

Päivi Ollila

ASSESSMENT OF CARIES RISK IN TODDLERS

A LONGITUDINAL COHORT STUDY

FACULTY OF MEDICINE,
INSTITUTE OF DENTISTRY,
UNIVERSITY OF OULU



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**ASSESSMENT OF CARIES RISK
IN TODDLERS**

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Abstract

Dental caries in toddlers was studied in relation to several risk factors which were also determined by salivary tests in a longitudinal design. Another specific aim was to reveal the effect of prolonged pacifier sucking on caries development.

The study population consisted of 183 pre-school children. At the baseline, salivary microbiological tests were taken from children whose average age was 2.5 years. Risk factors for colonisation of salivary lactobacilli and yeasts were determined from a questionnaire filled in by the parents. At the 2-year follow-up, caries in primary teeth was studied against the possible risk factors recorded at the baseline. At the 7-year follow-up, the risk factors identified at the age of two were analysed against caries development in primary molars and in first permanent molars. Also the long-term predictive value of salivary microbiological tests was investigated.

At the baseline, the use of pacifier and nocturnal use of nursing bottle were associated with colonisation of salivary lactobacilli and yeasts. Prolonged pacifier sucking and use of nursing bottle at nights were shown to be associated with caries development in children at the 2-year follow-up. Consumption of sweets, lack of daily tooth brushing and nocturnal use of nursing bottle at the age of two were associated with caries onset in both primary and permanent molars at the seven-year follow-up. The use of fluoride tablets reduced the risk of caries onset in primary molars. Children who were colonised by salivary lactobacilli or yeasts at the baseline were susceptible to caries in primary molars. Early colonisation of lactobacilli was associated with caries in permanent molars.

The results suggest that the risk of caries is possible to assess in toddlers by identifying caries-related habits early, already at the age of two. Microbiological tests may also have some value. Assessment of caries risk in toddlers enables both prevention and early intervention, and thereby prevention of caries development in children.

Keywords: breastfeeding, children, dental caries, lactobacilli, nursing bottle, pacifier, risk factors, yeasts

Ollila, Päivi, Kariesriskin määrittäminen taaperoilla. Pitkäaikainen seuranta tutkimus

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Tiivistelmä

Tämän tutkimuksen tarkoituksena oli pitkäaikaisseurannassa selvittää taaperoikäisiltä lapsilta otettujen sylkitekstiä ja muiden määritettyjen riskitekijöiden mahdollista yhteyttä karieskehitykseen. Erityisesti haluttiin myös tutkia pitkittyneen tutin käytön merkitystä.

Tutkimusaineisto koostui 183:sta alle kouluikäisestä lapsesta. Aloitusvaiheessa otettiin mikrobiologiset sylkitekstit lapsilta, joiden keski-ikä oli 2,5 vuotta, ja määritettiin anamnestisesti mahdolliset riskitekijät laktobasillien ja hiivasienten kolonisaatiolle. Kahden vuoden seurannan jälkeen tutkittiin riskin toteutumista maitohampaiden karioitumisessa. Seuraavassa seitsemän vuoden seuranta tutkimuksessa tutkittiin karieskehitystä maitomolaareissa ja ensimmäisissä pysyvissä molaareissa kahden vuoden iässä määritetyissä riskiryhmissä. Myös syljen mikrobiologisten testien avulla määritetyn riskin toteutumista selvitettiin seitsemän vuoden seurannan päätyttyä.

Tutkimuksen aloitusvaiheen sylkitekstitulokset osoittivat, että tutin käyttö ja tuttipullon käyttö öisin olivat yhteydessä syljen karieskehen liittyviin mikrobeihin, ja ne voivat siten lisätä kariesaktiivisuutta maitohampaistossa. Kahden vuoden seuranta tutkimuksessa todettiin, että karieskehitys oli yhteydessä aikaisemmin määritettyihin riskitekijöihin: pitkittyneeseen tutin käyttöön ja tuttipullon käyttöön öisin. Seitsemän vuoden seuranta tutkimuksen tulokset osoittivat, että makeisten käyttö kaksivuotiaana, puutteellinen hampaiden harjaus ja tuttipullon käyttö öisin ovat riskitekijöitä sekä maitomolaarien että ensimmäisten pysyvien molaarien karieskehitykselle. Fluoritablettien käyttö vähensi maitomolaarien karioitumista. Myös varhain tehdyillä syljen mikrobiologisilla testeillä oli pitkäaikainen ennustusarvo.

Tulokset osoittavat, että riskin määrittäminen taaperoikäisten lasten karieskehitykselle voidaan tehdä varhaisessa vaiheessa sekä mikrobiologisten sylkitekstiä avulla että määrittelemällä muiden mahdollisten riskitekijöiden esiintyvyys. Varhaisen kariesriskin määrittämisen avulla voidaan ehkäisevät hoitotoimenpiteet kohdistaa ajoissa riskiryhmille ja siten ehkäistä karieskehitystä ja korjaavan karieshoidon tarvetta lapsilla.

Asiasanat: hiivasieni, imetys, karies, laktobasilli, lapset, riskitekijät, tutti, tuttipullo

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Oulu, April 2010

Päivi Ollila

Abbreviations and definitions

Abbreviations

CFU	colony forming unit
dft	decayed and filled primary (deciduous) teeth
DFT	decayed and filled permanent teeth
dmft	number of decayed, missing, filled primary (deciduous) teeth
DMFT	number of decayed, missing, filled permanent teeth
dmfs	number of decayed, missing, filled primary (deciduous) surfaces
DMFS	number of decayed, missing, filled permanent surfaces
EAPD	European Academy of Paediatric Dentistry
ECC	early childhood caries
OR	odds ratio
PBR	practice-based research
RR	relative risk
MS	mutans streptococci
STAKES	Sosiaali- ja terveystieteiden tutkimus- ja kehittämiskeskus (National Research and Development Centre for Welfare and Health)
toddlers	children aged from 1 to 3 years
WHO	World Health Organisation
95% CI	95% confidence interval

Definitions

Caries experience

past and present caries in terms of DMF index values

Prevalence

in epidemiology, prevalence of a disease is defined as the proportion of cases with the disease in the population at a given time. In the present study, 'proportion of caries-free subjects' (dmfs=0 and/or DMFS=0) refer to the proportion of healthy subjects and 'proportion of subjects with dmfs>0 or DMFS>0' to prevalence of caries

Risk factor

factors for which a causal association with the outcome has been established. In the present study the term is used also for factors with no causal relationship

Sensitivity

the proportion of those who were believed to have a high risk among those whose actual caries increment during the follow-up was high

Specificity

the proportion of those who were believed to have a low risk among those whose actual caries increment was low

List of original papers

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals I–IV.

- I Ollila P, Niemelä M, Uhari M & Larmas M (1997). Risk factors for colonization of salivary lactobacilli and candida in children. *Acta Odontol Scand* 55: 9–13.
- II Ollila P, Niemelä M, Uhari M & Larmas M (1998). Prolonged pacifier-sucking and use of a nursing bottle at night: possible risk factors for dental caries in children. *Acta Odontol Scand* 56: 233–237.
- III Ollila P & Larmas M (2007). A seven-year survival analysis of caries onset in primary second molars and permanent first molars in different caries risk groups determined at age two years. *Acta Odontol Scand* 65: 29–35.
- IV Ollila P.S.H. & Larmas M.A. (2008). Long-term predictive value of salivary microbial diagnostic tests in children. *Eur Arch Paediatr Dent* 9: 25–30.

This thesis also contains unpublished data.

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1 Introduction

The decline in the level of dental caries among children in Western Europe during the last decades has been commonly reported (Nordblad *et al.* 2004, Marthaler 2004, Downer *et al.* 2005, Hugoson *et al.* 2008). However, there is a trend in many Nordic countries for the experience of caries to increase again, especially among young children (Haugejorden & Birkeland 2002, Stecksén-Blicks *et al.* 2004).

Polarisation of caries in children is also a very notable change in the last 20 years (Anderson 2002). In a Finnish study of 5-year-old children, eight per cent of the children were shown to be responsible for 76 per cent of all decayed teeth (Vehkalahti *et al.* 1997).

Children who have caries in their primary teeth during infancy or as toddlers tend to develop additional decay in their primary teeth during their pre-school years (Grindeffjord *et al.* 1995ab, Wendt *et al.* 1999, Pienihäkkinen *et al.* 2004). A Finnish national report on oral health in children (Nordblad *et al.* 2004) reported a high increase in the experience of caries at the age of 5 years as compared to 3-year-old children.

Many studies have shown that caries in primary teeth means also an increased risk for caries development in permanent teeth (Raadal & Espelid 1992, Mejare *et al.* 2001, Vanobbergen *et al.* 2001, Li & Wang 2002, Peretz *et al.* 2003, Skeie *et al.* 2006). Information collected at the toddler age may therefore be of great value in determining the risk for caries as well as when planning the future of children's dental health care.

It is generally known that prolonged sucking of pacifier is connected with malocclusion (Larsson 1975, 1986, Lindner & Modeer 1989), but still pacifier sucking has been reported to be very common in small children (Larsson *et al.* 1992). Some recent studies suggest that pacifiers may have some effect on dental activity as well (Ersin *et al.* 2006, Vázquez -Nava *et al.* 2008). Because pacifier sucking is a general habit, it is important to study the possible effect of this habit on caries development.

2 Review of the literature

2.1 Public dental records in epidemiology of dental caries

2.1.1 Use of dental records in research

All Nordic countries have their own comprehensive dental health recording system that is based on individual records. This information is often used in research, but specific knowledge and methods are required in order to benefit from the data recorded in the system. In Finland, adequate record keeping is essential, as illustrated by oral health authorities (National Board of Health 1986, Nordblad *et al.* 2001). The Finnish authorities (Ministry of Social Affairs and Health 2001) have issued instructions on record keeping to dentists continuously during the last decades (National Board of Health 1986, Ministry of Social Welfare and Health 1993).

In Finland, all children and adolescents from birth until the age of 18 are entitled to free dental care in public dental clinics. The coverage of the Public Oral Health Service is very good. For instance, in 1985, 95% of all 7- to 16-year-olds used this service (Milén *et al.* 1988). Caries is normally recorded in annual examinations, and restoration is undertaken if a lesion has reached dentin. This information is documented in standard patient records, together with information on preventive measures, fluoride therapies, and other procedures (Härkänen *et al.* 2002).

Public health dentists conduct the clinical investigations, and thus it is not possible to assess the objective data concerning the criteria for restoration. However, these criteria, which were defined during the bi-annual national conferences of the chief dentists of health centres, can be assumed relatively uniform in Finland (Larmas *et al.* 1995). Caries data collected from the Finnish Public Health records have been shown to be almost equal to the data from examinations conducted by trained examiners (Hausen *et al.* 2001). Recently, Korhonen *et al.* (2009) reported that practising dentists find significantly more caries in their new patients than their old patients, but this applies to patients over the age of 20, not to children.

Practice-based research (PBR) comprises a new branch of science that is performed in daily clinical practice (Niederman & Leitch 2006). This requires a kind of a large-scale case series approach, leading to knowledge on how science works in practice. PBR is becoming more common also in dental science because of the need for this real-life data and to guide the decisions of practitioners on

the treatment and the prevention of diseases. In PBR, observations are made by dentists in clinical settings where the population receives its dental care (Mjör *et al.* 2005, Mjör 2007, 2008).

2.1.2 Prevalence of caries in children

The prevalence of caries among 3-, 5-, 6-, 9- and 12-year-olds in Finland was followed in the period 1976–2000 through nation-wide reports to the National Board of Health and Welfare. The proportion of “caries-free” children increased until the middle of the 1990s, as shown in Figure 1 (Nordblad *et al.* 2004, STAKES). ‘Caries-free children’ were determined as children with healthy teeth in both primary and permanent dentition (dmft/DMFT =0).

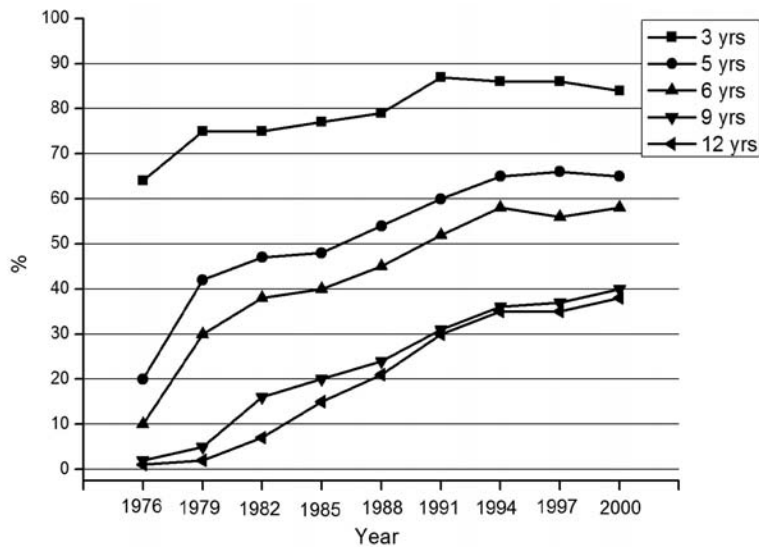


Figure 1. Proportion of caries-free 3-, 5-, 6-, 9-, and 12-year-olds in Finland from 1976 to 2000 (STAKES 2004).

In Finland, dental health has not improved since the 1990s (Figure 1). The decline in caries has stopped or even reversed slightly in recent years. In 1994, 86% of the 3-year-olds and 58% of the 6-year-olds were free of caries. In 2000, the corresponding values were 84% and 58%, respectively. In addition, important information about the trend of caries distribution between different age groups in

children is noticed. The decrease in the number children with no caries lesions in primary teeth after the age of three years is very strong, as can be seen from Figure 1. This outcome truly indicates a need for early risk assessment.

In the other Nordic countries as well, no important changes in caries prevalence have taken place in the last 15 years. In Sweden, the decline in caries experience in 4-year-olds discontinued after 1987 (Stecksén-Blicks *et al.* 2004). A report from Norway indicates evidence for a reversal of the caries decline among 5-year-old children in 1997–2000 with a statistically significant increase in the experience of caries (Haugejorden & Birkeland 2002). At the same time, national data of dental caries in 5-year-old children in 1988–2001 from Denmark show an almost unchanged distribution (Poulsen & Malling Pedersen 2002). The number of caries-free 12-year-olds has continued to increase until the year 2000 in Finland, Sweden and Norway, but since then, this development has virtually stopped (Table 1).

Table 1. Proportion of caries-free 12-year-olds in Finland, Sweden and Norway in 1985–2005 (STAKES 2004, Socialstyrelsen 2006, Sosial- og helsedirektoratet 2006).

Country	1985	1990	1991	1994	1995	2000	2004	2005
Finland	15	-	30	35	-	38	-	-
Sweden	22	40	-	-	50	61	-	58
Norway	18	33	-	-	40	48	41	-

2.2 Determinants of dental caries

Dental caries has a multifactorial aetiology, in which there is an interplay between the three principal factors, the host, the microflora, and the substrate, and the fourth factor, time. There is no single test that takes into consideration all these factors and can accurately predict an individual's susceptibility to caries. The risk of dental caries can best be evaluated by analysing and integrating several causative factors. (Reich *et al.* 1999.)

2.2.1 Microbiological factors

Dental caries is an infectious disease of bacterial origin (van Houte 1994). The disease is a result of a shift in the balance of the resident microflora, driven by a change in local environmental conditions. In the case of dental caries, repeated conditions of low pH in plaque following frequent sugar intake will favour the growth of acidogenic and aciduric species and predispose a site to caries (Marsh &

Nyvad 2008). Hence, high proportions of mutans streptococci and/or other aciduric bacteria may be considered biomarkers of particularly rapid caries progression (Takahashi & Nyvad 2008).

Clinical studies have shown that caries initiation is associated with an increase in the proportions of oral acidogenic and aciduric bacteria, especially mutans streptococci and lactobacilli, which are capable of demineralising enamel. These bacteria can rapidly metabolise dietary sugars to acid, creating locally a low pH. Even though mutans streptococci are strongly implicated with caries, the association is not unique: caries can occur in the apparent absence of these species, while mutans streptococci can persist without any evidence of detectable demineralisation. (Marsh 2006.)

Nevertheless, mutans streptococci and lactobacilli are certainly among the most pathogenic bacteria causing dental caries, the mechanism of which has been demonstrated in many *in vitro* studies. Furthermore, many cohort studies have clinically shown an association between the incidence of dental caries and levels of oral streptococci and lactobacilli (Nomura 2001). Mutans streptococci have a central role in initiation of caries and lactobacilli are implicated as important contributory bacteria in tooth decay (Tanzer *et al.* 2001).

Since the 1920s, many microbiological studies have searched the aetiological role of different microbes in the development of caries (Clarke 1924, Hadley 1933). Later many cross-sectional studies have demonstrated the role of mutans streptococci as the principal bacteria in the early development of dental caries in the primary dentition (Aaltonen *et al.* 1985, Holbrook *et al.* 1989, Weinberger & Wright 1989, Fujiwara *et al.* 1991, Matee *et al.* 1992, Grindefjord *et al.* 1993, Stenudd *et al.* 2001, Olak *et al.* 2007). The association of bacterial adhesion with high and low caries experiences in children showed that adhesion of mutans streptococci was among the factors correlating with high caries experience (Stenudd *et al.* 2001). Thus, bacterial adhesion may modulate susceptibility and resistance to dental caries.

Longitudinal studies have also clinically shown the role of mutans streptococci in caries increment (Alaluusua & Renkonen 1983, Köhler *et al.* 1988, Grindefjord *et al.* 1995b, Thibodeau *et al.* 1995, Kirstilä *et al.* 1998). A longitudinal study of caries onset in initially caries-free children showed that children who were caries-free and had high levels of salivary mutans streptococci at the baseline would be at a greater risk, and thereby more susceptible to caries onset, at any given time compared to caries-free children who had low levels of salivary mutans streptococci at the baseline (Kopycka-Kedzierawski & Billings 2004).

A systematic review summarised the literature assessing the association of mutans streptococci and dental caries in pre-school children (Thenish *et al.* 2006). The results showed that the presence of oral mutans streptococci, both in plaque or saliva of young caries-free children, appears to be associated with a considerable increase in caries risk. However, lack of adjustment for potential confounders in the original studies limits the extent to which interpretations can be made in practice.

The number of lactobacilli usually increases after the initiation of the carious process, and they are generally agreed to be secondary invaders of the cariogenic bacteria. Carlsson *et al.* (1975) found lactobacilli only occasionally in children younger than two years, but after the age of two years, the amount of lactobacilli increased and the presence of those microbes was associated with the presence of caries in children aged 2–5 years. Oral lactobacilli are also abundant in subjects consuming a sucrose-rich diet, indicating an asiduric oral environment. Salivary lactobacilli predicted dental caries better than salivary mutans streptococci in 4–5-year-old children in a cross-sectional study by Granath *et al.* (1994).

The role of lactobacilli in microbial caries risk assessment has been shown in many cross-sectional studies (Matee *et al.* 1992, Grindefjord *et al.* 1993, Hallonsten *et al.* 1995, Brambilla *et al.* 1999). A significant association was demonstrated between relatively low cariogenic bacterial levels of both mutans streptococci and lactobacilli and dental caries in infants and toddlers (Ramos-Gomez *et al.* 2002). Brambilla *et al.* (1999) provided information of a clear positive relationship between selected micro-organisms (mutans streptococci and lactobacilli) in saliva and caries in 9- and 13-year-old children, in spite of a relatively low prevalence of the disease.

Longitudinal studies on the significance of early colonisation by lactobacilli in the oral cavity on early caries development are comparatively few (Schröder *et al.* 1994). Roeters *et al.* (1995) showed in 2–5-year-old children that correlations between the clinical caries score and lactobacilli in saliva and mutans streptococci in plaque or saliva were highly significant. In a two-year cohort study on the association of human salivary anti-microbial agents and cariogenic micro-organisms, mutans streptococci and lactobacilli correlated positively with both baseline caries and caries increment (Kirstilä *et al.* 1998). Lactobacilli were associated with caries and they are dependent on retentive sites being available in high numbers, and hence lactobacillus counts have been successfully used to predict the increment of new caries lesions (Smith *et al.* 2001).

In a recent review about the ecology of lactobacilli in the oral cavity, also the relation of salivary lactobacilli and caries was summarised (Badet & Thebaud

2008). A strong correlation was established between the salivary lactobacillus count and dental caries: the higher the DMF index, the higher the number of children harbouring a high lactobacillus count.

The standard laboratory method for determining the number of lactobacilli includes the use of a selective medium, Rogosa SL-agar. Chairside methods for detecting lactobacilli have also been developed since the Dentocult® LB method in 1975 (Larmas 1975). Dip slide tests of salivary bacterial counts, for example of mutans streptococci and lactobacilli, are presently widely used in everyday practice in caries risk assessment. The current simple tests have been suggested to be useful for estimating caries risk due to unfavourable dietary habits (Dentocult-LB) and for establishing the presence of colonization of mutans streptococci (Dentocult-SM) and salivary yeasts for the determination of reduced salivary flow and thereby the patient's medical condition (Dentocult-Ca). (Larmas 1992.)

However, these tests may also be limited in their applicability for the assessment of caries risk and in caries prediction (van Palenstein-Helderman *et al.* 2001, Pinelli *et al.* 2001). In every respect, they can be effective in a group of persons with high or low caries experience (Bowden 1997). Different sampling methods for recovery of cariogenic bacteria in children from 9 to 36 months were evaluated by Barsamian-Wunsch *et al.* (2004). Tongue and plaque specimens collected on cotton swabs and stimulated saliva were diluted and plated on selective and non-selective media. The results showed that all sampling methods were adequate for microbial risk assessment of children under three years of age and that mutans streptococci was a stronger indicator of caries status than lactobacilli.

Yeasts have been shown to be more prevalent in the saliva and the plaque of caries-active subjects than of caries-free ones (Krasse 1954). The *Candida* species have been found to be the most common yeasts in the mouth, and the prevalence of carriers of yeasts varies from 21% to 70% (Martin & Wilkinson 1983, Pienihäkkinen *et al.* 1985, Starr *et al.* 2002) and depends on the salivary flow rate (Parvinen & Larmas 1981). Orthodontic appliances increase the densities of yeasts in saliva (Addy *et al.* 1982), as does the use of antibiotics. Russell *et al.* (1990, 1991) found a significant correlation between yeasts and caries incidence, and one-year caries increment. Pienihäkkinen *et al.* (1987) showed salivary yeasts to predict a high number (>3) of caries lesions compared to low numbers (<3) better than lactobacilli.

The level of yeasts was more easily assessed and appeared to be more useful indicator of caries risk than the lactobacilli count in a study where the relationship between yeast and lactobacilli levels and dental caries experience in children was

tested (Coulter *et al.* 1993). An association between dental caries and salivary microbial levels in 12-year-old schoolchildren showed that the DMFS scores were especially related to mutans streptococci, lactobacilli and yeasts (Beighton *et al.* 1996).

During the last years, the association between oral yeast levels and risk for early caries development has been studied frequently. The prevalence of carriage of oral yeasts in children who were fed with both breast milk and bottle milk or other fluids was 18.5%, while in children fed only with breast milk it was 0% (Kadir *et al.* 2005). *Candida albicans* was observed in the oral cavity of healthy children with high comparable rate in school and pre-school age children, and *Candida albicans* was isolated with high comparable rate from carious lesions in pre-school and schoolchildren (Rozkiewicz *et al.* 2006). The frequency of oral yeasts in children from 4- to 6-year old with moderate and high dft-indices was statistically significantly higher than in caries-free children (Ugun-Can *et al.* 2007). This research group concluded that mycologic examination may constitute a contributory role for epidemiologic studies of dental caries especially in children under seven years of age. Cerqueira *et al.* (2007) showed that dentinal caries lesions may be associated with yeast species colonisation in HIV-infected children. A significant association between the presence of *Candida albicans* and early childhood caries was determined also recently (de Carvalho *et al.* 2006).

A dip slide system using the Nickerson medium (1953) Oricult-N is also available for the measuring of oral yeast infection (Parvinen & Larmas 1981). Its clinical use has been suggested to include mainly determining the presence of yeast infection in the oral cavity and diagnosing the possible hyposalivation status of a patient, because the yeast infection and the number of salivary yeasts depend on the salivary flow (Larmas 1992).

Klinke *et al.* (2009) showed that due to strong acid production, yeasts make a significant contribution to caries pathogenesis in caries-active children. Clinical data of the proportions of microorganisms present in early childhood caries lesions, the contribution of oral lactobacilli and yeasts acid formation appears to be important.

Dental plaque is an example of a biofilm; its presence is natural and it supports the host in its defence against invading microbes. In health, the microbial composition of dental plaque is diverse and remains relatively stable over time (microbial homeostasis). Under certain circumstances, this microbial homeostasis can break down and diseases such as caries can occur. (Marsh 2009.)

2.2.2 Dietary habits

Sucrose-rich diets increase the growth of many oral bacteria and change the composition of the microflora in a caries-promoting manner (Marsh & Nyvad 2008). The high and frequent consumption of sugar has been known to be an aetiological factor in caries for several decades (Zero *et al.* 2008).

Many studies have shown that frequent consumption of sweets and sugar-containing beverages, particularly sweetened liquid in a feeding bottle, during the first years of life were associated with caries development during pre-school years (Wendt & Birkhed 1995, Grindefjord *et al.* 1996). In addition, Guthrie & Morton (2000) showed that sweetened drinks constitute the primary source of added sugar in children's daily diet. Marshall *et al.* (2003, 2007) have suggested that contemporary changes in beverage intake, particularly the increase in soft drink consumption, have the potential to increase dental caries rates in children. Frequent sugar consumption, particularly in-between meals, has been reported to associate to caries development in pre-school children (Stecksén-Blicks & Holm 1995, Paunio *et al.* 1993b, Karjalainen *et al.* 2001). Furthermore, consumption of sweets more than once a week was more common in children with caries than without (Grindefjord *et al.* 1993). Excessive sugar consumption increases the risk of caries even if the correlation between sugar intake and dental health has weakened due to exposure to fluoride (Karjalainen 2007).

Longitudinal studies on the influence of dietary factors on dental caries in young children are scarce. Person *et al.* (1985), Grytten *et al.* (1988) and Hallonsten *et al.* (1995) found that caries incidence in later childhood (>3 years) was associated with dietary habits at 12 or at 18 months of age. A Finnish follow-up study, where food consumption was prospectively recorded from infancy to the age of ten, showed that a persistently high sucrose intake increases the risk of dental caries in children (Ruottinen *et al.* 2004). A recent longitudinal follow-up study showed that frequent consumption of snacking products during early childhood appears to be a risk indicator for caries at the age of 15 in Sweden (Alm *et al.* 2008a).

Bottle-feeding during nights is suggested to be associated with carious lesions in the incisors of infants (Johnsen 1982, Roberts *et al.* 1993, Schwartz *et al.* 1993). Several studies have also reported strong associations between consumption of sugar-containing liquids at night-time before the age of one and caries development before the age of three (Paunio *et al.* 1993ab, Alaluusua & Malmivirta 1994, Wendt & Birkhed 1995).

Mattila *et al.* (2005) analysed behavioural and demographic factors during early childhood and dental health at the age of ten and found that daily sugar consumption, drinking of juice at the age of 18 months and daily consumption of sweets at the age of three, were associated with poor dental health as late as at the age of ten years.

Results of the association between breastfeeding habits and caries prevalence are contradictory. Alaluusua *et al.* (1990a) and Roberts *et al.* (1993) concluded that normal breastfeeding alone does not correlate either to an increased or to a decreased caries prevalence. However, many studies have reported an association between prolonged breastfeeding and nursing caries (Eronat & Eden 1992, Matee *et al.* 1994, Hallonsten *et al.* 1995).

Tinanoff's (2005) review about the association of diet with dental caries in pre-school children updates the evidence linking dietary factors to dental caries in pre-school children and recommends dietary approaches to reduce caries risk.

A recent review of early childhood caries with particular reference on nomenclature, case definition, epidemiology, aetiology, and risk assessment, states that dietary factors related to sugar consumption predispose to early MS colonisation and establishment and increase the risk for ECC development, as part of the causal chain. Inappropriate bottle- and breastfeeding behaviours also increase the risk, without showing a direct causal relationship. (Vadiakas 2008.)

2.2.3 Tooth brushing habits

Lack of good oral hygiene in young children has been found to be associated with the development of caries in many studies. Wendt *et al.* (1994) and Alaluusua & Malmivirta (1994) reported that visible plaque in 12-month-old children was associated with caries at three years of age. Tooth brushing habits of young children are associated with the parents' tooth brushing routines (Paunio 1994). Children who had their teeth brushed regularly had significantly lower dmf values than those with no regular tooth brushing routine in Swedish 4-year-old children (Stecksén-Blicks *et al.* 1989). Children with an established daily tooth brushing habit at twelve months of age were more likely to be caries-free at the age of three (Wendt *et al.* 1994). The parents having problems brushing their child's teeth was one important risk factor for early childhood caries in a study on Canadian pre-school children (Tiberia *et al.* 2007).

Presence of visible plaque was significantly associated with caries in Swedish 3-year-old children (Wennhall *et al.* 2002). Attitude to oral hygiene and the age when started, oral hygiene habits were one of the important caries risk indicators among 5-year-old children in Norway (Skeie *et al.* 2006). The caries experience among 2- to 3-year-olds was associated with visible plaque in a group of Swedish pre-school children (Bankel *et al.* 2006). Lack of oral hygiene, consumption of sugar containing snacks and enamel hypoplasia are significant factors for both MS infection and caries lesion initiation in 21- to 75-month-old children (Law & Seow 2006). Lack of regular tooth brushing was strongly linked to caries experience of schoolchildren in England (Levine *et al.* 2007). The improvement in caries prevalence of 4-year-old children had resulted from decreased intake of sugar in between-meal products and increased tooth brushing frequency (Stecksén-Blicks *et al.* 2008).

Some longitudinal studies support the notion that good oral hygiene reduces caries in children. In a two-year follow-up study by Curnow *et al.* (2002), high-caries-risk children, whose average age was 5.3 years, had significantly less caries after participating in a supervised tooth brushing programme compared to control group involving no intervention. Infrequent tooth brushing at the age of three years seemed to be a risk factor for poor dental health at ten years of age in Finland (Mattila *et al.* 2005). Good oral hygiene habits, established in early childhood, provide a foundation for low experience of approximal caries in adolescents in Sweden (Alm *et al.* 2008b). Self-reported tooth brushing with fluoride toothpaste at least twice a day reduced caries in the 3.4-year follow-up period of 11–12 year-old Finnish schoolchildren (Hietasalo *et al.* 2008).

Many systematic reviews on risk factors for dental caries in children have shown the effect of good oral hygiene being beneficial. In a recent review, Roberts (2008) showed that improving oral hygiene practices also remain essential in the prevention of dental caries. However, little information is available about the maintenance of good oral health factors over time, from the toddler age to adolescence, and its effect on caries development later in life.

2.2.4 Use of fluorides

In caries prevention, fluoride works primarily via topical mechanisms, which include inhibition of demineralisation at the crystal surfaces inside the tooth, enhancement of remineralisation at the crystal surfaces and inhibition of some bacterial enzymes (Featherstone 1999).

The caries preventive effect of fluoride tablets on oral health in children has been shown in many studies. Low caries prevalence was found in those children that took fluoride tablets regularly (Holbrook 1993, Widenheim & Birkhed 1991). Deterioration of dental health of 5-year-old Norwegian children after 1997 was associated with the reduction in the sale of fluoride tablets, whereas the increased sales of fluoride tablets after 1998 improved dental health among pre-school children in 2003 (Haugejorden & Birkeland 2005). The caries preventive effect of an oral health programme for pre-school children in Sweden showed that parents' daily assistance with tooth brushing and administering fluoride tablets was significantly better in the intervention group than in the reference group. This study demonstrated that the early start of an oral health programme had a significant beneficial effect on caries experience after three years (Wennhall *et al.* 2008).

The systematic review of the caries-preventive effect of fluoride toothpaste reinforced the importance of daily tooth brushing with fluoridated toothpaste for preventing dental caries (Twetman *et al.* 2003). The results suggested strong evidence (1) for the caries-preventive effect of daily use of fluoride toothpaste compared to placebo on young permanent dentition; (2) that toothpaste with 1,500 ppm fluoride had a superior preventive effect compared to standard dentifrices with 1,000 ppm F on the young permanent dentition; and (3) that a better preventive effect was recorded in studies with supervised tooth brushing compared with non-supervised. However, insufficient evidence was found on the effect of fluoride toothpaste on primary teeth. (Twetman *et al.* 2003.)

The use of fluoride has a well-documented effect on caries prevention. The current recommendations suggest that good oral hygiene and use of fluoride toothpaste twice a day is a major part of comprehensive preventive programmes to control dental caries in children. Tooth brushing with a tiny amount of fluoride toothpaste should start already when the first teeth erupt. (Twetman 2009, Käypä hoito 2009.)

2.2.5 Sucking habits

The use of a pacifier during infancy is common in Western countries (Larsson *et al.* 1992, Paunio *et al.* 1993b). Larsson *et al.* (1992) showed that approximately 70% of the Swedish infants had a pacifier-sucking habit. The corresponding figures for 2- and 3-year-olds were approximately 57% and 46%, respectively.

Paediatric dentists are generally well aware of the oral implications of non-nutritive sucking (NNS). NNS via digit or pacifier can cause occlusal anomalies,

such as an open bite, excessive overjet, and possibly posterior crossbite (Larsson 1971, Köhler & Holst 1973, Helle & Haavikko 1974, Ravn 1976, Adair *et al.* 1995, Warren *et al.* 2001a). There is some evidence that pacifiers are less harmful, particularly if pacifier-sucking habits are spontaneously shed at about 2 to 4 years of age. Digit-sucking habits are more likely to persist into the school-age years and can require appliance therapy for discontinuation. Thus, some authorities suggest that pacifiers be recommended for infants who engage in NNS (Adair 2003).

The relationship of this common pacifier-sucking habit and caries development have not interested the researchers of paediatric dentistry. However, some evidence suggests that pacifier may have some effect on caries activity (Larsson 1975, Wendt & Birkhed 1995). An association between pacifier use and bacteria or yeast infections was found (Manning *et al.* 1985, Sio *et al.* 1987, Brook & Gober 1997, Ersin *et al.* 2006), as well as a significant association with dental caries occurrence in primary dentition (Vázquez-Nava *et al.* 2008). A recent longitudinal study showed that prolonged sucking habits increased dental caries in children (Yonezu & Yakushiji 2008).

Dental research has focused on changes created by pacifier on the occlusion and perioral tissues. Paediatric dentists should be aware of other published risks and benefits of pacifier use. These include an association with protection against sudden infant death syndrome (SIDS). Mitchell *et al.* (1993) indicated that pacifier use during sleeping was associated with a greater than 50% reduction in risk of SIDS.

A relationship between pacifier use and increased risk for otitis media and other infectious diseases has also been reported in many studies, first by Niemelä *et al.* (1994, 1995) among Finnish pacifier users, and later by other researchers as well (Jackson & Mourino 1999, Warren *et al.* 2001b).

An association of pacifier sucking with reduced prevalence and reduced duration of breastfeeding has also been reported (Barros *et al.* 1995, Howard *et al.* 1999). Aarts *et al.* (1999) found that duration and prevalence of breastfeeding in a Swedish population was adversely related to pacifier sucking, but not thumb sucking. Other studies have also blamed pacifier use for shorter duration or lower prevalence of breastfeeding (Paunio *et al.* 1993b, Kloeblen-Tanver 2001).

The only review on the topics of pacifier use did not suggest a strong or consistent association between pacifier sucking and early childhood caries (Peressini 2003). Although the use of pacifier is very common in toddlers, very little information is available about the effect of prolonged use of pacifier on caries development. Therefore, proper research on the matter is needed.

2.2.6 General health and medication

Many children suffer from several infectious diseases during the first three years of life. Of 3-year-old children, 20–30% suffered from recurrent respiratory tract and ear infections (Sipilä *et al.* 1987). Syrup medications for these conditions in children contain often fermentable sugars. Associations have been found between the syrups and the occurrence of caries (Roberts & Roberts 1981, Holbrook *et al.* 1989). Furthermore, it may be difficult to maintain tooth brushing routines if the child is sick, and the illness may indirectly and temporarily increase the caries risk (Storhaug 1985). However, no relationship was found between caries prevalence in 3-year-old children and long-term illnesses during the first years of these children's lives; neither did short-term antibiotic medication influence the caries prevalence (Paunio *et al.* 1993c, Schröder *et al.* 1994).

Asthmatic children may have a higher caries risk than healthy children, due to both their medical condition and the physiological effects of their pharmacotherapy. Kankaala *et al.* (1998) showed that factors related to the asthmatic condition may increase the risk of caries. Many other studies have reported the same caries risk (Reddy *et al.* 2003, Wogelius *et al.* 2004, Wierchola *et al.* 2006). Bimstein *et al.* (2006) showed that children with systemic diseases like asthma had a higher prevalence of toothache episodes and higher plaque, calculus and caries indices than healthy children had. Stensson *et al.* (2008) reported that pre-school children with asthma had higher caries prevalence than healthy children. The factors discriminating for caries in asthmatic children were higher intake of sugary drinks, mouth breathing and immigrant background.

Some studies concerning the relationship between ear infections and dental caries are contradictory. Alaki *et al.* (2008) showed that the occurrence of middle ear infections or respiratory tract infections during the first year of life was associated with significantly increased risk for developing early childhood caries, whereas Nelson *et al.* (2005) found no association.

In a review on the management of drooling in children with mental and physical disabilities, it was recommended that if pharmacological or surgical treatment is carried out, careful monitoring for the development of dental caries and other problems is essential (Hussein *et al.* 1998).

2.2.7 Socioeconomic background

The socioeconomic status of the family has been shown to influence the caries risk in children in many European countries. Immigrant background and maternal education were found to be independent indicators of caries risk in primary dentition in Sweden (Grindefjord *et al.* 1995b). Same results were found also in a dental survey carried out in Netherlands, where the results showed both ethnicity and maternal education to be indicators of caries risk in the primary dentition (Verrips *et al.* 1995).

In Sweden, socioeconomic differences in oral health and the use of dental care were most marked in older adults (45–64 years), but they were also significant in young adults and, in terms of oral health, in children as well (Hjern *et al.* 2001). High prevalence of caries in 3-year-old children was found in a low socioeconomic, multi-cultural population in Sweden (Wennhall *et al.* 2002), and was also associated with the progression of caries at the age of 12–14 years independently of a previous history of caries (Källestål & Wall 2002).

Association between the dental health of children and current socioeconomic status of the families is not found in all studies in Finland (Ruottinen *et al.* 2004). However, in a recent study the socioeconomic status was significantly associated with caries at the age of five also in Finland (Meurman *et al.* 2009).

In a systematic review on the effect of the socioeconomic status, there was a fairly strong evidence for inverse relationship between the socioeconomic status and the prevalence of caries among children less than twelve years of age. The evidence for this relationship is weaker for older children and for adults, perhaps because of the relatively small number of studies and methodological limitations. (Reisine & Psoter 2001.)

2.3 Assessment of caries risk in children

Due to the multifactorial nature of caries aetiology, it is expected that multivariable approaches rather than the use of single parameters may improve caries risk assessment for individuals as well as groups of subjects (van Houte 1993). The prediction of low caries risk may be more reliable than that of high caries risk. In prediction studies, the main question is how accurately the individual studied can be classified into those having a high and those having a low risk for a disease. (Hausen 2008.)

Caries risk assessment at the toddler age can provide indication of possible preventive intervention detectable before visible caries has manifested. The Swedish studies by Grindefjord *et al.* (1995a, 1996) indicated that in multivariate analyses, several variables determined in 12-month-old children were associated with caries at three and half years of age. These variables included immigrant background, mother's education, consumption of sugar-containing beverages, mutans streptococci, and consumption of sweets. In a systematic review of the risk factors for dental caries in young children, the evidence suggests that children are most likely to develop caries if mutans streptococci are acquired at an early age, although this may be partly compensated by other factors, such as good oral hygiene and a non-cariogenic diet. (Harris *et al.* 2004.) Diet and oral hygiene may interact such that if there is a balance of "good" habits by way of maintaining good plaque control and poor habits by way of having a cariogenic diet, the development of caries may be controlled.

Clinical variables, especially past caries experience, are confirmed as the most significant predictors of future caries development. Alaluusua *et al.* (1990ab) showed the power of caries-related salivary tests and a test based on past caries experience (baseline DFS) to select persons at high risk for caries. Among the tests, DFS was the most sensitive and specific as predictor. A combination of microbial test and DFS was more efficient to select persons at risk than various alternatives alone. Alaluusua (1993) also showed that the baseline caries experience was better than or as powerful as salivary tests in predicting future caries increment at comparable screening and validation levels. The past caries experience was the most powerful predictor of caries in both low-fluoride and high-fluoride areas, too (Mattiasson-Robertson & Twetman 1993). The findings indicated that the natural fluoride exposure had a limited influence on caries risk assessment and the caries predictive ability of salivary bacterial test and past caries in populations with a low level of disease.

Raitio *et al.* (1996a) developed a multifactorial model for the prediction of 11-month caries increment in adolescents. The risk indicators consisted of past caries experience, white spot lesions, visible plaque, gingivitis, salivary secretion rate, buffer effect, sucrase, salivary mutans streptococci, lactobacilli and yeasts. Past caries experience, salivary yeasts and sucrase were the most powerful.

Pienihäkkinen *et al.* (2004) showed in a three-year follow-up that in two-year-old children, the combination of two or three risk indicators (Dentocult-MS strip, incipient caries lesions, and consumption of candies) might have caries-predictive

power enough for clinical implications. The results of the study, investigating caries risk profiles in two-year-old children in Sweden, showed that the frequency of sugar consumption was the most pertinent factor in the children's risk profiles (Stecksén-Blicks *et al.* 2007).

Some longitudinal studies have reported that caries in permanent teeth is correlated with caries in primary teeth. The relationship between caries in the primary dentition at the age of five and permanent dentition at the age of ten was studied in a longitudinal study by Skeie *et al.* in Norway (2006). The results showed that more than two surfaces with caries in primary second molars at the age of five years was a clinically useful predictor for having caries at the age of ten. Furthermore, an eight-year cohort study by Li & Wang (2002) demonstrated that caries status in primary teeth can be used as a risk indicator for caries in the permanent teeth.

The conclusion of a systematic review by the Swedish Council on Technology Assessment in Health Care (SBU 2007) was that past caries experience is the best factor for predicting future caries development among pre-school children, schoolchildren and adolescents. Identification of children and adolescents at very low risk of developing caries in the following 2–3 years is easy, but it is more difficult to determine accurately which individuals are at high risk of developing caries. (SBU 2007.)

Also a recent textbook of cariology concludes that the multifactorial aetiology of dental caries makes it likely that even the most sophisticated models using the known risk factors and risk markers cannot predict future caries development very accurately. Clinical examination and proper dental history are the most important sources of information for a decision. (Hausen 2008.)

The latest textbook of paediatric dentistry concludes that based on numerous clinical studies with single predictors, the probability of identifying true high-risk individuals is lower (sensitivity) than the probability of correctly identifying subjects with low caries risk (specificity). However, models that combine several predictors may significantly increase the predictive power. (Koch *et al.* 2009.)

3 Aims of the present study

The inevitable increase in caries prevalence after the age of three supports early assessment of caries risk in toddlers. The skew distribution, with a large group of caries-free children and a smaller group with high caries experience, is a challenge to dental professionals. Therefore, early identification of children with caries risk is needed.

The hypothesis was that in toddlers, the risk of caries could be assessed by identifying caries-related habits early and by using salivary microbiological tests.

The detailed aims of the present study were

1. to analyse the effect of pacifier sucking and sweet-intake habits on the colonisation of salivary lactobacilli and yeasts
2. to study the effect of sucking, sweet-intake and tooth brushing habits, the use of fluoride tablets, and the carriage of lactobacilli and yeasts on the dental health of children after 2- and 7-years of follow-up and
3. to assess long-term caries predictive value of salivary lactobacilli and yeasts

4 Subjects and methods

4.1 Subjects (I, II, III and IV)

This study was originally a part of a large infection survey, which investigated the connection of pacifier sucking to susceptibility to otitis media infections. The size of the study population (n=183) was determined according to the prevalence of otitis media infections. The average age of the children in the study cohort was two and a half years old (ranging from 0.7 to 4.3 years). One hundred and eighty-three children aged one to four years were included in the study, which was conducted at 11 day care centres in the city of Oulu, Finland. These day care centres were chosen based on their willingness to participate in the survey and the number of children looked after in them. Seventeen of the children were subsequently excluded either because they neglected to return the questionnaire or because it proved impossible to obtain a proper sample of saliva. The baseline analysis was therefore performed on 166 children (88 boys and 78 girls). The average age of children at the onset was 2.5 years (range 0.7–4.3 years). (I)

In the 2-year follow-up study, only those children who could be microbiologically tested at the baseline were followed. Information about the cariological status at the baseline and at the 2-year follow-up was available on 152 (80 boys and 72 girls) children of the original 166 children. The average age was 4.7 years (range 2.7–6.5 years). (II)

In the 7-year follow-up study, the data on the same 183 children was collected first at the baseline and then after approximately seven follow-up years (range 2.0–8.9 years), when the average age of the children was 9.6 years (range 3.1–12.7 years). Information about the tooth-specific survival analysis of dental health was available on 175 of the original 183 children. (III, IV)

The flow chart of the enrolled and completed study groups and dropouts with the reasons for their exclusion are shown in Figure 2.

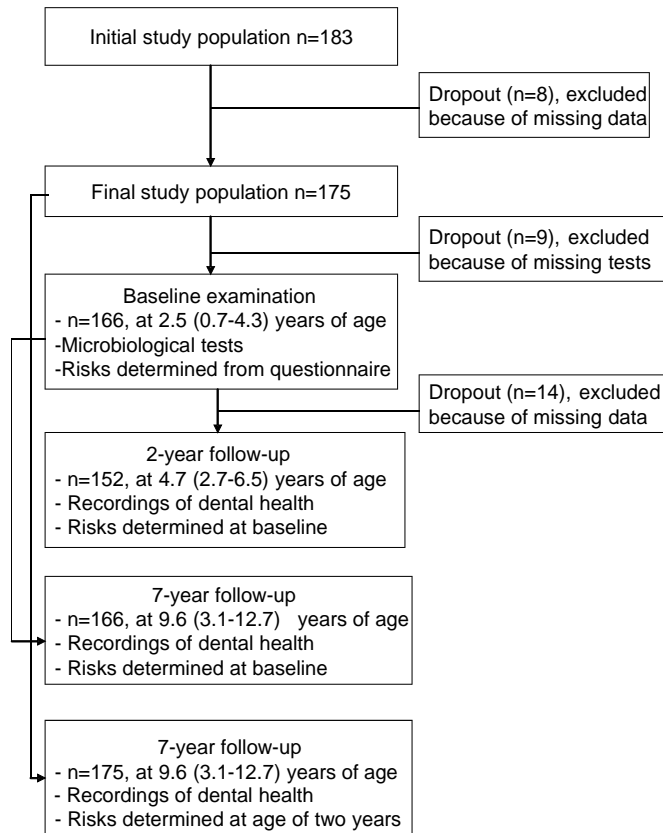


Figure 2. Flow chart of the study population.

4.2 Structured questionnaire at the baseline and recordings at child health clinics (I, II, III and IV)

At the baseline, i.e. when the children's mean age was 2.5 years, information of the possible risk factors was collected from a structured questionnaire filled in by the parents for the current purpose (I). The questionnaire included questions about pacifier and thumb sucking, breastfeeding, bottle-feeding at night, and symptoms and/or signs of respiratory infections at the time of saliva sampling. The symptoms included rhinitis, cough, fever $> 38^{\circ}\text{C}$, and conjunctivitis. The symptoms were classified as positive if at least one of the symptoms existed. Information about antibiotic therapy at the time of sampling was obtained from the day care centre. The questionnaire is available in English in Appendix 1.

Each child's socioeconomic status was classified, based on the Central Statistical Office of Finland's classification (1989), on a scale from one to seven and determined according to the parent with the higher socioeconomic status (II). Of the parents, 25% were upper-lever employees, 53% lower-lever employees, 17% manual workers, and 3% were students. The socioeconomic status of the parents was unknown in 2% of the cases.

In the 7-year follow-up study, information on the possible risk factors was collected from questionnaires filled in by the parents at the baseline of this cohort study and from the children's dental health records in the files of child health clinics in Oulu (III). At the 2-year visit, parents are routinely questioned about the child's tooth brushing habits (daily/occasionally), use of sweets (\leq once a week/ $>$ once a week), use of fluoride tablets (regularly/occasionally), pacifier, finger and nursing bottle sucking habits (ongoing/discontinued), and breastfeeding (ongoing/discontinued). Oral examinations of young children at child health clinics in Oulu were done by dentists or oral hygienists.

Possible risk factors of the study population at the baseline (mean age 2.5 years) and at the age of two are presented in Table 2.

Table 2. Possible risk factors registered at the baseline (mean age 2.5 years) for the 2-year follow-up (II) and at the age of two for the 7-year follow-up (III).

	Yes		No		Total number
	%	n	%	n	
Data collected from the questionnaire at the baseline					
Pacifier sucking	39	65	61	101	166
Thumb sucking	4	6	96	160	166
Breast feeding	2	3	98	157	166
Nursing bottle at nights	28	46	72	120	166
Symptoms of respiratory infections	63	104	37	62	166
Antibiotic therapy	8	13	92	152	166
Data collected at the child's 2-year-visit to the child health clinic					
Pacifier sucking	55	92	45	74	166
Breast feeding \geq 12 months	13	22	87	151	173
Nursing bottle at night	21	36	79	135	171
Daily tooth brushing	88	117	12	16	133
Sweets $>$ 1x/week	24	31	76	99	130
Fluoride tablets in use	74	115	26	41	156

4.3 Microbial sampling (I)

Saliva samples for lactobacilli and yeast counts were taken from unstimulated saliva sublingually with cotton swabs. The sample size was large enough for an immediate cultivation over one side of Dentocult[®]LB and Oricult[®]N slides (Orion Diagnostica, Espoo, Finland). The samples were incubated at 37°C for two days (Oricult[®]N) or four days (Dentocult[®]LB), and the presence of colonies was determined, the cut-off value being one colony-forming unit (CFU). The results with one or several CFUs were categorised as “positive”, while tests without any CFUs were categorised as “negative” test results.

4.4 Registration of dental caries (II, III and IV)

The information on the development of caries during the follow-up was collected from the children’s dental health records in the files of dental health care clinics in Oulu (II, III and IV). The data on 183 children was collected first at the baseline, then after two years and finally after seven years. The personnel of the clinics were not aware that the 7-year follow-up and analysis was in progress.

Dental examinations were carried out annually by the local health centre dentists according to normal routines, and the findings were recorded on standard national oral health patient documents. X-ray pictures were not routinely taken, but were taken when considered necessary. The criteria used for caries diagnosis were those issued by the National Board of Health in Finland following the criteria made by the World Health Organisation in Oral Health Surveys (1997). According to these instructions, caries was recorded as initial when it was restricted to the enamel and could probably be arrested with preventive means. Manifest caries lesions were defined as those that extended to the dentin and needed restorative dental care.

4.5 Statistical procedures (I, II, III and IV)

4.5.1 Statistical analyses by cross-tabulation and logistic regression analyses (I and II)

At the baseline, the associations between the occurrence of microbes and the possible caries risk factors were evaluated by both univariate analysis and logistic multivariate regression modelling (I). The same method was used also in the 2-year

follow-up study, where associations between caries development and risk factors registered at the baseline were studied (II).

Direct univariate associations were studied by cross-tabulation of the dichotomous variables. The strength of association was tested for statistical significance using the chi-square test. As the present study was designed as a cohort study defined by exposure, possible risk factors were analysed by calculating their relative risks (RR) and 95% confidence intervals (CI). To control the effect of age, the analyses were also performed separately on two age groups at the baseline, i.e. children younger than two years and children two years or older at the baseline (I).

Stepwise logistic multivariate modelling was used to control confounding variables. Age (I and II) and socioeconomic status of the parents (II) were included in the multivariate analysis.

The data for all subjects on whom information was available were used in the univariate analysis, whereas logistic multivariate modelling included only the subjects on whom data regarding all the variables in the model were available. The statistical analyses were performed using SPSS, version 6.0.

4.5.2 Statistical analyses applying survival analysis method (III and IV)

In the 7-year follow-up, the survival analysis method was used (III and IV). Survival probabilities, i.e. time to caries onset leading to dental restoration, were estimated using the Kaplan-Meier survival analysis method. Information for the survival analysis was available on 175 of the original 183 children. In the statistical analysis of thesis, the index teeth were all primary second molars and permanent first molars and two index teeth in the original article (III).

The survival time for each individual tooth was the time elapsing between the birth of the child and the first restoration due to dental caries – that is the time when caries had progressed to a stage when a dentist made a decision to perform a restoration (Larmas *et al.* 1995, Virtanen & Larmas 1995, Härkänen *et al.* 2002). In order to avoid the dependence problem inside the oral cavity, the Kaplan-Meier estimates for survival time were produced separately for each tooth in different risk groups. The estimates represented the proportion of new restorations at a certain point in time and were expressions of the speed and timing of caries attacks.

The dates of caries onset and placement of restorations were interval-censored, i.e. recorded to have occurred at the first examination following the diagnosed caries. This means that the birth of the child was recorded as the first “examination”.

In the 7-year follow-up study on oral health habits (III), no manifest caries was registered at the baseline in primary molars, which were the index teeth in the analysis. In the 7-year follow-up study on microbial tests (IV), manifest caries was found only in four teeth in the primary molars, which were index teeth in this survival analysis and left censoring was not done. Right censoring was used for the teeth that were sound at the end of follow-up. We followed the entire study cohort as long as information was available. We did not exclude any subjects outside the study, because survival analysis provides a summary curve of individuals at their actual ages as long as they are followed (Hannigan 2004).

The survival analyses were performed applying the Kaplan-Meier method and using the SAS version 9.1 statistical package. The comparisons between the survival curves were tested using the log-rank test. The statistical significance was set at $p < 0.05$. The outcome variable evaluated was the time from the birth of the subject to caries, defined as the first restoration due to caries in the primary molars and permanent first molars. To examine the covariates simultaneously, the data was also analysed by Cox proportional hazards regression analysis with the survival time of an individual tooth as the outcome (III).

In the 7-year study on oral health habits (III), the relationships between different possible risk factors were studied also by cross-tabulation. Direct univariate associations were analysed by cross-tabulation of the dichotomous variables. Possible associations were analysed by calculating the odds ratios (OR) and 95% confidence intervals (CI). Fisher's exact test and the chi-square test were used to compare differences. The predictive value of microbiological tests was also computed (Table 6). The difference between lactobacilli or yeasts and caries development at different age groups was tested by using Fisher's exact test. The statistical analyses were carried out using SPSS statistical software (SPSS, versions 11.5 and 16.0).

4.6 Ethics (I, II, III and IV)

The study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Oulu. The Municipal Health Board of Oulu approved the follow-up study protocol and granted permission to use the children's dental health records. Additionally, informed consent for participation in this study was obtained from the parents.

5 Results

5.1 Baseline observations (I)

At the baseline, lactobacilli were found in 18% of the cultures from the children's saliva and yeasts in 24%. The use of a pacifier was significantly associated with a positive salivary lactobacilli test (RR, 3.1; CI, 1.5–6.2) and yeast test (RR, 2.7; CI, 1.5–4.9). In addition, the use of nursing bottle at nights and antibiotic therapy were associated with the occurrence of these microbes. Thumb sucking, breastfeeding and symptoms of respiratory infections were not related to the occurrence of either microbe (Table 3).

Table 3. Sucking and feeding habits, symptoms of respiratory infections and antibiotic therapy in children with positive or negative lactobacilli and yeast tests with relative risks (RR) and 95% confidence intervals (CI) at the baseline (I) by univariate analysis.

Variables	Lactobacilli test			Yeast test		
	Positive	Negative	RR (CI)	Positive	Negative	RR (CI)
	n=30 % (n)	n=136 % (n)		n=39 % (n)	n=127 % (n)	
Pacifier sucking	67 (20)	33 (45)	3.1 (1.5–6.2)***	64 (25)	32 (40)	2.7 (1.5–4.9)***
Thumb sucking	0	4 (6)	0.3 (0.02–5.8)	5 (2)	3 (4)	1.4 (0.4–4.5)
Breastfeeding	0	2 (3)	0.6 (0.03–11.6)	3 (1)	2 (2)	1.4 (0.2–7.5)
Nursing bottle at nights	47 (14)	24 (32)	2.2 (1.2–4.2)**	41 (16)	24 (30)	1.8 (1.0–3.1)*
Symptoms of respiratory infections	67 (20)	62 (84)	1.1 (0.5–2.3)	67 (26)	61 (78)	1.1 (0.6–2.1)
Antibiotic therapy	23 (7)	4 (6)	3.5 (1.8–6.6)***	16 (69)	6 (7)	2.1 (1.1–4.2)*

* p < 0.05, ** p < 0.01, *** p < 0.001

The variables that most strongly associated with the occurrence of lactobacilli according to multivariate logistic regression analysis were pacifier sucking (RR, 2.9; p = 0.01; CI, 1.1–7.0) and antibiotic therapy (RR, 4.6; p = 0.01; CI, 1.2–16.9). When a positive yeast test was the dependent variable, pacifier sucking was the only significant risk factor (RR, 4.8; p = 0.0001; CI, 2.1–10.7).

5.2 Two-year follow-up of primary teeth (II)

The caries prevalence at the baseline was 14.5% (n=22). Of the children, 11.2% (n=17) had initial caries and 3.3% (n=5) manifest caries. Two years later at the follow-up examination, 30.3% (n=46) of the children, whose average age now was 4.6 years, exhibited one or more initial (n=22) or manifest (n=24) carious lesions.

In univariate analysis caries at the 2-year-follow-up was significantly associated with the use of a nursing bottle at nights (p=0.014) and the use of a pacifier (p=0.001) at or over two years of age. The relative risk for a child to have caries at the follow-up was almost double if the child had used nursing bottle at nights for over two years (RR, 1.9; CI, 1.2–3.0) and was increased (RR, 2.5; CI, 1.4–4.4) if the child had sucked a pacifier for over two years compared to children without the habit (Table 4).

Pacifier sucking ≥ 2 years was the variable most strongly associated with caries in the multivariate logistic regression analysis (RR, 3.5; CI, 1.5–8.2; p=0.003). The use of a nursing bottle at nights ≥ 2 years was also a risk factor for caries development, but less significant than pacifier sucking (RR, 2.6; CI, 1.1–6.4; p=0.03). Socioeconomic background of the family had no effect on caries development of children.

Table 4. Children with or without caries at the follow-up of two years in relation to caries-associated variables registered at the baseline in univariate analysis with relative risks (RR), 95% confidence intervals (CI) and p-values (II).

	Caries at the follow-up		RR	CI	P-value
	yes (n=46) %	no (n=106) %			
Positive lactobacilli test	27	15	1.6	0.9–2.7	0.111
Positive yeast test	29	19	1.5	0.8–2.5	0.189
Nursing bottle at nights ≥ 2 years	33	15	1.9	1.2–3.0	0.014
Pacifier sucking ≥ 2 years	76	46	2.5	1.4–4.4	0.001
Breastfeeding ≥ 12 months	13	13	1.0	0.5–2.0	0.978

The harmful effect of pacifier sucking on teeth was apparent when the sucking lasted over two years. Of the children with the prolonged sucking habit, 42.5% exhibited caries at the follow-up examination compared to 21.4% of the children with the habit lasting less than two years or to 15.6% of the children with no sucking habit (Figure 3).

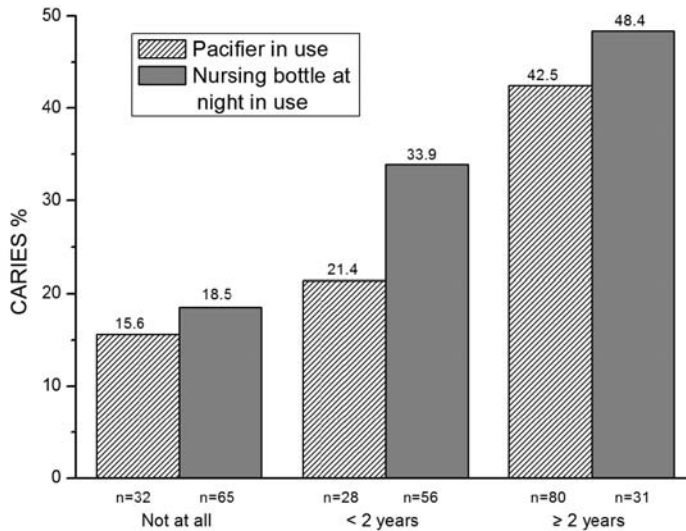


Figure 3. Proportion of the children with caries at the two-year follow-up, by the duration of pacifier sucking and the use of nursing bottle at nights (II).

5.3 Seven-year follow-up (III and IV)

The onset for the 7-year follow-up revealed that at the age of two years 23.8% of the children consumed sweets more than once a week, 21.1% used a nursing bottle at nights, and 55.4% were still sucking a pacifier. On the other hand, 73.7% were using fluoride tablets and 88% brushed their teeth daily. Thirteen per cent of the children had been breastfed for twelve months or longer (Table 2). At the age of two, the caries prevalence was 10.8% (n=17), but as many as 16 had initial caries and only one had manifest caries. Four years later, at the age of six, 33.7% of the children exhibited one or more manifest carious lesions (n=57) in the primary dentition. At the age of seven, the mean DMFT was 0.2 (SD=0.90), and at the age of ten, the mean DMFT was 1.1 (SD=1.7).

The effect of different risk factors registered at the age of two years on caries onset in primary second molars in terms of survival estimates are shown in Figure 4. The survival of the primary second molars decreased faster during the whole follow-up of children who consumed sweets more than once a week ($p<0.001$), used a nursing bottle at nights ($p=0.001$), whose teeth were not regularly brushed ($p<0.001$), or who did not use fluoride tablets ($p<0.001$) at the age of two years (Figure 4). The probability of survival for the primary second molars expressed by

Kaplan-Meier curves was about 70% at the age of six years among children who used sweets, compared with 97% among children who did not (Figure 4). Shorter survival times were also seen among children who used a pacifier at two years of age, but this difference was not statistically significant. Prolonged breastfeeding (≥ 12 months) had no effect on caries onset in terms of survival estimates.

Differences in the rate of caries onset were seen also in permanent first molars (Figure 5). The survival times were significantly shorter among children who used a nursing bottle at nights ($p=0.008$), who did not brush their teeth daily ($p<0.012$), or who consumed sweets more than once a week ($p=0.009$). The use of fluoride tablets and pacifier at the age of two years and breastfeeding for more than a year did not affect the Kaplan-Meier estimates of caries onset in the first permanent molars (Figure 5).

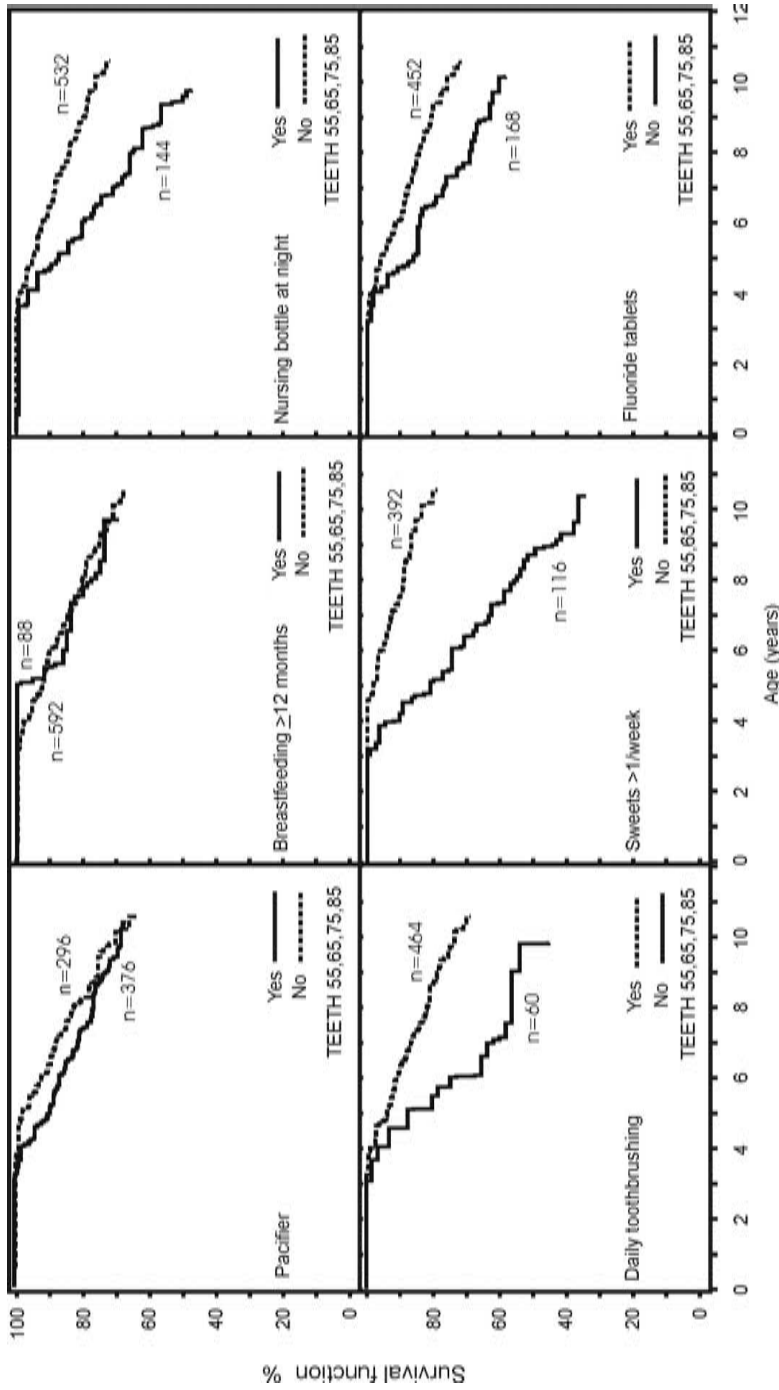


Figure 4. Survival curves for caries onset in primary second molars calculated by chronological age in different risk groups registered at the age of two (III).

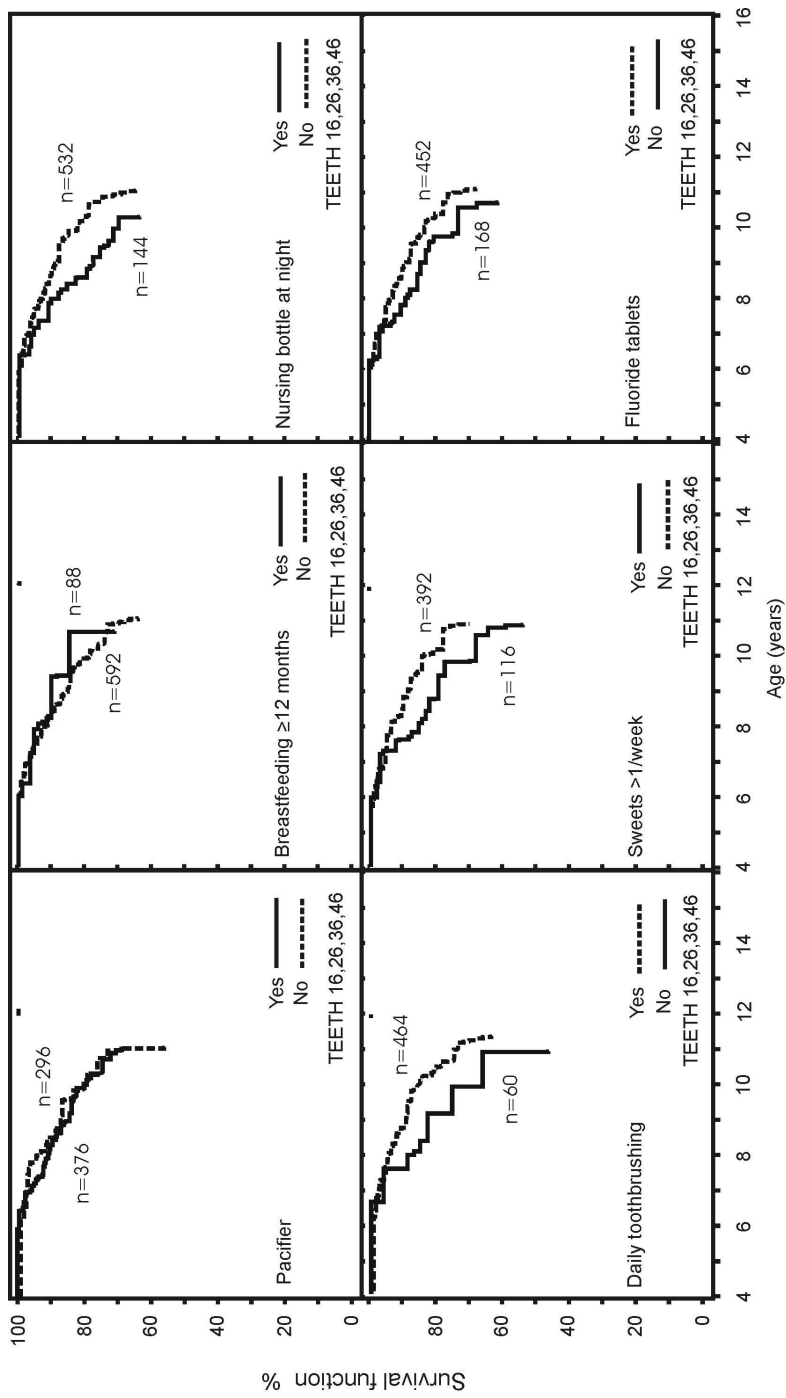


Figure 5. Survival curves for caries onset in permanent first molars calculated by chronological age in different risk groups registered at the age of two (III).

The analysis of the relationships between different risk factors showed that the use of a pacifier was significantly associated with breastfeeding. The children who still used a pacifier at the age of two were breastfed for less than one year (Table 5). When prolonged use of a nursing bottle at nights was analysed, more associations with other caries-related habits were found. According to the univariate analysis, children who at the age of two still used a nursing bottle at nights consumed sweets more often, did not use fluoride tablets, and did not brush their teeth daily (Table 5).

Table 5. Associations between prolonged pacifier sucking and use of nursing bottle at nights with other risk factors reported at the age of two with odd ratios (OR), 95% confidence intervals (CI) and p-values (III).

	Pacifier in use		OR	CI	P-value
	Yes, %	No, %			
	(n=91)	(n=73)			
Sweets more than once a week	30	18	2.0	0.8–4.6	0.117
Breastfeeding < 12 months	96	75	7.3	2.4–22.8	< 0.001
Fluoride tablets in use	71	75	1.3	0.6–2.6	0.542
Daily tooth brushing	89	86	0.7	0.3–2.1	0.584

	Nursing bottle at nights in use		OR	CI	P-value
	Yes, %	No, %			
	(n=91)	(n=73)			
Sweets more than once a week	38	19	2.5	1.0–6.6	0.054
Breastfeeding < 12 months	92	86	1.8	0.5–6.6	0.346
Fluoride tablets in use	57	77	2.6	1.1–6.0	0.021
Daily tooth brushing	75	92	3.6	1.2–11.4	0.021

The evaluation of the predictive value of microbiological tests was made with respect to caries. The positive and negative predictive values of salivary microbiological tests for caries development are shown in Table 6. The results show that at the baseline and in the first follow-up years the negative values are high but later the positive values of both tests are growing. Differences between lactobacilli and caries development was statistically significant at the age of five years (p=0.030), seven years (p=0.001) and ten years (p=0.034).

Table 6. Predictive values of microbiological tests registered at the baseline for caries development at the baseline and at the follow-up examinations.

Positive microbiological test at the baseline	Caries at the							
	baseline		Caries at 5 years		Caries at 7 years		Caries at 9 years	
	PV+	PV-	PV+	PV-	PV+	PV-	PV+	PV-
Lactobacilli	0.14	0.89	0.50	0.86	0.72	0.63	0.71	0.52
Yeasts	0.24	0.93	0.44	0.81	0.50	0.58	0.61	0.50

PV+, positive predictive value

PV-, negative predictive value

The long-term predictive values of the two salivary microbial tests were also tested for the development of caries in primary and permanent molars after the 7-year follow-up (IV).

Survival estimates for caries onsets in primary molars were consistently lower among those children who were colonised by salivary lactobacilli or yeasts at the baseline (Figure 6). The difference was statistically significant in primary first molars, when lactobacilli positive and negative groups were compared (Figure 6A, $p < 0.001$). At the age of eight, the survival of primary first molars was 70% in the group colonised by lactobacilli and 90% among children not colonised with lactobacilli. A clear difference in survival curves for primary first molars was seen also in yeast positive or negative groups (Figure 6D, $p = 0.02$).

In primary second molars, statistically significant relationship was found with onset of caries among children colonised by both lactobacilli and yeast (Figure 6B and E). The survival of primary second molars caries-free was 60% at the age of nine in the group colonised by lactobacilli and 80% in those children who were not (Figure 6B, $p < 0.001$). Significant difference in survival of primary second molars was seen also in children with positive *Candida* tests as compared to negative tests (Figure 6E, $p = 0.001$).

In permanent first molars a significant difference was seen in children colonised by lactobacilli (Figure 6C, $p = 0.004$), but colonisation by yeasts did not cause any difference in survival function (Figure 6F, $p = 0.689$).

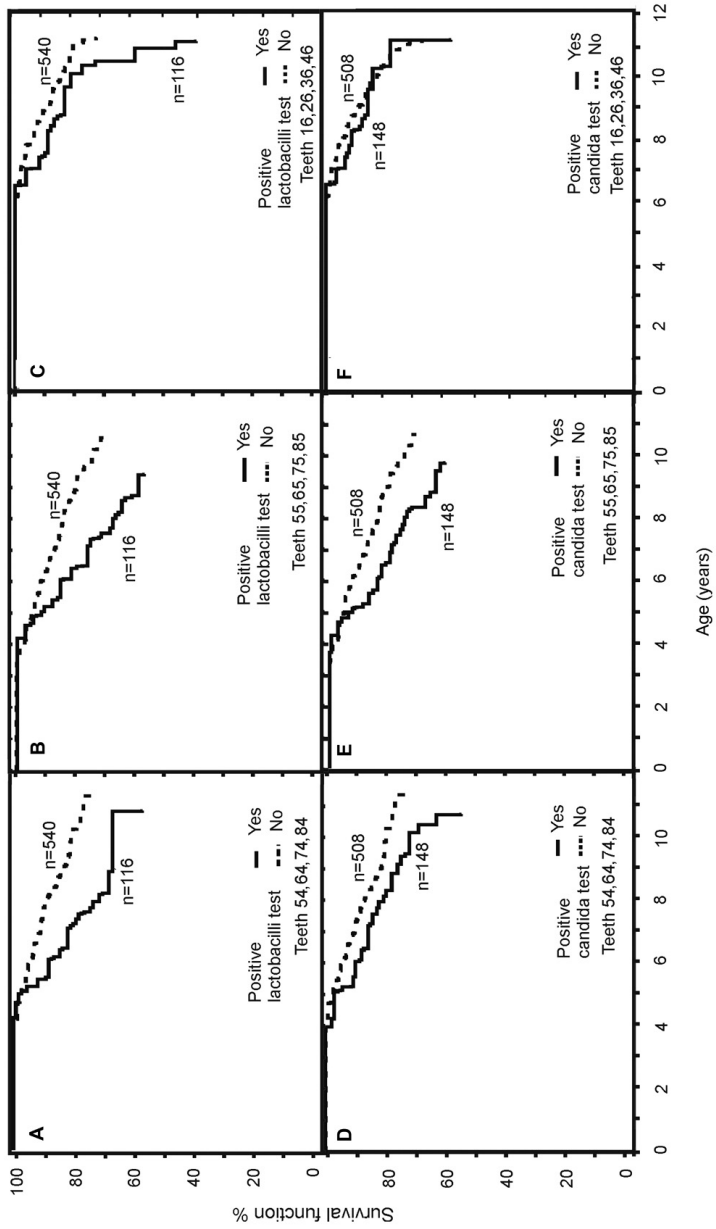


Figure 6. Survival curves of the primary first (A,D) and second (B,E) molars and permanent first molars (C,F) with positive or negative lactobacilli and yeast tests at the baseline (IV).

The relationship between caries experience in primary teeth and permanent first molars is shown in the survival curves in Figure 7. The survival curves of permanent first molars were significantly shorter ($p < 0.001$) among children with caries in primary dentition at the age of five. The differences in survival curves of primary first molars show that caries in primary teeth can be considered as a risk indicator for caries development in permanent teeth.

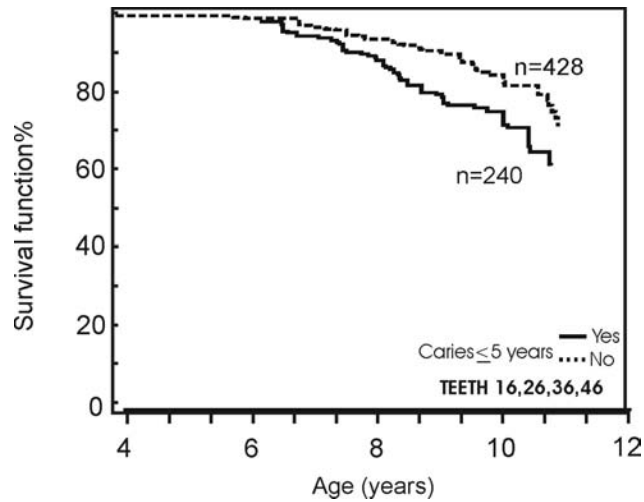


Figure 7. Survival curves of permanent first molars in children with or without caries in primary teeth at the age of five.

6 Discussion

6.1 Methodological considerations

6.1.1 Study cohort, validity and reliability of data

A cohort study is a study in which subjects who presently have a certain condition are followed over time and compared with another group who is not affected by the condition under investigation. The validity of data refers to the degree to which a study accurately reflects or assesses the specific concept that the researcher is attempting to measure. The reliability of the data consists of entering the reliability of the equipment at different times or stages.

The size of the population of this study cohort was determined based on the prevalence of otitis media infections, which was the same order of magnitude at the pre-school age as the prevalence of caries in our study (Niemelä *et al.* 1994). To control the wide age range of the study cohort and to improve the validity of data, we performed the baseline analyses separately on two age groups (I). To control the validity and reliability of the data, age was also included in multivariate analyses both in the cross-sectional study and in the follow-up studies (I and II).

All Finns under the age of 18 are entitled to dental services in municipal health centres free of charge. As most subjects use this privilege, the Finnish dental care system enables retrospective analyses of dental data. Usually dental examinations are performed annually. The measured risk indicators/factors were available from the records of the child health clinics and the clinical outcomes – dental caries and restorative treatment – were available from the records of the health centres.

6.1.2 Background information and caries registration

Information on possible risk factors was collected using questionnaires filled by the children's parents at the baseline. Such information was also partly obtained from dental health records of the public health centres in Oulu. Thus, the study protocol represents partly a controlled clinical survey and partly a practice-based research approach (PBR) (Niederman & Leitch 2006).

Information of caries development was collected from public health records. Several dentists made the examinations, and intra- and inter-examiner variations were not analysed. The variation may have had an effect on the results. On the

other hand, Hausen *et al.* (2001) showed that data collected from public health records are not decisively inferior to those obtained from examinations by trained examiners. The fact that the criteria in our study was manifest caries needing restorative treatment reduces the proportion of most likely errors, i.e. the amount of false positive findings. This may assure that prevalence of caries is not too high and is clinically relevant. In fact, the dmfs- and DMFS indices as outcome measures are relatively valid measures of the amount of restorative treatment need for subjects.

In Finland, dental radiographs are not routinely taken of children for caries examination. As our study had a practice-based research approach, these terms were accepted. At the same time, we have to accept possible underestimation of approximal lesions (Clark & Curzon 2004, Anderson *et al.* 2005). The lack of radiographic examination reduces the prevalence of caries, hence lengthening the survival time in our findings.

6.1.3 Statistical procedures

We used the survival analysis to demonstrate the manifestation of caries risk in primary teeth and in permanent first molars. The benefits of this method, especially in longitudinal caries research, have been shown in many studies (Larmas *et al.* 1995, Virtanen & Larmas 1995, Hannigan *et al.* 2001, Härkänen *et al.* 2002).

The exact timing of caries onset is always impossible to determine. At one examination, the tooth or a tooth surface can be categorized relatively reliably to be either healthy or decayed. The possibility of censoring the data is one of the main benefits of survival analysis (Collet 1994). In case the tooth or the tooth surface is decayed at the onset of the follow-up, the data is usually left censored. In case a healthy tooth or a surface is recorded as decayed at the following examination, the data is interval censored. If the birth of the subject is the first “examination”, interval censoring is often appropriate in young children also at the onset of the follow-up, because the real onset and censored onset are so close to each other. Censoring also occurs when incomplete information about survival time is available for some individuals. Survival analysis has an advantage over conventional statistical methods, as it includes censored observations in the analysis of the data. (Hannigan 2004.) In our study, the right-censored observations are comprised of subjects who did not reach a disease endpoint, i.e. they did not develop caries during the seven-year period of the study or they were left out of the follow-up. Left censoring was not done in the second follow-up of microbiological tests due to the young age of the children.

Because survival analysis needs independent data for the statistical significance determination, which is not the case between individual teeth in the oral cavity, we solved the problem first by analysing the caries onset of each tooth separately. We noticed that no differences existed between right and left contralateral teeth. In addition, we noticed that no significant differences existed between maxilla and mandible in primary and permanent molars and therefore we selected certain teeth as indicators of the oral health in that oral cavity (III and IV). This procedure was performed only in the figures to make them more stable. In the statistical analysis, the number of subjects, not the degree of teeth, was always used in comparing different risk groups in the significance testing.

6.2 Discussion on the results

6.2.1 Prolonged pacifier sucking as a risk factor for caries

The use of a pacifier during infancy is common in Western countries. Larsson *et al.* (1992) showed that about 70% of Swedish children have had a pacifier sucking habit some time during their early childhood. The proportion of pacifier users of 2- and 3-year-olds was approximately 57% and 46%, respectively. Our results are in line with those results: 79% of our subjects had sucked a pacifier and 56% of them were still using a pacifier at the age of two.

The effect of prolonged use of pacifier on caries development was a new and a very important result of our studies. Although the prevalence of pacifier sucking is known to be high among small children (Larsson *et al.* 1992), the relation of that common habit to caries development has not interested the researches of paediatric dentistry. However, before our studies there have been some suggestions in a few studies (Larsson 1975, Wendt & Birkhed 1995) that pacifiers may have some effect on caries activity. After the publication of our studies, also other researchers have become interested in studying the effect of pacifier sucking on caries development in children (Gizani *et al.* 1999, Peressini 2003, Ersin *et al.* 2006, Peres *et al.* 2007). Recent studies have demonstrated similar results as our studies as concerns the relationship between pacifier sucking and caries-related micro-organisms lactobacilli and yeasts (Ersin *et al.* 2006, Comina *et al.* 2006). In addition, the effect of pacifiers on the risk of dental caries in children has been shown in some new studies (Vázquez-Nava *et al.* 2008, Yonezu & Yakushiji 2008). However, some results of the studies on the relationship between the use of pacifiers and dental caries have been controversial (Deery 2004).

We found that both microbes analysed were related to the use of pacifier at the baseline. The reason for the increased frequency of oral lactobacilli and yeasts among children using a pacifier may be the change in local environmental conditions in the mouth. A pacifier may affect oral sugar clearance in the same way as removable dentures, which have been shown to contribute to less effective clearance (Hase & Birkhed 1991). Reduction in clearance would prolong conditions of low pH in plaque and thereby favour the selection of aciduric micro-organisms (Bradshaw *et al.* 1989). In the study by Arendorf & Addy (1985), a significant fall in salivary pH was found in the presence of a removable orthodontic appliance together with an associated increase in both the frequency and density of yeast colonisation.

A pacifier may also increase the number of receptor sites available for microbial adhesion. It may directly affect plaque accumulation, increasing it in the same way as when a partial denture is placed in a patient's mouth (Addy & Bates 1977). Such appliances could provide a suitable surface on which oral yeasts and lactobacilli could adhere to and become established. It has also been suggested that a pacifier interferes with the mucous membrane, favouring yeast colonisation (Sio *et al.* 1987).

Prolonged use of a pacifier was associated only with the duration of breastfeeding, a finding also seen in the study by Paunio *et al.* (1993b). They found out that children who had been breastfed for a shorter time had a significantly higher need to suck a pacifier. Pacifier sucking was not associated with other behavioural factors. Therefore, it may be an independent factor that alters the circumstances in the oral cavity into a more caries-active direction, as was the case with the present study cohort at the age of two, when aciduric lactobacilli and yeasts increased in saliva (I). In contrast to the pacifier use, prolonged use of a nursing bottle at nights was related to several other risk factors, confirming that dental caries in children is mainly a lifestyle disease caused by various caries promoting habits (Wendt *et al.* 1996, Mattila *et al.* 2005, Leroy *et al.* 2005).

In the follow-up studies, we found a relationship between the prolonged duration of pacifier sucking (≥ 2 years) and caries development. If the sucking habit lasted less than two years, the effect was not that harmful (III, Figures 3 and 4).

The effect of prolonged use of pacifier on caries onset was principally shown in the two-year follow-up, when the total caries development was registered. Initial caries lesions were included in this data as well (II). So the whole experience of caries was estimated, and especially the effect of pacifier sucking on the development of caries in primary incisors was shown. Caries is primarily found on the buccal, lingual and proximal tooth surfaces of maxillary incisors up to three

years of age (Wendt *et al.* 1992, Grindefjord *et al.* 1993). Later, other tooth surfaces are affected, such as occlusal surfaces of second molars (Grindefjord *et al.* 1995b), however, they are probably not as susceptible as incisors are. In the longer, 7-year follow-up, when primary and permanent molars were the index-teeth, the effect was not significant. However, the survival time was shorter in primary molars among children with prolonged use of pacifier (III).

The duration of pacifier sucking has also been demonstrated to increase the risk of suffering recurrences of acute otitis media infections among children aged 2–3 years (Niemelä *et al.* 1995). Furthermore, it is generally known that sucking habits are connected with malocclusions and disturbances in dentofacial development (Larsson 1986, Lindner & Modéer 1989). To prevent the harmful effect of pacifier sucking on occlusal development, it has been suggested that the use of pacifier should be stopped by the age of two (Modéer *et al.* 1982). To prevent early caries development, the same recommendation seems to be justified.

6.2.2 Oral health habits at two years of age as risk factors for caries

The main factor associated with early manifestation of caries in both dentitions was the consumption of sweets at the age of two. This is in agreement with results of a previous Finnish study, which reported that the main risk factor for caries development in primary dentition of children from three to six years of age was daily sucrose intake that had started already at three years of age (Karjalainen *et al.* 2001). Consumption of sweets more than once a week was an important predictor for developing manifest caries between 2.5 and 3.5 years of age also in an earlier Swedish study (Grindefjord *et al.* 1996).

Prolonged use of a nursing bottle at nights was a significant risk factor for caries development in both primary and permanent molars in our study. Similar findings in primary teeth have been reported also by other researchers in different countries (Wendt *et al.* 1996, Hunter *et al.* 1997, Hallet & O'Rourke 2002).

Our studies have shown that caries onset was significantly associated with the lack of daily tooth brushing. These findings are in line with other studies, showing that oral hygiene is strongly related to caries development in children (Alaluusua & Malmivirta 1994, Wendt *et al.* 1996, Grindefjord *et al.* 1996, Karjalainen *et al.* 2001, Vanobbergen *et al.* 2001).

Our findings are also in agreement with studies showing that the use of fluoride tablets is associated with dental health in primary teeth, but no associations have been found in permanent molars (Wang & Riordan 1999, Leroy *et al.* 2003).

Perhaps the positive effect of fluoride at two years is not as long lasting as the negative effects of the possible risk factors.

We found no association between prolonged breastfeeding (≥ 12 months) and children's dental health in our study, which is in line with some earlier studies that have found no correlation between breastfeeding and caries prevalence (Alaluusua *et al.* 1990a, Roberts *et al.* 1994). However, some studies have demonstrated an association between prolonged breastfeeding and caries (Williams & Hargreaves 1990, Wendt & Birkhed 1995).

We conclude that consumption of sweets more than once a week at the age of two, inadequate tooth brushing, prolonged (≥ 2 years) use of a nursing bottle at nights, pacifier sucking, and neglecting the use of fluoride tablets are important risk factors for caries development in both primary and permanent molars. In addition, caries in primary molars was shown to be a significant risk factor for caries development in permanent molars.

Assessment of caries risk in toddlers enables early intervention and prevention. Early intervention is known to have a beneficial effect on the dental health of young children (Wennhall *et al.* 2005, 2008). The results of the study by Pienihäkkinen *et al.* (2002) showed also that as concerns young children, risk-based management of caries seems practical and prevention of caries can be targeted efficiently to individuals at risk. Success in risk-based prevention enables also successful work division, and consequently economic effectiveness (Pienihäkkinen *et al.* 2005).

6.2.3 Lactobacilli and yeasts as risk factors for caries

We found a borderline association between early colonisation of oral lactobacilli and yeasts and development of dental caries in young children. This finding is in accordance with earlier studies that have demonstrated a relationship between caries development and cariogenic microflora (Grindefjord *et al.* 1993, Granath *et al.* 1994, Roeters *et al.* 1995).

The prevalence of lactobacilli (18%) in our study was of the same magnitude as that reported by Grindefjord *et al.* (1993), who found that 22% of children of 2.5 years of age were colonised with lactobacilli. They also demonstrated an association between early colonisation of cariogenic microflora, mutans streptococci and lactobacilli and development of dental caries in young children. Carlsson *et al.* (1975) found lactobacilli only occasionally in children younger than two years, but after the age of two, the lactobacilli count increased, and the presence of these microbes was associated with caries in children aged 2–5 years.

Candida genera have been found to be the most common yeast in the mouth (Martin & Wilkinson 1983). The proportion of yeast carriers among children varies from 21% to 70% (Hodson & Craig 1972, Pienihäkkinen *et al.* 1985, Starr *et al.* 2002) and increases with age (Somerville 1969). Carriage of salivary yeasts and carriage of both lactobacilli and yeasts predict significantly the three-year caries increment (Pienihäkkinen *et al.* 1987). We found oral yeasts in 24% of the children whose average age was 2.5 years, and we noticed that pacifier sucking increased the occurrence of the carriage of yeasts. Salivary lactobacilli counts were also elevated among children using a pacifier. Both microbes are considered aciduric (Bratthall & Carlsson 1989) and thus unfavourable to dental health.

Chairside salivary microbial tests have been developed for normal dental clinics but they can also be used in practice-based research (PBR) to select patients with high caries risk (Larmas 1992). The present study investigated the ability of chairside diagnostic methods to categorise and predict caries development in children. The commonly used dip slide techniques for microbial screening in saliva require collection of stimulated saliva, which may be difficult to obtain from small children. This has been remedied by direct inoculation of agar media by tongue depression used to collect salivary specimens from the tongue (Weinberger & Wright 1990), or by licking the dip slide (Gabre *et al.* 1999). In the present study, saliva samples were taken from unstimulated saliva by cotton swabs and were immediately cultivated over the medium. The results showed that this method was an easy microbial screening system at the toddler age.

In the follow-up study by Raitio *et al.* (1996b), significant associations were observed between caries increment and salivary lactobacilli and yeasts in adolescents. This is in agreement with our 2-year follow-up study, where we found a positive association between salivary microbial counts and caries development (II). Our results are also in line with those reported by Twetman *et al.* (2002), who stated that high levels of salivary lactobacilli was one of the most influential determinants for high caries development during the three-year follow-up period in young type 1 diabetes mellitus patients.

Many studies have shown that past caries experience is a better predictor of caries increment than salivary bacterial counts (Alaluusua 1993). Salivary bacterial counts have been reported not to be a useful additional caries predictor for mixed dentition, when a combination of caries related variables are used (van Palenstein-Helderman *et al.* 2001). Our previous studies may explain this: salivary microbial counts have an association with other caries risk factors, such as prolonged use of a pacifier and nursing bottle at nights (I & II). It is well known that bacteria represent

only one single causative factor in the multifactorial caries process but high levels may reflect caries-active circumstances in the mouth caused by other aetiological factors. In small children without visible signs of caries, microbiological screening could be of great value in predicting caries activity. Especially to be able to prevent or arrest caries development before manifest caries lesions are established. It is important to identify children with high caries risk as early as possible and arrange extra preventive intervention for them.

At the baseline, 14.5% of our study population had initial or manifest caries (II). Naturally, caries lesions may have increased the level of microbial counts. As mentioned before, past caries experience alone has a good predictive value, but our study showed that bacterial tests used as indicators of caries active conditions predict caries in young pre-school children (IV).

We studied the value of microbial counts to predict caries risk. Our results showed that the negative predictive values were greater especially at the baseline. This study together with many others (van Houte 1993, Llena-Puy *et al.* 2000, Pinelli *et al.* 2001) indicate that prediction of low caries risk by microbiological tests may be more reliable than the same tests predicting high caries risk. In other words, microbial tests may be more effective in identifying healthy children than those needing treatment.

Although mutans streptococci and lactobacilli have been implicated as major etiological agents of dental caries, many new studies have shown that also non-mutans streptococci and Actinomyces spp likely play important roles in caries progression (Aas *et al.* 2008, Takahashi & Nyvad 2008). Therefore, further studies of the potential etiologic roles and also the possible predictive value of these diverse bacterial communities are needed.

6.2.4 Clinical implications

Dental health of children has not improved in Finland during the last years (Nordblad *et al.* 2004). Past caries experience has usually been the most powerful single predictor of future caries increment (Hausen 2008). To enhance the possibility to prevent caries in children, it is important to do early risk assessment, even before any lesion exists.

The results of our study indicate that it is possible to assess risk factors for early caries development in children by oral history and salivary microbial tests. This will enable early intervention when needed. These results support the new decree of the Ministry of Social Affairs and Health on welfare clinical services,

school and student health services, and preventive health services for children and the youth (2008). For children with high caries risk, intensified prophylactic public health care has to be arranged. All these findings also support the Uniform criteria for access to non-emergency treatment in the reports of the Ministry of Social Affairs and Health (2009), early childhood intervention was also shown important to remain free of the caries disease.

7 Summary and conclusions

1. The results of the present study suggest that the use of a pacifier may increase the occurrence of caries-associated micro-organisms, and later the manifestation of caries lesions. Pacifier sucking long after the eruption of teeth may be a risk to dental health.
2. Consumption of sweets more than once a week and inadequate tooth brushing at the age of two are important risk indicators for caries development in both primary and permanent molars. Prolonged (≥ 2 years) use of a nursing bottle at nights, pacifier sucking and neglected use of fluorides may also contribute to the risk of caries.
3. Assessing the level of salivary lactobacilli and yeasts may help in planning preventive programmes for children.

In conclusion, variables associated with caries were consumption of sweets more than once a week, inadequate tooth brushing, prolonged use of nursing bottle at nights, neglected use of fluorides and prolonged use of pacifier. These important indicators are collected routinely at Finnish child health clinics of two-year old children. Therefore, information collected at the toddler age is of great value when planning the future dental health care of children.

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Appendix 1

Questionnaire for the parents

Has your child sucked a pacifier?

Yes, since _____ months old.

- Yes, still does.
- Yes, but stopped at _____ months.
- No, never.

If your child sucks/has sucked a pacifier, does/did she or he use it

- only when going to sleep, or
- also while awake?

Has your child had a thumb-sucking habit?

- Yes, still has.
- Yes, but it stopped at _____ months.
- No, never.

Has your child had any respiratory infection symptoms in the past two weeks?

- No.
- Yes.

If yes, which symptoms and for how long?

- Clear nasal discharge for ____ days.
- Yellow or green nasal discharge for ____ days.
- Nasal congestion for ____ days.
- Cough for ____ days.
- Fever over 38°C for ____ days.
- Eye discharge for ____ days.

Was your child breastfed?

- () No, not at all.
- () Yes, until ___ months old.
- () Yes, still is.

Has your child had a habit of sucking a nursing bottle when going to sleep?

- () No, not at all.
- () Yes, until ___ months old.
- () Yes, still has.

How many ear infections has your child had?

___ ear infections.

Have your child's tonsils been removed?

- () No.
- () Yes. When? ___/ ___ - ___ Where? _____

Does your child have ear tubes inserted?

- () No.
- () Yes. When? ___/ ___ - ___ Where? _____

How long has your child been attending this day care centre?

Since ___/ ___ - ___ .

Please, return this form to the day care centre.

Thank you for your input!

Original papers

- I Ollila P, Niemelä M, Uhari M & Larmas M (1997). Risk factors for colonization of salivary lactobacilli and candida in children. *Acta Odontol Scand* 55: 9–13.
- II Ollila P, Niemelä M, Uhari M & Larmas M (1998). Prolonged pacifier-sucking and use of a nursing bottle at night: possible risk factors for dental caries in children. *Acta Odontol Scand* 56: 233–237.
- III Ollila P & Larmas M (2007). A seven-year survival analysis of caries onset in primary second molars and permanent first molars in different caries risk groups determined at age two years. *Acta Odontol Scand* 65: 29–35.
- IV Ollila P.S.H. & Larmas M.A. (2008). Long-term predictive value of salivary microbial diagnostic tests in children. *Eur Arch Paediatr Dent* 9: 25–30.

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