# Caries Prevalence and Caries Associated Measures in Children Living in a Rural Romanian Village

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**ABSTRACT**

A cross-sectional study was performed with the aim to investigate the caries prevalence and associated factors in 6 to 15 year old school children living in a village outside Cluj-Napoca, Romania. All children were offered to be included and examined for dental caries according to World Health Organisation (WHO) criteria. Caries was scored at the tooth and surface levels D/d=decayed, M/m=missing, F/f=filled, T/t=tooth, S/s=surface (DMFT/DMFS/dmft/dmfs) for the permanent and deciduous dentition, respectively. Presence of dental plaque was evaluated according to Silness-Löe, infection with the caries associated bacterial species *Streptococcus mutans* and *Streptococcus sobrinus* by polymerase chain reaction (PCR), and general information collected with a questionnaire. Totally, 71 children, 69 children were clinically examined. Mean DMFT was 5.7 and DMFS 9.8, and the Significant-Caries-Index (SIC), *i.e.* the mean (DMFT-value) in the highest tertile based on caries distribution, was 10.8. *S. mutans* was detected in 86% and *S. sobrinus* in 49% of the children, and 46% were infected with both species. Five children (7%) were neither infected by *S. mutans* nor *S. sobrinus.* Among all children the mean number of reported daily intakes of sweet products was 4.8, and large amounts of dental plaque was generally seen. In conclusion, the caries prevalence among school children in the village was very high, oral hygiene inadequate, daily sugar intake high, and prevalence of *S. mutans* high, combined with no access to dental care and caries preventive programs.

## INTRODUCTION

Dental caries is an infectious, multifactorial disease characterized by loss of dental tissues. In fact, untreated caries was the most prevalent medical condition among close to 300 hundred conditions compared in a recent systematic review (Marcenes *et al*., 2013). In its more advanced stages the disease results in a cavity or fracture of the tooth. The main disease determinants are acidogenic and aciduric bacteria and their by-products from carbohydrate fermentation, but several factors modify disease outcome. Thus, disease development is influenced by (*i*) host related factors, such as saliva flow and composition, dental biofilm composition (numbers and type of bacteria), (*ii*) lifestyle and environmental factors, such as, diet, oral hygiene, availability of fluoride, and (*iii*) socioeconomic conditions (WHO, 2001a). Among the acidogenic and aciduric bacteria, *Streptococcus mutans*, *Streptococcus sobrinus* and lactobacilli are commonly found associated with dental caries, through their ability to survive at low pH and effective production of lactic acid at sugar exposure. However, any bacterial species that lowers pH in the tooth biofilm to a critical level may contribute to the disease. The present concept, the ecological plaque hypothesis, takes the balance in the entire microbiota into consideration rather than focusing on single species (Takahashi and Nyvad, 2008; 2011).

Romania is a country in eastern Europe, with approximately 22 million inhabitants (WHO, 2014). The country had communistic regime from 1947 until 2007 when it became a democratic republic. Poverty has decreased lately and today Romania is classified as an upper middle-income country (The World Bank, 2012).

According to the Oral Health Profile database at Malmö University, the caries prevalence (Decayed, Missing, Filled Teeth, DMFT) in 12-year olds in Romania was 2.8 in 2000, and 3.3 in 2007 in the Constanta district. In comparison, DMFT in German and Swedish 12-year-olds was 0.7 and 0.8 in 2009 and 2010, respectively (Malmö University, 2014a). Besides this, no information on the

caries situation in Romania was available until a recent publication showed that the caries prevalence remains very high in schoolchildren in Bucharest and that a large portion of the caries is untreated (Funieru *et al*., 2013).

The purpose of the present study was to survey caries prevalence and caries associated factors in a village in Romania where no regular dental care or preventive measures were available. Since this is a descriptive study no hypothesis was formulated, but our expectation was that dental caries prevalence and levels of various caries risk factors would be very high in the examined children.

## MATERIALS AND METHODS

### Study design and study subjects

The present study is a cross-sectional population study performed in June 2013 in a rural village in Romania. The village is located in Cluj county in north-western Romania. Approximately 100 subjects were within the age range to go to school (6 to 15 years). The socioeconomic standard in the village was low with limited dental treatment and health counselling. The only dental care that was provided was by a volunteer dentist, who did emergency treatments on an irregular basis.

All children attending school in the village were invited to participate. The invited children were from mixed socio-economic, ethnical and religious backgrounds. Children, for whom the parents consented to participate, came to a fully equipped private dental clinic located approximately 100 m from their school. At the clinic, the oral examinations took place when the child sat in a dental chair equipped with good artificial light. Sterilized probes and mirrors were used and caries was scored according to WHO criteria (WHO, 2013).

**Self reported information**

Information on health status, oral hygiene, dietary habits and family situation was obtained by a questionnaire written in their native language (Appendix 1). The questionnaires were handed out to the parents two weeks before the examination, and returned when the child attended the clinic. At the clinic one of the examiners tried, in collaboration with the child, to solve ambiguities or fill in missing answers in the questionnaire.

**Collection on bacterial biofilm**

The children had been instructed not to brush their teeth the morning they attended the clinic. After biofilm registration, bacteria biofilm was sampled from random incisors, premolars and molars using sterile toothpicks. The samples were transferred to Eppendorf tubes with 100 µl Tris-EDTA (TE)-buffer, kept cool, and transferred to Umeå University.

**Plaque registration**

Plaque was registered on six selected teeth (16, 12, 24, 36, 32 and 44) according to the Silness-Löe index (Silness and Löe, 1964). If a permanent tooth was missing the corresponding deciduous tooth was chosen, and if both a deciduous and permanent tooth were missing or not fully erupted, a neighbouring tooth was selected. Plaque amount was registered on a four level scale (Table 2). Four surfaces were scored on each tooth (distal, mesial, buccal and palatine or lingual).

**Caries registration**

Caries was registered visually under good artificial light by probing with new probes and non-magnifying mirrors. No x-rays were taken. The teeth were not systematically cleaned before scoring, but if necessary debris was removed with the probe.

Decayed (D/d, including enamel (initial) and dentin (manifest) caries), missing (M/m) and filled (F/f) teeth (T/t) or surfaces (S/s) were recorded. The criterion for initial caries was a chalky white spot with an unbroken surface. The criteria

for manifest caries were a visually detectable cavity, or a catch of the blunt probe under slight pressure for fissures and approximal surfaces.

DMFT and DMFS were calculated for the permanent teeth, dmft and dmfs for the deciduous teeth according to (Klein *et al.,*1938). Third molars and initial caries lesions were not included in the calculation, secondary caries lesion were registered as decayed, and a missing tooth was calculated as four surfaces for an incisor and five surfaces for a premolar/molar.

**Oral hygiene instructions**

When the oral examinations were completed each child got a toothbrush and a tube of fluoridated toothpaste. Individual instructions on tooth cleaning were given by demonstration on a model, in the mouth and by hands-on training. When the child had no previous technique or knowledge on tooth brushing, he/she was instructed to use the modified Bass-method (Poyato-Ferrera *et al*., 2003). It was confirmed that the children understood the instructions, and expressed motivation to improve oral hygiene.

**PCR for detection and qPCR for enumeration of *S. mutans* and *S. Sobrinus.***

Conventional polymerase chain reaction (PCR) was used to detect presence of *S. mutans* and *S. sobrinus*, and quantitative polymerase chain reaction (qPCR) to quantify DNA from these bacteria (Tanner *et al.,* 2012). According to manufactures instructions, dental biofilm DNA was extracted using the Gen Elute Bacterial Genomic DNA kit (Sigma-Aldrich, St Louis, MO, USA), and the amounts measured with a Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). For conventional PCR the KAPA2G Robust HotStart PCR Ready Mix (2´) kit (Kapa Biosystems, Boston, MA) and *S. mutans* and *S. sobrinus* specific primers (Yano *et al.*, 2002) were used. DNA (2 µL) was added to a 25-µL PCR reaction, and standard PCR conditions applied, *i.e.* initial denaturing at 95°C for 15 min followed by 30 cycles at 95°C for 30 min, 60°C for 30 s, 72°C for 1 min, and a 72°C extension for 5 minutes. PCR products were verified by electrophoresis on 1 % agarose gels and ethidium bromide staining.

For qPCR, the same primers and the KAPA SYBR Fast qPCR Master Mix Universal kit (KAPA Biosystems, Boston, MA) was used.
The qPCR reaction mixture contained, 1 µl DNA template, 10 µl KAPA SYBR Fast Master Mix (X2) (Invitrogen, Life Technologies, Carlsbad, CA), and 0.5 µM primers. The qPCR conditions were initial denaturation at 95°C for 1 min, followed by a 40-cycle amplification program with denaturation at 95°C for 60 s and annealing and extension at 68°C for 1 min. The critical threshold cycle (Ct), *i.e.* the cycle in which fluorescence is detectable above the background fluorescence, was monitored for extracted DNA with its increasing amounts of *S. mutans* strain Ingbritt (CCUG 27624, Culture Collection, University of Göteborg, Sweden) and *S. sobrinus* strain OMZ176 (CCUG 28076), respectively. Standard curves plotting Ct-values against increasing bacteria DNA quantities were plotted, and sample Ct-values converted to number of DNA molecules.

**Ethical considerations**

At the time the project was performed, no formal routine for ethical approval was defined in Romania or the study region. Hence, the guidelines in the Helsinki declaration, including information to the parents and children, informed consent from the parents and the child, confidentiality and demands of usefulness, was considered. Thus, a written description of the project and a form for consenting for the child´s participation was given to the parents before the child was invited to participate. The teachers of the children had been informed about the project and could answer questions from the parents or children.

Study participation was voluntary with possibility to withdraw whenever the child wanted, and there was no dependence between the examiners and the children. The applied clinical procedures are not associated with any risk, and the risk that obtained information could be connected to an individual was eliminated by using consecutive number codes only. The results are presented on a group level, and the groups are too large for any individual subject to be identified. The children benefitted from participation by getting a careful

instruction how to maintain healthy teeth. This was possible to do since one of the investigators (EW) speaks the language.

**Data handling**

SPSS (version 20) was used for descriptive analyses and testing of associations. First the distribution of various variables was tested by the Kolmogorov-Smirnov test. If a variable was normally distributed, data are presented as means with 95% confidence interval and parametric tests, *i.e.* ANOVA and *t*-test, were applied to compare group means. If non-normally distributed, medians with interquartile values, and non-parametric tests, such as Mann Whitney U-test for comparison of ranking distribution between groups, were used. Spearman correlations were used to test association between variables, and Chi-square test to test differences in frequency distributions. A p-value <0.05 was considered statistically significant.

The number of caries affected surfaces per tooth (DMFS+dmfs/number of teeth) was calculated, and the children were ranked into tertile groups based on their caries/tooth distribution. The caries prevalence data (DMFS+dmfs/number of teeth) were not normally distributed, but logarithmic transformation improved normality (Figs. 1a,b). The Significance caries Index (SIC), which is mean caries among the individuals in the upper third of the caries distribution in the study population, was calculated (Bratthall, 2000).

Literature search was made in PubMed and The Cochrane Library with the search terms: dental caries, rural area, cross sectional studies, children, Romania, caries prevalence, Europe, qPCR, PCR analysis, cariogenic bacteria and mutans streptococci. This resulted in thirty articles and twelve were used. Additional references were from reference lists in read papers, the World Wide Web, and the textbook Dental Caries - the disease and its clinical management.

## RESULTS

From approximately 100 children expected to attend the village school, 70 children were included. Among the 30 non-participating students one was temporarily diseased and could not be examined, one did not want to participate, and the other 29 did not attend school on a regular basis. All parents consented for their child to participate in the project. The children answered the questionnaire before the clinical examination. The response rate among the 70 included children was 92.9%.

The mean (95% CI) age of the study participants was 11.8 (11.2-12.3) years (Table 1). The sex distribution was 48.5% girls and 51.5% boys. Mean (95% CI) number of teeth (deciduous + permanent teeth) in all children was 24.7 (24.2-25.3), and in those with a mixed dentition 22.7 (22.2-23.3) and with a permanent dentition 26.7 (26.3-27.1) (Table 2). None had deciduous teeth only.

When children with a mixed dentition were compared with those with a permanent dentition, age, sex, illness last year, medication and daily intake frequency of sugar snacks differed significantly ($p<0.05$, Table 1). Other characteristics, *i.e.* number of family members, number of children in the family, physical activity in leisure time, daily tooth brushing, problems with teeth, visited a dentist last year, and never visited a dentist did not differ between these two groups (all $p>0.05$).

The study participants where split into a high caries group with those with a caries prevalence above the median value of dmfs+DMFS/number of teeth (≥0.67), and a low caries group with a caries prevalence below the median value. Most characteristics, like sex, number of family members, number of children in the family, physical activity in leisure time, illness last year, medication last year, problems with teeth, visited a dentist last year, daily tooth brushing, never visited a dentist and daily intake frequency of sugar snacks did not differ between the two caries groups, but children in the low caries group tended to be older than those in the high caries group ($p=0.056$, Table 1).

Adjustment for type of dentition was evaluated, but found to have very little impact in these comparisons (Table 2). Therefore, unadjusted data are presented throughout the paper.

**Caries prevalence**

Besides the traditional dmfs and DMFT indices, the dmfs+DMFS/number of teeth was calculated and all calculations involving caries prevalence were tested both with crude and log transformed data. Crude data are presented in Table 2 to facilitate readability. For some calculations we standardized for type of dentition as a proxy for cariogenic exposure time (Table 2).

Among all children mean (95% CI) DMFT was 5.7 (4.65-6.74), DMFS 9.8 (7.7-12.0), dmft 3.3 (2.2-4.4), and dmfs 9.3 (6.1-12.5), respectively. The corresponding SIC-values, *i.e.* values for highest tertile, were 10.8 (6-16) for DMFT. As expected dmfs/DMFS and caries/tooth were significantly higher in the high compared to the low caries group ($p<0.001$, Table 2, Fig. 1c). These measures were also higher in children with a mixed dentition compared to those with permanent teeth only ($p\leq0.001$, Table 2). Using logarithmically transformed data or adjusting for type of dentition did not alter the outcome.

**Plaque by Silness-Löe index**

A weak, but not statistically significant, positive correlation was seen between amount of plaque and prevalence of caries/tooth ($r_{Spearman}= 0.145$, $p=0.233$; Fig. 2). Mean plaque index did not differ significantly between the high and low caries groups or children with mixed or permanent dentition (Table 2).

**Prevalence of *S. mutans* and *S. sobrinus* infection**

In total, only five children out of 69 children (7%) were neither infected by *S. mutans* nor *S. sobrinus*. *S. mutans* was detected in 86% of all children with no difference by dentition, but significantly higher prevalence in the high versus low caries children (94% and 77%, respectively, $p=0.036$). *S. sobrinus* was detected in 49% of all children, with no difference by dentition or caries group

(Table 2). All children infected with *S. sobrinus* were also infected with *S. mutans*. Among these children, 61% were in the high and 39% in the low caries group (p=0.113, data not shown).

Spearman correlation between bacterial counts. *i.e.* species specific DNA, and prevalence of caries per tooth was 0.355 (p=0.003), whereas no correlation was seen with measures for *S. sobrinus.* However, mean values for *S. mutans* or *S. sobrinus* DNA did not differ significantly by caries or dentition group (Table 2, Figures 3a,b).

**Oral hygiene habits**

Among all study participants 45% claimed to brush their teeth twice daily. The corresponding proportions were 40% and 50% in the high and low caries groups, respectively (p=0.359). Similarly, proportions claiming to brush twice daily did not differ by dentition group (p=0.737).

**Dietary habits**

Among the children 48.2% reported that they ate breakfast daily, 93.7 % lunch and 88.9% dinner, with no difference between the high and low caries groups (data not shown). The mean (95% CI) number of reported daily intakes of sweet products was 4.8 (3.8-5.7) among all children. Reported intake frequency of sweet products (intakes/day) tended to be lower in the high than the low caries group 3.8 (2.6-5.0) versus 5.7 (4.1-7.2), p=0.062, respectively). Children with a mixed dentition reported fewer sweet intakes than those with a permanent dentition 3.7 (2.5-4.9) versus 5.7 (4.2-7.2), p=0.037, respectively.

## DISCUSSION

In the present study children living in Ciurila, Romania were examined in order to estimate the caries prevalence and levels of caries risk factors in an area where dental care was negligible and no previous studies had been made. The main findings were that the caries prevalence was high, oral hygiene inadequate, sugar intake frequent, prevalence of caries-associated bacteria high, and access to operative or preventive dental care, including use of a

fluoride containing toothpaste, virtually non-existing. These findings were fully in accordance with our assumptions. This documentation forms a foundation for a treatment program and follow-ups in the area.

The caries prevalence in Ciurila was alarmingly high. The prevalence was more than two times higher than reported for 12-year olds in other parts of Romania (DMFT=2.7 Romania in 2001) and seven times higher than in 12-year olds in Sweden (DMFT=0.8, 2010-2011). Mean SIC-value, *i.e.* mean values in the upper tertile, was 10.8, and the only countries reporting similarly high SIC-levels are Croatia 10.9 and Slovakia 14.3 (Malmö University, 2014a,b,c). Thus, the presently found caries prevalence is higher than other available data for 12-year olds in Romania. There are several plausible explanations for this; (*i*) the prevalence was actually this high in this rural village, (*ii*) the M component may be overestimated since the children had a mixed dentition and we could not distinguish if a tooth was lost due to caries or exfoliated. However, even in the latter case it may be assumed that the tooth was affected by caries, but maybe to a lower degree than by imputation. Explanation (*i*) is supported by the facts that caries prevalence in the present study was close to twice as high as recently reported for central Bucharest, and that Luca *et al*. (2003) reported approximately twice as high caries prevalence in rural compare to urban areas (Luca *et.al*., 2003), and a similar tendency for central Bucharest versus peripheral areas (Funieru *et al.,* 2013).

The strength of the present study is that it was conducted in an unexplored area where the natural history of caries development could be monitored. Additional strengths are that all oral examinations was done by the same examiners, the entire child population in the village was invited, and that the examinations were performed in a dental clinic with good light and not with sunlight or a flashlight. It is also a strength that the other examiner could communicate in the children´s mother language, and that molecular methods could be used for bacteria enumeration since no facilities for culturing were available.

The weaknesses are that it was not possible to take x-rays, which may have lead to underestimation of caries symptoms. However, most caries lesions were so extensive that visual registration was sufficient, and probing and x-ray would not add much information. Another weakness is that the study group is mixed from an age and dentition point of view, and that the numbers do not allow for proper stratification by age, age/gender or dentition. This was partly circumvented by calculating caries per tooth and by evaluating what adjustment for dentition did. It is also a disadvantage that we could not obtain any information on the non-attending children, but this was not possible since they did not come to school. However, we see no reason to believe their dental status would differ largely from those who attended the study.

According to self-reported questionnaire information, 45% of the children claimed to brush their teeth daily, but the clinical examination revealed large amounts of plaque on the teeth in most children. The children were told not to brush their teeth the same morning as the examination, but lack of oral hygiene for approximately 14 hours could not explain the extensive amounts of plaque registered. Besides a likely over-reporting of brushing frequency, observations during the tooth brushing training indicated that heavy plaque amounts were probably due to inaccurate brushing technique, short brush time, and lack of motivation and knowledge about the consequences of poor oral hygiene. Further, probably none of the children used a fluoridated toothpaste (or any other toothpaste), since one shop already had, and the other was about to, stop selling toothpaste since the request was virtually non-existent. It was also noticed that the fluoride amount in the locally available toothpastes was 1,000 ppm fluoride, which is 2/3 rds of the common content in Sweden and most other parts of Europe (European Commission, 2010).

Surprisingly, the reported daily intake of sweet products tended to be less frequent in the high versus low caries group. The difference was not statistically significant, but it cannot be excluded that this reflects an under-reporting due to awareness that sweets are bad for your teeth.

Among all children 86% where infected with *S. mutans* and 49% with *S. sobrinus* as detected by regular PCR. Detection was significantly more prevalent in the high compared to in the low caries group regarding infection with *S. mutans,* but not *S. sobrinus*. These findings are in accordance with previous studies associating both *S. mutans* and *S. sobrinus* with development of dental caries, and that *S. mutans* infection is more prevalent that than *S. sobrinus* infection (Takahashi and Nyvad, 2008, 2011; Fejerskov and Kidd, 2008). In contrast, qPCR detected amounts of *S. mutans* and *S. sobrinus* DNA did not differ significantly between the high and low caries group, though high levels were only seen in the high caries group. The most likely explanation for this is that the study was under-powered due to the large variation within the high caries group. Interestingly, among Swedish adolescents both detection prevalence by regular PCR and DNA levels by qPCR are significantly lower than in the present Romanian group (Prof. I. Johansson personal communication).

The present data combined with other available data show that there is a major caries problem in Romania. The high caries prevalence is especially alarming in rural areas with potentially severe consequences for the inhabitants. It is, however, not straight forward to suggest a plan for how this problem could be solved since both tradition/attitudes and economics must be considered. Such a plan should include actions to reduce both disease causing factors, as well as impact the attitude towards the importance of having healthy teeth and not seeing a dentist only when there is tooth ache. The latter aspect includes both the individual and governmental authority level. Based on our experience we suggest that the primary aim should be to establish nationwide preventive programs targeting tooth brushing with a 1500 ppm fluoridated toothpaste and fissure sealing. Such a program should involve all levels of actors, *i.e.* local dentists, school personnel and parents. During the work with the present project we have learned that there is a similar project run by Rotary in Göteborg (Gothenburg), Sweden in collaboration with local dentists, and the most positive

effect from our study is that the village Curilia will be included in this project. In summary, the caries prevalence was very high among children living in the studied village in Romania, but activities to meet the situation may be on its way.

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**Table 1.** Characteristics of study participants.

| | | Caries group[1,2] | | | Dentition[1,3] | | |
|---|---|---|---|---|---|---|---|
| | All children[1] (n=70) | high (n=34) | low (n=36) | p-value | mixed (n=34) | permanent (n=35) | p-value |
| Age (mean (95%CI))[4] | 11.8 (11.2-12.3) | 11.1 (10.1-12.1) | 12.3 (11.7-12.8) | 0.056 | 9.9 (9.2-10.5) | 13.2 (12.7-13.7) | <0.001 |
| Sex (% girls)[5] | 48.5 | 39.4 | 57.6 | 0.139 | 35.5 | 60.0 | 0.047 |
| Family members (mean (95%CI)[4] | 5.5 (4.9-6.0) | 5.9 (4.9-6.9) | 5.1 (4.5-5.7) | 0.162 | 5.6 (4.7-6.4) | 5.5 (4.7-6.2) | 0.865 |
| Children in family (mean (95%CI)[4] | 3.5 (2.9-4.0) | 3.8 (2.9-4.8) | 3.2 (2.6-3.8) | 0.256 | 3.6 (2.7-4.5) | 3.4 (2.7-4.2) | 0.772 |
| Physical activity in leisure time (% yes)[5] | 43.3 | 43.3 | 43.3 | 1.000 | 48.3 | 38.7 | 0.455 |
| Illness latest year (% yes)[5] | 59.4 | 54.8 | 63.6 | 0.474 | 44.8 | 71.4 | 0.031 |
| Medication latest year (% yes)[5] | 65.6 | 58.1 | 72.7 | 0.217 | 51.7 | 77.1 | 0.033 |
| Problem with teeth (% yes)[5] | 70.5 | 70.0 | 71.0 | 0.934 | 64.3 | 75.8 | 0.328 |
| Visited a dentist last year (% yes)[5] | 25.4 | 16.7 | 31.3 | 0.180 | 25.0 | 23.5 | 0.893 |
| Never visit a dentist (% who did not)[5] | 12.9 | 13.3 | 12.5 | 0.607 | 10.7 | 14.7 | 0.555 |
| Daily tooth brushing (% twice daily)[5] | 44.9 | 40.0 | 50.0 | 0.359 | 40.9 | 48.1 | 0.737 |
| Daily intake frequency of sugar snacks (mean (95%CI))[6] | 4.8 (3.8-5.7) | 3.8 (2.6-5.0) | 5.7 (4.1-7.2) | 0.062 | 3.7 (2.5-4.9) | 5.7 (4.2-7.2) | 0.037 |

CI=confidence interval; IQR=interquartile range.

1) The numbers vary slightly for different variables due to single missing values, *e.g.* one child did not participate in the oral examination.
2) High and low caries group refers to children with a caries prevalence (dmfs+DMFS/tooth) above or below the median value for. Median (IQR) 0.67 (0.72).
3) Mixed dentition includes children with both deciduous and permanent teeth and permanent dentition those with permanent teeth only.
4) Differences between group means were tested with Student´s t-test.
5) Differences in sampling distributions were tested with Chi-square test.
6) Daily intake frequency of sugar snacks (mean (95%CI)).

**Figure 1. a,b and c**

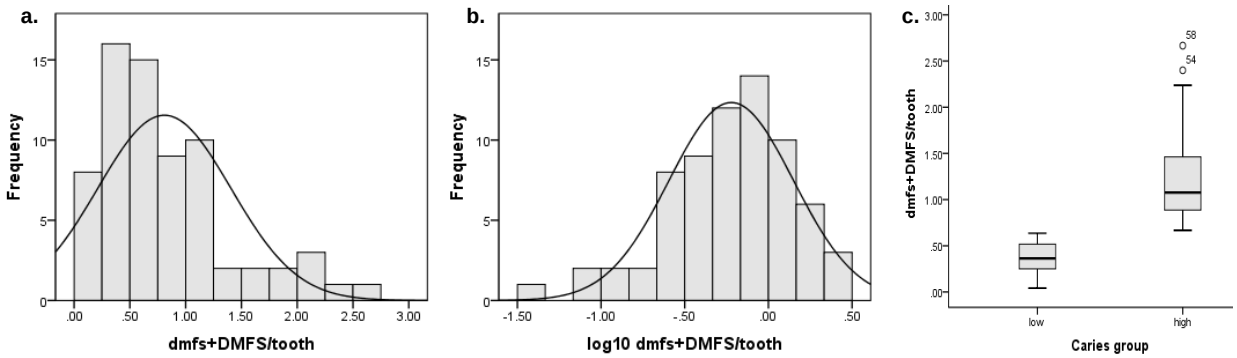


**Figure 1a.** Distribution of dmfs+DMFS/tooth.

**1b**. Distribution of dmts+DMFS/tooth with logarithmic transformation.

**1c**. Distribution of high and low caries group.

**Figure 2.**

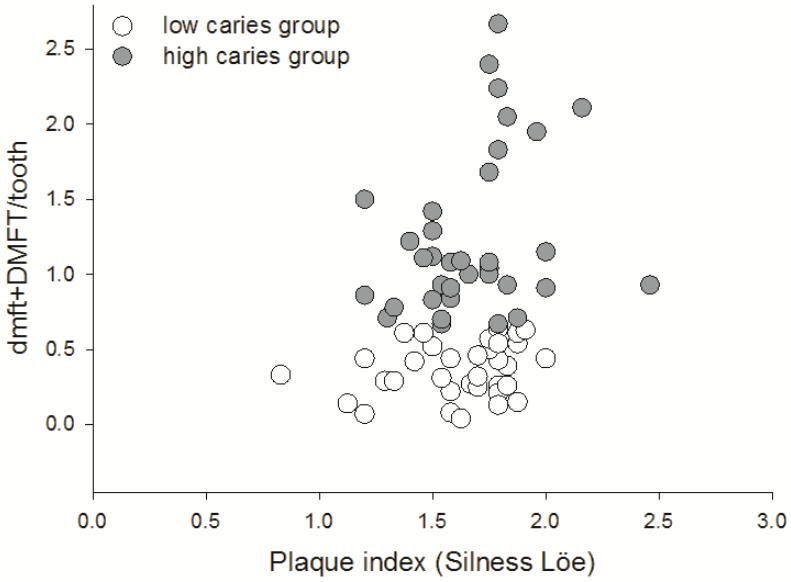


**Figure 2**. Scatter plot of amount of plaque and prevalence of caries/tooth. A weak, but not statistically significant, positive correlation was seen between amount of plaque and prevalence of caries/tooth ($r_{Spearman}$= 0.145, p=0.233, and mean plaque index did not differ significantly between the high and low caries groups or children with mixed or permanent dentition.

**Figure 3. a and b.**

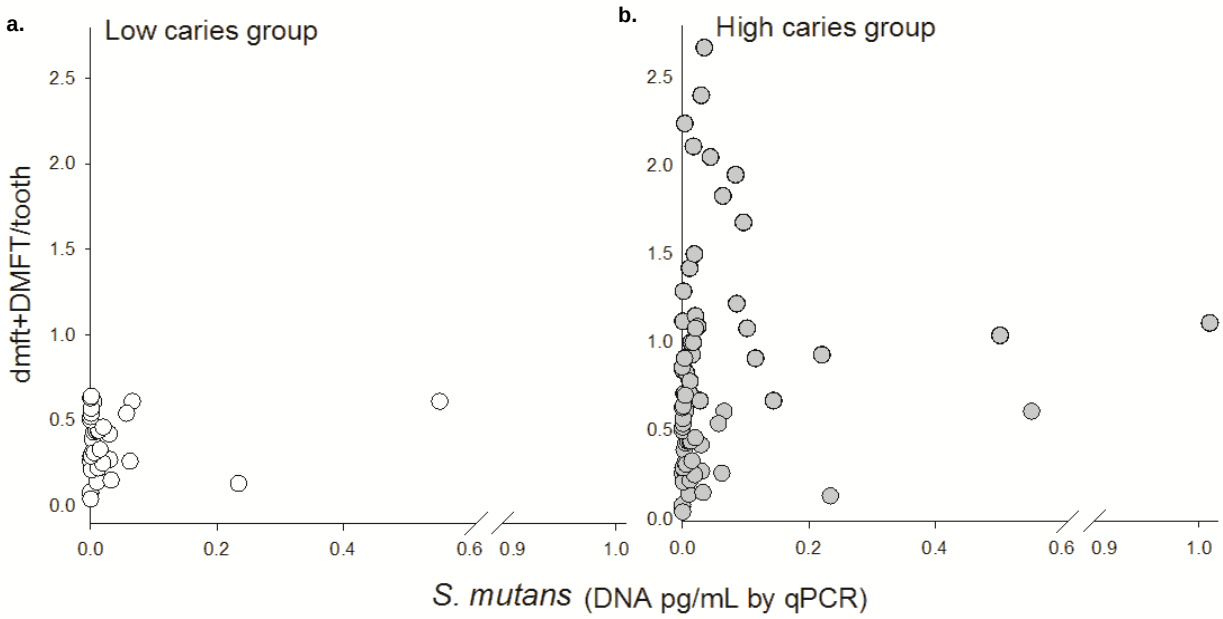


**Figure 3. a and b.** Scatter plot of bacterial counts (expressed as species specific DNA) and prevalence of caries per tooth. Spearman correlation between bacterial counts was 0.355 (p=0.003).

**Supplementary Table 2.** Caries status in the study group.

| | | Caries group | | | Dentition | | |
|---|---|---|---|---|---|---|---|
| | All children[1] (n=69) | high[1] (n=34) | low[1] (n=35) | p-value | mixed | permanent | p-value |
| Number of teeth (mean (95%CI)) | 25(24.2-25.3) | 24(22.9-24.6) | 26(25.1-26.5) | <0.001 | 23(22.2-23.3) | 27(26.3-27.1) | <0.001 |
| deciduous teeth | 4(2.6-4.9) | 5(3.4-6.9) | 2(1.0-3.6) | 0.01 | 8(6.3-8.9) | - | |
| permanent teeth | 21(19.4-22.6) | 19(16.1-21.0) | 23(21.6-25.3) | 0.002 | 16(13.6-16.7) | 27(26.2-27.1) | <0.001 |
| Caries prevalence | | | | | | | |
| dmfs | 9.29(6.1-12.5) | 15.8(10.3-21.2) | 2.6(1.1-4.2) | <0.001 | 18.9(14.1-23.6) | - | |
| DMFS | 9.8(7.7-12.0) | 12.9(9.0-16.7) | 6.7(5.2-8.1) | 0.004 | 4.4(3.2-5.6) | 15.1(11.7-18.4) | <0.001 |
| Caries prevalence/tooth | 0.8(0.67-0.95) | 1.2(1.1-1.4) | 0.4(0.3-0.4) | <0.001 | 1.1(0.8-1.3) | 0.6(0.4-0.7) | 0.001 |
| Plaque | | | | | | | |
| Silness-Löe PlI | 1.6(1.6-1.7) | 1.7(1.6-1.8) | 1.6(1.5-1.7) | 0.346 | 1.7(1.6-1.7) | 1.6(1.5-1.7) | 0.706 |
| *S. mutans* | | | | | | | |
| PCR | 85.5% | 94.3% | 76.5% | 0.036 | 88.2% | 82.9% | 0.526 |
| qPCR | 0.590(0.023-0.095) | 0.080(0.015-0.014) | 0.35(0.0003-0.70) | 0.228 | 0.53(0.017-0.088) | 0.63(-0.002-0.127) | 0.783 |
| *S. sobrinus* | | | | | | | |
| PCR | 49.3% | 54.3% | 44.1% | 0.398 | 52.9 | 45.7 | 0.548 |
| qPCR | 0.033(0.012-0.055) | 0.050(0.008-0.91) | 0.018(0.008-0.027) | 0.134 | 0.47(0.008-0.086) | 0.022(0.002-0.041) | 0.239 |

1) The numbers vary slightly for different variables due to single missing values, *e.g*. one child did not participate in the oral examination.

0 = no visible plaque, 1 = plaque present at the gingival margin, 2 = plaque more than one third but less than two thirds of the tooth surface, and 3 = more than two thirds of the tooth surface was covered with plaque.

**Appendix 1.**

# CIURILA PROJECT

Surname Name Date of birth

Date

How many are you in the family?

How many siblings do you have?

If you have siblings are you youngest, oldest or middle child? Youngest
Middle
Oldest

Have you been sick the last year? Yes No

Have you eaten medication? Yes No

Do you have hereditary diseases in the family?
(example Diabetes)

What do you usually do after school?

How far approximately (km) do you have to school?

How do you usually get to school?

Do you eat breakfast? Yes No Sometimes
If yes, what do you usually eat for breakfast?

Do you eat lunch? Yes No Sometimes

Do you eat dinner? Yes No Sometimes

What do you usually drink with the food? Tap water Water in bottle
Milk Soda Soda light Juice
Fruit syrup

What do you usually eat/drink between meals?

Fruit Sandwich
Cake/Cookies
Candy Icecream
Chocolate
Water Soda Juice
Coffee/tea Milk

The last 6 months, how often have you eaten the mentioned food below?

| | Never | 1/week | 2-3/week | 1/day | 2-3/day |
|---|---|---|---|---|---|
| Butter | | | | | |
| Oil | | | | | |
| Yellow cheese | | | | | |
| White cheese | | | | | |
| Yoghurt, sour milk, kefir | | | | | |
| Meat | | | | | |
| Fish | | | | | |
| Chicken | | | | | |
| Cabbage | | | | | |
| Tomatoes, cucumber | | | | | |
| Carrots, beans | | | | | |
| Potatoes | | | | | |
| Rice | | | | | |
| Pasta, Spagetti | | | | | |
| Jam, marmelade | | | | | |
| Icecream | | | | | |
| Cookies, cakes | | | | | |
| Chips | | | | | |
| Candy | | | | | |
| Honey | | | | | |
| Milk | | | | | |
| Lemonade, fruit syrups | | | | | |
| Coca-Cola | | | | | |
| Juice | | | | | |
| Coffee/tea | | | | | |
| Banana | | | | | |
| Apple/pear | | | | | |
| Berries | | | | | |

How often do you brush your teeth? More than 2 times / day
2 times / day
1 times / day
Few times / week
Few times / month

What do you brush your teeth with? Tooth paste
Something else

Is there fluoride in your tooth paste? Yes No
If you dont know if there is fluoride write the name of the product.

Have you had problems with your teeth? Yes No
If yes, please describe the problem.

Have you´ve visited the dentist? Yes No
If yes, when was the last visit?

Are you afraid of the dentist? Yes No