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Oral microflora in infants delivered vaginally and by caesarean section

METTE NELUN BARFOD[1], KERSTIN MAGNUSSON[2], MICHALA ORON LEXNER[3], SUSANNE BLOMQVIST[4], GUNNAR DAHLÉN[4] & SVANTE TWETMAN[1,2]

[1]Department of Cariology and Endodontics, Faculty of Health Sciences, Institute of Odontology, University of Copenhagen, Copenhagen, Denmark, [2]Maxillofacial Unit, County Hospital, Halmstad, Sweden, [3]Department of Paediatric Dentistry and Clinical Genetics, Faculty of Health Sciences, Institute of Odontology, University of Copenhagen, Copenhagen, Denmark, and [4]Department of Odontology/Oral Microbiology, The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden

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Background. Early in life, vaginally delivered infants exhibit a different composition of the gut flora compared with infants delivered by caesarean section (C-section); however, it is unclear whether this also applies to the oral cavity.

Aim. To investigate and compare the oral microbial profile between infants delivered vaginally and by C-section.

Design. This is a cross-sectional case–control study. Eighty-four infants delivered either vaginally ($n = 42$) or by C-section ($n = 42$) were randomly selected from the 2009 birth cohort at the County Hospital in Halmstad, Sweden. Medically compromised and premature children (<32 weeks) were excluded. The mean age was 8.25 months (range 6–10 months), and parents were asked to complete a questionnaire on socioeconomic factors, lifestyle, and hygiene habits. Saliva was collected and analysed using checkerboard DNA–DNA hybridization.

Results. A higher prevalence of salivary *Streptococcus salivarius*, *Lactobacillus curvata*, *Lactobacillus salivarius*, and *Lactobacuillus casei* was detected in infants delivered vaginally ($P < 0.05$). The caries-associated bacteria *Streptococcus mutans* and *Streptococcus sobrinus* were detected in 63% and 59% of all children, respectively.

Conclusion. A significantly higher prevalence of certain strains of health-related streptococci and lactobacilli was found in vaginally delivered infants compared with infants delivered by C-section. The possible long-term effects on oral health need to be further investigated.

Introduction

The oral microflora consists of numerous different species and genera. The colonization starts at birth, and the mother is considered to be the principle source[1,2]. Studies have shown that vaginally delivered infants exhibit a different microbial composition in the intestines compared with infants delivered by caesarean section (C-section) or with the aid of instruments[3,4]. By the age of 1, infants delivered by C-section in Western countries have a lower ratio of anaerobic to facultative anaerobic bacteria in the gut than vaginally delivered infants, possibly indicating a less mature microbiota[5]. Furthermore, it was suggested that infants delivered by C-section harbour lower proportions of the health-promoting strains of lactobacilli and bifidobacteria[6]. Possible consequences of the delivery mode are, however, sparsely investigated with regard to the oral microflora. One study of the early colonization of the oral cavity has shown that infants delivered by C-section acquired the caries-associated pathogen *Streptococcus mutans* almost 1 year earlier than vaginally delivered infants[7].

Data from the Swedish National Board of Health show an increase in C-sections during the last 30 years. In 1973, C-sections constituted 5.3% of all births compared with 17.2% in 2008[8], and this has resulted in a growing interest for the medical consequences. Previous studies have established an increased risk of diabetes, food allergies, and

Correspondence to:
M. Nelun Barfod, Faculty of Health Sciences, Institute of Odontology, Nørre Allé 20, DK 2200 Copenhagen N, Denmark. E-mail: stwe@sund.ku.dk

asthma among infants delivered by C-section[9–12]. These findings might correlate with the observations made by Schlinzig *et al.*[13] that infants delivered by C-section may have an impaired leucocyte function because of the lack of the pressure normally experienced at birth.

The exposure to a diverse bacterial community early in life may be decisive for the maintenance of oral health. Therefore, the aim of this study was to examine whether there is a variation in the oral microflora between infants delivered vaginally and by C-section. The null hypothesis was that no differences would be displayed between the groups.

Material and methods

Study population

The investigation was planned as a case–control study with a cross-sectional design. It was conducted in Sweden and included infants born at the County Hospital in Halmstad in 2009. A total of 1811 children were born at the hospital that year, and after exclusion of premature infants (born before week 32) and infants born with medical problems, they were divided into two groups: (i) delivered vaginally ($n = 1689$) and (ii) delivered by C-section ($n = 335$). From those between 6 and 10 months, 42 infants were randomly selected from each group with the aid of a computer programme. The size of the study groups was estimated based upon a power calculation with the risk for a Type-1 error set at $\alpha = 0.1$ and $\beta = 0.2$. This would allow a detection of a 35% difference between the groups, which was considered of clinical relevance. The study design was approved by the Regional Ethical Committee in Lund, Sweden (Dnr 2009/706).

Saliva sampling

All parents were contacted and informed about the project with an invitation to participate. After written informed consent, they received a saliva sample kit containing a sterile cotton bud, a plastic tube, and an information folder. Furthermore, a questionnaire with 14 questions regarding socioeconomic parameters, lifestyle (smoking), and oral hygiene habits (tooth brushing and fluoride) was distributed. The saliva samples were collected by the parents in the morning by placing the cotton bud in the mouth of the child, and when soaked, it was placed in the plastic tube and immediately returned to the laboratory by mail. Upon arrival, the samples were frozen and stored in −70°C until further analyses.

Checkerboard DNA–DNA hybridization

The presence and level of 20 oral species were determined with the aid of the checkerboard DNA–DNA hybridization methodology according to Wall-Manning *et al.*[14]. In brief, whole genomic DNA probes were prepared from the panel of bacterial species with strain designation as listed in the tables. DNA was extracted with mutanolysin and lysozyme as previously described[15], and the DNA quality was evaluated from the UV spectrum between 200 and 300 nm using a Gene Quant spectrophotometer (Pharmacia Biotech, Uppsala, Sweden). Probe DNA (1 μg) was labelled with deoxygenin using the DIG High Prime kit according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). A volume of 100 μL each of all plaque and saliva samples in alkali (0.25 M NaOH in 0.5 mM Tris-EDTA buffer) was boiled for 5 min, neutralized with 800 μL of 5 M ammonium acetate, transferred onto nylon membranes (Minislot device; Immunetics, Cambridge, MA, USA), and fixed by ultraviolet light (UV Stratalinker 1800; Stratagene, La Jolla, CA, USA). After 2 h of prehybridization at 42°C, the DNA probes (1–10 ng) were allowed to hybridize overnight in lanes vertically to the plaque and saliva samples using a Miniblotter device (Immunetics) at 42°C. Buffer-set 2[15] was used as buffers for prehybridization and hybridization. After a series of stringency washes at 70°C, hybrids were detected using phosphatase-conjugated antidigoxygenin antibodies and the signals were visualized using a chemiluminescent substrate (CDP Star; Roche Diagnostics). Evaluation of the number of bacteria in the samples was carried out by comparing the signals with the ones generated by pooled standard samples containing 10^6 and 10^5 cells of each species. The signals were scored and categorized in four scores as 0 = not detected ($<10^4$ CFU); 1 = low counts (approximately 10^4 CFU); 2 = moderate counts (approximately 10^5 CFU); and 3 = high counts ($>10^5$ CFU).

Statistical procedures

The Statistical Programme of Social Sciences (SPSS, version 18.0; Chicago, IL, USA) was used to analyse the results of the questionnaire and the checkerboard DNA–DNA hybridization. Differences between groups were analysed by chi-square test. The level of significance was set at 5%.

Results

In total, 83 questionnaires and saliva samples were received. One sample was delayed in the mail and therefore not received on time. Thus, the final study group included 42 infants delivered vaginally and 41 infants delivered by C-section with a mean age of 8.25 months (range 6–10 months). The background data from the questionnaires are shown in Table 1. The only significant difference between groups was 'parental smoking', which was slightly more prevalent in the C-section group ($P < 0.05$).

The results of the checkerboard DNA–DNA hybridization assay of all infants are summarized in Table 2. In general, *Streptococcus mitis, Streptococcus salivarius, Streptococcus sanguinis*, and *Veillonella parvula* were found to be the most common bacteria, whereas the strains of bifidobacteria, lactobacilli, and candida were relatively infrequently seen. Notably, *S. mutans* and *Streptococcus sobrinus* were detected in the saliva of more than half of the infants in both groups (63% and 59%), and no significant relation with the presence of teeth was displayed.

The oral microflora in relation to the mode of delivery is presented in Table 3. The salivary prevalence of *S. salivarius, S. sobrinus, Actinomyces odontolyticus,* and *Rothia denticariosa* was significantly higher ($P < 0.05$) in the vaginally delivered infants and similar strong tendencies were recorded for *S. sanguinis, V. parvula*, and *Prevotella intermedia*. Likewise, many of the lactobacilli species were more prevalent among vaginally delivered infants and these differences were statistically significant for *Lactobacillus curvata*, *Lactobacillus salivarius*, and *Lactobacuillus casei* ($P < 0.05$). It was also observed that infants delivered by C-section were colonized more often with *Enterococcus faecalis* than infants delivered vaginally ($P < 0.05$). No difference was observed between groups regarding the oral colonization of *S. mutans*.

Table 1. Description of characteristics within the two study groups.

Characteristics	Vaginal delivery (*n* = 42)	Caesarean section (*n* = 41)	*P**
Girls/boys	29%/71%	44%/56%	NS
Mean age	8.4 months	8.1 months	NS
Preterm (32–36 weeks)	10%	15%	NS
Antibiotics (yes)	10%	22%	NS
Probiotics (yes)	10%	10%	NS
Teeth present (yes)	74%	61%	NS
Siblings (>2)	14%	22%	NS
Mother born in Sweden (yes)	88%	80%	NS
Mother's education (low)	7%	12%	NS
Parental smoking (yes)	2%	15%	<0.05
Daily tooth brushing (yes)	71%	59%	NS
Fluoride toothpaste (yes)	62%	56%	NS
Finger sucking habit (yes)	29%	46%	NS

NS, not significant.

*Differences between groups subjected to chi-square tests, comparison of proportions.

Discussion

To our knowledge, this was the first study to investigate the oral microbiota in saliva from infants born with different modes of delivery. As the findings suggested an increased prevalence of certain groups of lactobacilli and streptococci among infants delivered vaginally, the null hypothesis was rejected. The results were not unexpected because they largely reflect previous observations in the gut

Table 2. Distribution of salivary microorganisms in the total study population (*n* = 83).

Species	OMGS*	Score 0** (%)	Score 1 (%)	Score 2 (%)	Score 3 (%)
Streptococcus mutans	2482	37	59	4	–
Streptococcus sobrinus	1214	41	57	2	–
Streptococcus mitis	1770	17	46	23	14
Streptococcus gordoni	2471	69	31	–	–
Streptococcus sanguinis	2478	34	64	2	–
Streptococcus salivarius	2473	31	63	6	–
Prevotella intermedia	2514	54	46	–	–
Fusobacterium nucleatum	2865	73	27	–	–
Actinomyces naeslundii	2466	88	11	1	–
Actinomyces odontolyticus	G67	71	28	1	–
Veillonella parvula	G186	30	69	1	–
Rothia denticariosa	1956	57	43	–	–
Bifidobacterium dentium	G174	99	1	–	–
Enterococcus faecalis	3632	73	27	–	–
Lactobacillus curvata	3181	37	48	15	–
Lactobacillus salivarius	3830	52	47	1	–
Lactobacuillus casei	3184	80	20	–	–
Lactobacillus rhamnosus	3179	92	8	–	–
Lactobacillus fermentus	3182	98	2	–	–
Candida albicans	3751	92	8	–	–

Values in table denote the number of subjects expressed as per cent.

*OMGS = culture collection Oral Microbiology, Gothenburg, Sweden, strain number.

**Score 0 = not detected; score 1 = low counts; score 2 = moderate counts; score 3 = high counts.

Table 3. Prevalence of salivary microorganisms in infants in relation to mode of delivery.

Species	Vaginal delivery (*n* = 42)	Caesarean section (*n* = 41)	*P**
Streptococcus mutans	69	52	NS
Streptococcus sobrinus	76	39	<0.05
Streptococcus mitis	82	76	NS
Streptococcus gordonii	26	34	NS
Streptococcus sanguinis	74	56	NS (*P* = 0.09)
Streptococcus salivarius	83	51	<0.05
Prevotella intermedia	55	34	NS (*P* = 0.06)
Fusobacterium nucleatum	34	29	NS
Actinomyces naeslundii	10	15	NS
Actinomyces odontolyticus	48	10	<0.05
Veillonella parvula	79	59	NS (*P* = 0.06)
Rothia denticariosa	57	27	<0.05
Bifidobacterium dentium	2	0	NS
Enterococcus faecalis	17	34	<0.05
Lactobacillus curvata	81	41	<0.05
Lactobacillus salivarius	62	32	<0.05
Lactobacuillus casei	31	10	<0.05
Lactobacillus rhamnosus	12	5	NS
Lactobacillus fermentus	5	0	NS
Candida albicans	7	10	NS

NS, not significant.

Values in table denote the number of subjects with detectable growth expressed as per cent.

*Differences between groups were subjected to chi-square tests.

flora[4,6]. Bearing the transient nature of the oral microflora in mind, it was interesting to note that obvious contrasts in the microbial composition were still persisting up to 8 months after delivery. As differences in the oral microflora probably equalizes over time, it is reasonable to expect an even greater variation in the microbial profiles of younger infants. The present cross-sectional design has, however, its limitations and an apparent shortcoming was the lack of information concerning breast feeding and diet. Additionally, to further elucidate acquisition and early biofilm colonization in relation to mode of birth, a prospective design is desired.

The hybridization methodology applied here has previously been considered to adequately reflect the microbiological profiles of caries-active subjects[16], but its low sensitivity and relatively high detection levels in saliva

($\geq 10^4$ CFU) remain a certain limitation. Compliance with the cross-sectional protocol was excellent. In fact, all the participating families managed to collect saliva from their offspring's and return the saliva sample and questionnaire on time. The selected groups were also found to be well balanced concerning the background factors except for the fact that more parents in the C-section group reported domestic tobacco smoke. It is well known that biological as well as several lifestyle factors such as smoking, diet, and hygiene may influence the composition of the oral microflora[17,18]. It should, however, be underlined that the absolute number of smoking parents was fairly low also in the C-section group ($n = 6$).

The main findings were that common oral streptococci and lactobacilli species were observed to be more prevalent in infants delivered vaginally compared with those delivered by C-section. The findings are in accordance with several studies concerning the early colonization of the infant gut that reported a higher proportion of health-related bacteria, especially bifidobacteria, in infants born vaginally compared with infants delivered by C-section[4,6]. Commensal streptococci such as *S. salivarius* and *S. mitis* are the first and dominating microbes to colonize the oral cavity of the newborn infant. The increased prevalence of lactobacilli in vaginally delivered infants may be attributed to the exposure and early colonization by vaginal lactobacilli, and the immune stimulation by microbial exposure early in life may protect against diseases such as allergy and eczema[3–6,19], but whether there is a similar relationship between the early colonization of the oral cavity and oral health later in life remains an open question.

Our analyses were able to demonstrate that the caries-associated bacterium *S. mutans* could be detected in predentate children. Previously, it was thought that *S. mutans* only colonized the oral cavity after tooth eruption[20], but more recent studies have shown the presence of the bacterium in infants as young as 2 months[21]. Recent findings that *S. mitis* and *S. sanguinis* have an antagonistic role to mutans streptococci and that an early colonization of *S. sanguinis,* or the mode of delivery, may be protective for the acquisition of *S. mutans*[7,22,23] were not verified here. Our findings that other bacteria, which are more or less associated with caries, such as *S. sobrinus*, *A. odontolyticus*, and *R. denticariosa*, were more prevalent in the vaginal delivery group were perhaps more unexpected. We have no immediate explanation for these findings, but a 'positive' interpretation would be that their presence indicates a more complex diversity of the oral microflora. The possibility of an unwanted cross-reaction with false-positive signals during the analysis, however, cannot be excluded. Likewise, the higher prevalence of *E. faecalis* in the saliva of infants delivered by C-section could be a finding by chance but also explained by an alteration of the intestinal colonization[6]. In paediatric medicine, it has been suggested that neonatal exposure to a commensal gut microflora and probiotics may counteract a low microbial diversity and thereby be protective and beneficial for the child's health[24]. Based on our present findings, further research is justified to elucidate whether this concept might be applicable for the oral health.

In conclusion, this study suggested that the mode of delivery had an influence on the salivary microbial profile in infants. A significantly higher prevalence of streptococci and lactobacilli associated with oral health was found in vaginally delivered infants when compared with the C-section group. The possible long-term effects of these findings need to be further investigated.

What this paper adds

- This cross-sectional study demonstrates that the mode of delivery may influence the acquisition and early colonization of the salivary microflora.

Why this paper is important to paediatric dentists

- The findings may support the hypothesis that neonatal exposure to a diverse bacterial community may be beneficial for oral health and open up for new strategies to prevent diseases.

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