indicators that may predict the development and progression of ECC, in a representative sample of preschoolers.

MATERIAL AND METHODS

Ethical considerations

This study was approved by the Ethical Committee in Research of Piracicaba Dental School/State University of Campinas (UNICAMP) under protocol number 015/2006 and the preschools also granted permission for the study. The children's parents signed a written informed positive consent.

Sample

All 3 to 4 years old children enrolled in the 6 public nurseries and 17 preschools in the urban area of Itatiba-SP/Brazil were invited to participate in the study. This age range was chosen because in this stage of life, all primary teeth are supposed to be erupted and no permanent teeth should be present in the mouth. The city of Itatiba has a population of about 91 000 habitants. It is considered one of the best cities of the State of São Paulo as for the quality of life and infrastructure, showing a human development index of 0.83. The majority of the habitants live in the urban area, where all households have access to public water supply with fluoride level of 0.7 ppm. Children from public nurseries and preschools in the city where the present study took place are from mid socioeconomic backgrounds.

A minimum sample size of 123 children was required to achieve a level of precision with a 0.07 standard error. The 95 percent confidence interval level and caries prevalence (0.72) found in a previous pilot study carried out with part of these children were used for the sample size calculation. It was decided to invite all 3 to 4 years old children in order to minimize eventual problems that would contribute to a sample size smaller than the minimum calculated. Preschoolers whose parents refused to sign the informed consent term, or who did not collaborate with the clinical exams were excluded from the study without any prejudice. Moreover, preschoolers whose responsibles did not attend the scheduled school meetings at entrance/exit time to understand the study's aims and/or its importance, or refused to fill a chart that was used to evaluate the dietary habits were also dismissed. Still, the children who were absent from school when biofilm was collected for the microbiological analysis were also lost. For all of these reasons, from the 546 children invited to participate only 176 were enrolled. Out of these 176 preschoolers, 7 were

excluded because they only presented fillings without decay or fillings with white chalky spot lesions.

Thus, the final sample comprised 169 children of both genders (88 females and 81 males) aging from 36 to 48 months. These children were divided in three groups:

- Caries-free children (CF) group (n=53): the number of decayed missing or filled surfaces (dmfs) scored 0. Children who presented no caries lesion.
- Children presenting early caries lesions (ECL) group (n=56): the number of dmfs ≥1. Preschoolers who presented white chalky spots lesions only.
- Children with cavitated caries lesions (CCL) group (n=60): the number of dmfs ≥1. Preschoolers who presented a minimum of one cavitated caries lesion.

Caries assessment

Dental examinations were conducted by one of the authors (T.M.P.) using a mouth mirror, gauze and a ball-ended probe under a focusable flashlight. Cross-infection control measures were followed rigorously. Before these procedures, clinically visible dental biofilm on maxillary incisors was recorded (Alaluusua and Malmivirta, 1994). The dental examiner sat behind the child, who was lying on a table, and was assisted by a scribe. Prior to the beginning of the study, replicate examinations were carried out, on a random sample of 23 preschoolers of the subjects studied, with a 1-week interval period, to avoid the dental examiner memorization. Intra-examiner agreement, taking into account all components from the diagnostic criteria, was of 0.78 measured using Kappa calculation at the surface level.

The criteria used for early childhood caries diagnosis in the present study was WHO + ECL (Nyvad *et al.*, 1999, Assaf *et al.*, 2006; Kassawara *et al.*, 2007) (Table 1). Thus, caries was recorded as manifested lesions if frank cavitations were present and as early caries lesions if white chalky spot lesions were present. In smooth surfaces the ECL were diagnosed if there were active white spot lesions, which were chalky and rough, usually adjacent or close to the soft tissue margin where the dental biofilm accumulates; in occlusal surfaces, caries was recorded on lesions extending along the walls of the fissure, where increased opacity and roughness were evident. Gentle probing was used to assess the ECL

roughness as well as to remove debris to enhance visualization. Gauze was employed in order to dry or clean the teeth favoring the ECL identification.

The units of evaluation used in the clinical exams were dmfs (decayed, missing and filled surfaces).

Dietary sugar consumption evaluation

In order to assess the children's daily frequency of meals containing sugar, the mothers and health agents of the preschools participating in the study were asked to fill a diet chart for 3 consecutive days, except for the weekends. This diet chart included the time of the day that the children ate and drank anything as well as the content of all meals and snacks. Using this dietary chart, the daily frequency of total sugar exposure was calculated. Additionally, the daily frequency of baby bottle consumption and its use with sweetened fluids before sleeping were estimated.

Questionnaire

The children's mothers were asked to fill a standardized questionnaire, with closed questions, assisted by two of the authors (T.M.P. and C.S.O.). The questionnaire encompassed information regarding family income, mother's education level and hygiene practices of the children. For income data, the question was "How much is the family income per month?" and the values were obtained in Brazilian currency (1 *real* ≈ ½ dollar); for the oral hygiene habits data the question included frequency of toothbrushing; for mother´s level of education the question addressed was "What is your level of scholar education?". In addition the child's ethnicity was also registered in the questionnaire by one of the researchers (T.M.P. or C.S.O.).

Collection of dental biofilm samples and microbiological analysis

Supragingival biofilm collection was performed from all buccal and lingual smooth surfaces, except to the cavities interior, at least one hour after food intake in the afternoon period. A sterilized plastic disposable handle (Greiner, Frickenhausen, Germany) with a circle opening of about 1 µl volume capacity on its extreme was used for the collection in order to standardize biofilm quantity. Biofilm samples were immediately placed in a caped microcentrifuge tube containing 1 ml of reduced transport fluid (Syed and Loesche, 1972).

These tubes were transported in refrigerated boxes (4ºC) to the Pediatric Dentistry Laboratory at the Piracicaba Dental School, where microbiological analysis was performed within 6 hours to maintain the cellular viability (Ersin *et al.*, 2006). Firstly, the tubes were vortexed for approximately 45 seconds and the suspension was serially diluted with saline solution 0.9%. For each dilution, 25 µl of the samples were placed in triplicate in three media: 1. Mitis Salivarius agar (Difco, Sparks, MD) with 0.2 units/ml bacitracin (Sigma, Poole, UK) to assess the number of colony-forming units (CFU) with typical morphology of mutans streptococci (MS); 2. Rogosa agar- (Difco, Sparks, MD) supplemented with 0.13 % glacial acetic acid to assess the presence of lactobacilli (LB); 3. Brain Heart Infusion agar (Difco, Sparks, MD) with 5% defibrinated sheep blood to assess the number CFU of total microorganisms (TM). The plates were incubated for 24 h at 37°C (Dasanayake *et al.*, 1993) in a candle-extinguish jar obtaining a 5-10% carbon dioxide atmosphere, except to the Rogosa agar plates that were incubated for 48h (Ersin *et al.*, 2006). The counts were performed using a stereomicroscope.

Statistical analysis

Data were analyzed using the Statistical Package for Social Science 9.0 (SPSS Inc., Chicago, IL, USA). Univariate analysis was initially performed, using the chi-squared test, between the caries lesions stages (dependent variable) and microbiological composition and presence of dental biofilm, dietary and social factors as well as oral hygiene habits (independent variables). The variables that showed a significant association ($p<0.05$) with the dependent variable were selected for multivariate logistic regression analysis, expressed by odds ratios (OR) with a confidence interval (CI) of 95%. For these analyses, all the independent variables were dichotomized based on their median values. The statistical tests were considered at the level of significance of 5%.

RESULTS

Table 2 evidences the association between early childhood caries stages and microbiological composition and presence of dental biofilm, dietary and oral hygiene habits as well as social variables. After univariate analysis, the factors that showed statistical

significance with regard to CF versus ECL group were: mutans streptococci counts, daily total sugar exposure and presence of dental biofilm on the maxillary incisors (Table 2); because of this, they were submitted to a multiple logistic regression analysis. After multivariate modelling, all these evaluated variables revealed statistically significant odds ratios ranging from 2.3 to 5.4 (Table 3). Considering CF versus CCL group, the univariate analysis has revealed that the significant factors were: total microorganisms counts, lactobacilli presence and daily total sugar exposure (Table 2); furthermore, the multivariate analysis has shown that total microorganisms counts and lactobacilli presence were strong indicators for cavitated lesions development, with odds ratios of 4.6 and 20.3, respectively (Table 4). Also, after chi-square tests, the statistically significant factors regarding ECL versus CCL group were: lactobacilli presence and oral hygiene frequency; however, the multivariate modelling has identified only lactobacilli as an impact caries risk indicator.

DISCUSSION

This study shows for the first time that there is a relationship between microbiological composition of dental biofilm and the stages of dental caries lesions in the early childhood. While mutans streptococci counts may be a good risk indicator for the development of early caries lesions, the lactobacilli counts may predict caries lesions progression. In this respect, caries-free children highly infected ($>10^6$ – Table 3) by mutans streptococci were 2.3 times more likely to have ECL when compared to those less infected ($\leq 10^6$ –Table 3). The literature has revealed several studies that used a regression model and has verified that MS was a significant factor for ECC (Milgrom *et al.* 2000; Mattos-Graner *et al.*, 2001; Ramos-Gomez *et al.*, 2002; Vachirarojpisan *et al*., 2004). However, there are no studies previously reported that compared a group of preschoolers with early lesions only and a group with cavitated ones. It was also observed in the present research that the high total microorganisms counts and the presence of lactobacilli have significantly influenced the development of cavitated lesions. It was verified that the presence of LB in the children's oral cavity without caries or with ECL was associated with 20.3 and 3.4 times more chance of a child to develop cavitated lesions, respectively (Table 4 and 5). A

possible explanation for these findings could be that cavities provide a favorable ecological niche for microorganisms colonization, such as a retentive area with low pH (Matee *et al.*, 1992; Fejerskov, 2004; Selwitz, 2007). Moreover, during the cyclic pH variation in the oral cavity, due to carbohydrate fermentation by mutans streptococci, acid products damage the protective exterior tooth surface, allowing the LB to colonize these areas producing more acid substrates and further damaged areas (Ramos-Goméz *et al.*, 2002). These findings strongly emphasize that the MS are related to caries initiation and LB with caries progression (Krishnakumar *et al.*, 2002).

As dental caries is a multifactorial disease, other important factors are involved in this process. In light of this, among the dietary habits, children without caries presenting high daily frequency of total sugar consumption were 5.4 times more likely to develop early caries lesions than caries-free children (Table 3). In this context, Milgrom *et al.* (2000) have verified that children who consumed cariogenic snack foods more frequently had 7.8 times (CI=2.45-25.16) more chance to develop white chalky spot lesions. The continuous sugar exposure over extended periods leads to MS accumulation to pathological levels, which enables the caries process initiation, and this was shown in this research, as previously mentioned. This happens because sucrose serves as a specific substrate to glucan production favoring mutans streptococci adherence (Loesche, 1986). Although the total sugar consumption was statistically significant in the univariate analysis ($p<0.05$ - Table 2) regarding CF versus CCL group, in the multivariate modelling, where it was analyzed simultaneously with the microbiological factors (Table 4), total sugar consumption did not achieve statistical significance. This result could evidence that total microorganism counts as well as lactobacilli presence might be stronger indicators for cavities development.

Even though the inappropriate feeding behavior intensifies the risk of caries, especially during the sleep time due to oral clearance and salivary flow decreases (Berkowitz, 2003), the literature reveals contradictory results. Whereas Hallett and O'Rourke (2002, 2003, 2006) have found that sleeping with a bottle at night have increased the risk of developing caries in 1.73 (CI=1.49-2.00), 1.9 (CI=1.5-2.4) and 1.5 times (CI=1.1-2.2) respectively, the study from Milgrom *et al.* (2000) that considered younger children, have not supported these findings and still have shown that baby bottle

consumption (BBC) decreased with age. Although BBC on free demand allows the sugar content to be in constant contact with the dental structures favoring tooth demineralization, it is important to emphasize that other sources of sucrose, particularly the sticky ones (Touger-Decker and van Loveren, 2003), are also able to demineralize teeth.

Considering the social factors including family income, mother's education level and ethnicity, none of these variables has achieved a significant level in the univariate analysis for early or cavitated lesions (Table 2). Other studies have already demonstrated that these social factors are related to ECC (Hallett and O' Rourke, 2003, 2006; Oliveira *et al.*, 2008). However, our study was conducted in a small city from a developing country with children attending public nurseries and preschools. Therefore, our sample comprised children from mid socioeconomic backgrounds without significant differences regarding income, level of education and ethnicity, being a very homogeneous sample.

The role played by toothbrushing in the development of ECC was also evaluated in this investigation. Despite the fact that the univariate analysis has revealed that children with ECL were significantly more likely to brush their teeth more than three times a day compared to children presenting cavities, this variable could not be pointed out as an impact risk indicator, because in the multivariate modelling, it did not achieve statistical significance (Table 5). A possible explanation could be that a high frequency of toothbrushing is not synonymous of high quality cleaning standard (Bellini *et al.*, 1981). Moreover, the fact that the questionnaires were answered by the supervision of two dentists-researchers, the mothers may not have felt comfortable in revealing the real situation. Considering the influence that oral hygiene habits have in biofilm presence, this clinical parameter was also evaluated. It was verified that caries-free children who presented biofilm accumulation on the maxillary incisors were 2.6 (CI=1.07-6.27) times more likely to develop early caries lesions than CF children (Table 3).

It is important to highlight that this is a cross-sectional study and, therefore, longitudinal investigations considering the child's response to a determined factor during the disease process are necessary to improve the knowledge about early childhood caries risk factors.

Mutans streptococci counts, daily total sugar exposure and dental biofilm presence may be good risk factors for the development of early caries lesions and total microorganisms counts and lactobacilli presence may predict caries lesions progression. However, a longitudinal study should be designed to evaluate these possibilities.

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Table 1. Summary of caries diagnosis criteria codes according to WHO + ECL criteria.

WHO + ECL Codes	
A	Sound, excluding early caries lesions
ECL	Early caries lesion (white chalky spot lesion)
B	Cavitated, without ECL
BECL	Cavitated+ECL
C	Filled+cronic cavity
CECL	Filled+cavity +ECL
D	Filled, without cavity
DECL	Filled+ECL
4	Missing, as a result of caries
5	Missing, any other reason

Adapted from Nyvad *et al.*, 1999, Assaf *et al.*, 2006 and Kassawara *et al.*, 2007.

Table 2. The relationship between early childhood caries and related factors.

Variables	CF x ECL		CF x CCL		ECL x CCL	
	0	1	0	1	0	1
	n(%)		n(%)		n(%)	
mutans streptococci counts (CFU)	p = 0.028*		p = 0.102		p = 0.534	
> 10^6	21(38)	34(62)	21(39)	33(61)	34(51)	33(49)
≤ 10^6	32(59)	22(41)	32(54)	27(46)	22(45)	27(55)
Total microorganisms counts (CFU)	p = 0.102		p = 0.006*		p = 0.268	
> 10^7	32(43)	42(57)	32(39)	50(61)	42(46)	50(54)
≤ 10^7	21(60)	14(40)	21(68)	10(32)	14(58)	10(42)
Lactobacilli	p = 0.094		p = 0.000*		p = 0.006*	
present	2(20)	8(80)	2(8)	22(92)	8(27)	22(73)
ausent	51(52)	48(48)	51(57)	38(43)	48(56)	38(44)
Daily frequency of baby -bottle	p = 0.163		p = 0.100		p = 0.894	
> 2	2(22)	7(78)	2(20)	8(80)	7(47)	8(53)
≤ 2	51(51)	49(49)	51(29)	52(71)	49(49)	52(51)
Put to sleep with a baby-bottle with sweetened liquid	p = 0.311		p = 0.369		p = 0.891	
yes	22(55)	18(45)	22(52)	20(48)	18(47)	20(53)
no	31(45)	38(55)	31(44)	40(56)	38(49)	40(51)
Daily solid sugar consumption	p = 0.614		p = 0.052		p = 0.145	
> 3	23(46)	27(54)	23(38)	37(62)	27(42)	37(58)
≤ 3	30(51)	29(49)	30(57)	23(43)	29(56)	23(44)
Daily liquid sugar consumption	p = 0.657		p = 0.616		p = 0.335	
> 3	29(47)	33(53)	29(49)	30(51)	33(52)	30(48)
≤ 3	24(51)	23(49)	24(44)	30(56)	23(43)	30(57)
Daily total sugar consumption	p = 0.007*		p = 0.049*		p = 0.383	
> 6	3(18)	14(82)	3(21)	11(79)	14(56)	11(44)
≤ 6	50(54)	42(46)	50(51)	49(49)	42(46)	49(54)
Ethnicity	p = 0.648		p = 0.673		p = 0.967	
Caucasian	39(50)	39(50)	39(48)	42(52)	39(48)	42(52)
Non-caucasian	14(45)	17(55)	14(44)	18(56)	17(49)	18(51)
Mother's education level	p = 0.251		p = 0.462		p = 0.660	
≥ complete first grade	42(52)	39(48)	42(49)	44(51)	39(47)	44(53)
≤ incomplete first grade	11(39)	17(61)	11(41)	16(59)	17(52)	16(48)
Family income per month	p = 0.679		p = 0.190		p = 0.080	
≥ R$ 1.200	33(47)	37(53)	33(52)	30(48)	37(55)	30(45)
< R$ 1.200	20(51)	19(49)	20(40)	30(60)	19(39)	30(61)
Daily oral hygiene frequency	p = 0.158		p = 0.599		p = 0.048*	
> 3	30(43)	39(57)	30(49)	31(51)	39(56)	31(44)
≤ 3	23(58)	17(43)	23(44)	29(56)	17(37)	29(63)
Dental biofilm	p = 0.002*		p = 0.068		p = 0.668	
present	30(41)	43(59)	30(41)	44(59)	43(49)	44(51)
ausent	23(64)	13(36)	23(59)	16(41)	13(45)	16(55)

* Significance evaluated by the chi-square test or by Fisher's exact test.
CFU: colony-forming units

Table 3. Multivariate modelling of caries-free children x children with early caries lesions.

Variable	Multivariate OR	95%CI	p-value
mutans streptococci counts (CFU)			
$> 10^6$	2.3	1.01-5.14	0.048
$\leq 10^6$	1		
Daily total sugar consumption			
> 6	5.4	1.42-20.88	0.013
≤ 6	1		
Biofilm			
present	2.6	1.07-6.27	0.034
ausent	1		

Model fitting information:-2 Log Likelihood (21.554), Chi-square (17.355), Freedom –degrees (3), Significance (0.001).

CFU: colony-forming units

Table 4. Multivariate modelling of caries-free children x children with cavitated caries lesions.

Variable	Multivariate OR	95% CI	p-value
Total microorganisms counts (CFU)			
$> 10^7$	4.6	1.56-13.74	0.006
$\leq 10^7$	1		
Lactobacilli			
present	20.3	4.03-102.51	0.000
ausent	1		
Daily total sugar consumption			
> 6	3.2	0.75-13.47	0.116
≤ 6	1		

Model fitting information:-2 Log Likelihood (15.119), Chi-square (33.815), Freedom –degrees (3), Significance (0.000).

CFU: colony-forming units

Table 5. Multivariate modelling of children with early caries lesions x children with cavitated caries lesions.

Variable	Multivariate OR	95%CI	p-value
Lactobacilli			
present	3.4	1.33-8.49	0.010
ausent	1		
Daily oral hygiene frequency			
> 3	1		
≤ 3	2.0	0.94-4.52	0.072

Model fitting information:-2 Log Likelihood (14.474), Chi-square (11.121), Freedom –degrees (2), Significance (0.004).

IV – CONCLUSÃO GERAL

1. A avaliação qualitativa dos artigos incluídos na revisão sistemática comprovou os níveis de SM são um forte indicador de risco para a cárie precoce da infância. Entretanto, estudos longitudinais com melhor delineamento, a fim se obter maiores níveis de evidência científica, são necessários para que os níveis de SM sejam apontados como fatores de risco de impacto.

2. A utilização de um critério para o diagnóstico da cárie precoce da infância que incluiu as lesões de mancha branca possibilitou a identificação precoce dos grupos de alto risco e atividade de cárie, o que viabiliza a implementação de medidas preventivas para o controle da doença.

3. A análise da influência da composição microbiológica do biofilme dentário, dieta, fatores sociais e hábitos de higiene bucal nos estágios de desenvolvimento da CPI permitiu apontar como possíveis fatores de risco para a iniciação desta doença: altos níveis de SM, alta freqüência de exposição ao açúcar total e presença de biofilme nos incisivos superiores. Adicionalmente, a alta contagem de microrganismos totais e a presença de lactobacilos podem ser apontados como fatores de risco para a progressão da CPI. Contudo, um estudo longitudinal torna-se necessário para que estas possibilidades sejam comprovadas.

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* De acordo com a norma da UNICAMP/FOP, baseadas na norma do International Committee of Medical Journal Editors – Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

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ANEXO 1

INFORMAÇÃO CCPG/OO2/06[6]

Tendo em vista a necessidade de revisão da regulamentação das normas sobre o formato e a impressão das dissertações de mestrado e teses de doutorado e com base no entendimento exarado no Parecer PG n° 1985/96, que trata da possibilidade do formato alternativo ao já estabelecido, a CCPG resolve:

Artigo 1º - O formato padrão das dissertações e teses de mestrado e doutorado da UNICAMP deverão obrigatoriamente conter:

I. Capa com formato único ou em formato alternativo que deverá conter informações relativas ao nível (mestrado ou doutorado) e à Unidade de defesa, fazendo referência à Universidade Estadual de Campinas, sendo o projeto gráfico das capas definido pela PRPG.
II. Primeira folha interna dando visibilidade à Universidade, a Unidade de defesa, ao nome do autor, ao título do trabalho, ao número de volumes (quando houver mais de um), ao nível (mestrado ou doutorado), a área de concentração, ao nome do orientador e co-orientador, ao local (cidade) e ao ano de depósito. No seu verso deve constar a ficha catalográfica.
III. Folha de aprovação, dando visibilidade à Comissão Julgadora com as respectivas assinaturas.
IV. Resumo em português e em inglês (ambos com no máximo 500 palavras).
V. Sumário.
VI. Corpo da dissertação ou tese dividido em tópicos estruturados de modo característico à área de conhecimento.
VII. Referências, formatadas segundo normas de referenciamento definidas pela CPG da Unidade ou por critério do orientador.
VIII. Todas as páginas deverão, obrigatoriamente, ser numeradas, inclusive páginas iniciais, divisões de capítulos, encartes, anexos, etc... As páginas iniciais poderão ser numeradas utilizando-se algarismos romanos em sua forma minúscula.
IX. Todas as páginas com numeração "impar" serão impressas como "frente" e todas as páginas com numeração "par" serão impressas como "verso".

§ 1º - A critério do autor e do orientador poderão ser incluídos: dedicatória; agradecimento; epígrafe; lista de: ilustrações, tabelas, abreviaturas e siglas, símbolos; glossário; apêndice; anexos.

§ 2º - A dissertação ou tese deverá ser apresentada na língua portuguesa, com exceção da possibilidade permitida no artigo 2° desta Informação.

§ 3º - As dissertações e teses cujo conteúdo versar sobre pesquisa envolvendo seres humanos, animais ou biossegurança, deverão apresentar anexos os respectivos documentos de aprovação.

Artigo 2º - A critério do orientador e com aprovação da CPG da Unidade, os capítulos e os apêndices poderão conter cópias de artigos de autoria ou de co-autoria do candidato, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, escritos no idioma exigido pelo veículo de divulgação.

§ único - O orientador e o candidato deverão verificar junto às editoras a possibilidade de inclusão dos artigos na dissertação ou tese, em atendimento à legislação que rege o direito autoral, obtendo, se necessária, a competente autorização, deverão assinar declaração de que não estão infringindo o direito autoral transferido à editora.

Artigo 3º - Dependendo da área do conhecimento, a critério do orientador e com aprovação da CPG da Unidade, a dissertação ou tese poderá ser apresentada em formato alternativo, desde que observados os incisos I, II, III IV, V e VII do artigo 1º.

Artigo 4º - Para impressão, na gráfica da Unicamp, dos exemplares definitivos de dissertações e teses defendidas, deverão ser adotados os seguintes procedimentos:

§ 1º - A solicitação para impressão dos exemplares de dissertações e teses poderá ser encaminhada à gráfica da Unicamp pelas Unidades, que se responsabilizarão pelo pagamento correspondente.

§ 2º - Um original da dissertação ou tese, em versão definitiva, impresso em folha tamanho carta, em uma só face, deve ser encaminhado à gráfica da Unicamp acompanhado do formulário "Requisição de Serviços Gráficos", onde conste o número de exemplares solicitados.

§ 3º - A gráfica da Unicamp imprimirá os exemplares solicitados com capa padrão. Os exemplares solicitados serão encaminhados à Unidade em, no máximo, cinco dias úteis.

§ 4º - No formulário "Requisição de Serviços Gráficos" deverão estar indicadas as páginas cuja reprodução deva ser feita no padrão "cores" ou "foto", ficando entendido que as demais páginas devam ser reproduzidas no padrão preto/branco comum.

§ 5º - As dissertações e teses serão reproduzidas no padrão frente e verso, exceção feita às páginas iniciais e divisões de capítulos; dissertações e teses com até 100 páginas serão reproduzidas no padrão apenas frente, exceção feita à página que contém a ficha catalográfica.

§ 6º - As páginas fornecidas para inserção deverão ser impressas em sua forma definitiva, ou seja, apenas frente ou frente/verso.

§ 7º - O custo, em reais, de cada exemplar produzido pela gráfica será definido pela Administração Superior da Universidade.

Artigo 5º - É obrigatória a entrega de dois exemplares para homologação.

Artigo 6º - Esta Informação entrará em vigor na data de sua publicação, ficando revogadas as disposições em contrário, principalmente as Informações CCPG 001 e 002/98 e CCPG/001/00.

Campinas, 13 de setembro de 2006

Profa. Dra. Teresa Dib Zambon Atvars
Presidente
Comissão Central de Pós-Graduação

ANEXO 2

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Associação entre cárie precoce na infância e variáveis clínicas, bioquímicas, microbiológicas e comportamentais em pré-escolares de 24 a 60 meses**", protocolo nº **015/2006**, dos pesquisadores **THAÍS MANZANO PARISOTTO e MARINÊS NOBRE DOS SANTOS UCHÔA**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 31/03/2006.

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**Association between early childhood caries and clinical, biochemical, microbiological and behavioral variables in preschool children aging 24 to 60 months**", register number **015/2006**, of **THAÍS MANZANO PARISOTTO and MARINÊS NOBRE DOS SANTOS UCHÔA**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for researching in human subjects and was approved by this committee at 31/03/2006.

Profa. Cecília Gatti Guirado
Secretária
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

ANEXO 3

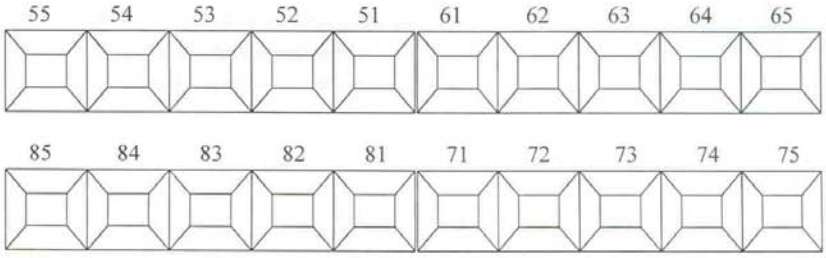
FICHA CLÍNICA

Nome:_____Nº da Ficha:_____
Creche:_____Data exame:_____

Data nasc.:_____Idade (meses):_____Sexo: (F) (M) Raça: (B) (N) (P)

A: hígido;
W: mancha branca ativa
B: cariado com lesão crônica;
BW: cariado com lesão ativa;
C: cariado com lesão crônica de cárie;
CW: cariado com lesão ativa de cárie;
D: restaurado sem lesão de cárie;

DW: restaurado com mancha branca;
4: perdida devido à cárie;
5: perdido por outra razão;
F: selante de fissura;
FW: selante de fissura com mancha branca;
7: coroa
T: trauma (fratura)



Biofilme clinicamente visível:_____
0: ausência
1:presença

ANEXO 4

Figura 1: Realização do exame clínico para determinação do índice de cárie

Figura 2: Instrumentos utilizados para a realização do exame clínico.

Figura 3: Manifestação clínica inicial (A) e lesão já cavitada (B) da cárie precoce da infância nos incisivos superiores. As manchas brancas ativas foram incluídas no critério de diagnóstico empregado nesse trabalho.

ANEXO 5

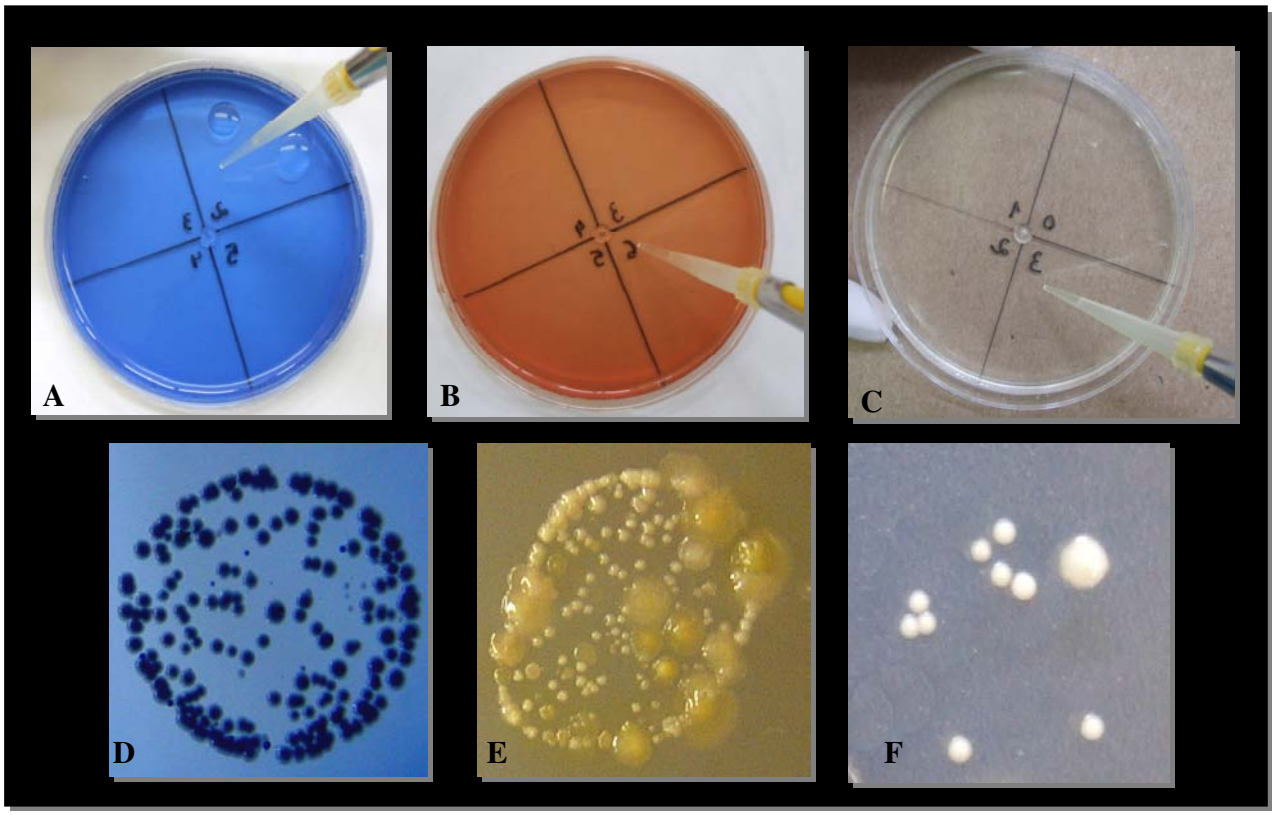


Figura 4: Plaqueamento do biofilme após a diluição em série decimal nos meios de cultura: Mitis Salivarius + Bacitracina (A) , Ágar Sangue (B) e Rogosa (C). Colônias de estreptococos do grupo mutans (D), microrganismos totais (E) e lactobacilos (F), crescidas após incubação à 37°C.

ANEXO 6

QUESTIONÁRIO

Creche/pré-escola: _____Data:_____

Nome (filho): _____Data nasc.: -_____ Raça: (B) (N) (P)

Nome (mãe): _____Data nasc.:_____

Estado civil:_____

1) Grau de instrução da mãe:

a- ( ) sem instrução

b- ( ) primeiro grau completo

c- ( ) primeiro grau incompleto

d- ( ) segundo grau completo

e- ( ) segundo grau incompleto

f- ( ) superior

2) Renda familiar

a- ( ) menos de 1 salário mínimo

b- ( ) 1 a 2 salários mínimos

c- ( ) 3 a 4 salários mínimos

d- ( ) 5 a 6 salários mínimos

e- ( ) 7 a 8 salários mínimos

f- ( ) Outro_____

3) Com que idade começou a escovar os dentes do seu filho?

a- ( ) assim que os primeiros dentes nasceram

b- ( ) durante o primeiro ano de idade

c- ( ) durante o segundo ano de idade

d- ( ) durante o terceiro ano de idade

4) Quantas vezes por dia você acha que seu filho deveria escovar os dentes por dia?

a- ( ) 1 vez por dia
b- ( ) 2 vezes por dia
c- ( ) 3 a 4 vezes por dia
d- ( ) às vezes
e- ( ) não deveria escovar

5) Você tem automóvel/carro?

a- ( ) sim
b- ( ) não

6) Você tem plano de saúde?

a- ( ) sim
b- ( ) não

7) Quem escova os dentes do seu filho?

a- ( ) mãe ou responsável

b- ( ) seu filho escova sozinho

c- ( ) não escova

8) Quem você acha que deveria escovar os dentes do seu filho?

a- ( ) mãe ou responsável

b- ( ) seu filho sozinho

c- ( ) não escova

9) Quantas vezes por dia seu filho escova os dentes em casa?

a- ( ) 1 vez por dia

b- ( ) 2 vezes por dia

c- ( ) 3 a 4 vezes por dia

d- ( ) às vezes

e- ( ) não escova

10) Qual a pasta dental utilizada?

a- ( ) não utiliza pasta
b- ( ) Tandy
c- ( ) Colgate
d- ( ) Sorriso
e- ( ) Outra_____

11) Ao nascimento a criança:

a- ( ) só foi amamentada no peito

b- ( ) só foi amamentada com mamadeira

c- ( ) foi amamentada com peito e mamadeira

12) Por quanto tempo a criança foi amamentada?

a- ( ) menos de 6 meses

b- ( ) 6 meses

c- ( ) 12 meses

d- ( ) mais de 12 meses

13) A criança usa o peito como chupeta?

a- ( ) sim
b- ( ) não

14) A criança usa a mamadeira como chupeta?

a- ( ) sim
b- ( ) não

Antecedentes médicos:

1. Saúde da criança:

( ) boa ruim ( )

2. Se ruim, qual o problema?_____

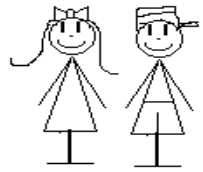
3. A criança consultou-se com algum médico nos últimos 6 meses?

( ) sim ( ) não

Se sim, por quê?_____

ANEXO 7

Diário de Dieta



Nome da criança:_____
Escola:_____
Telefone:_____

	_____/_____ _____- feira	_____/_____ _____- feira	_____/_____ _____- feira
Café da manhã			
Lanche da manhã			
Almoço			
Lanche da tarde			
Jantar			
Antes de dormir			