

Mode of delivery, mutans streptococci colonization, and early childhood caries in three- to five-year-old Thai children

K. Pattanaporn¹, P. Saraithong²,
S. Khongkhunthian³, J. Aleksejuniene¹,
P. Laohapensang⁴, N. Chhun⁵, Z. Chen⁵
and Y. Li^{5*}

¹Department of Oral Health Sciences,
Faculty of Dentistry, University of British
Columbia, Vancouver, BC, Canada,

²Department of Biology, Faculty of Science,
Chiang Mai University, Chiang Mai,
Thailand, ³Department of Restorative

Dentistry and Periodontology, Faculty of
Dentistry, Chiang Mai University, Chiang
Mai, Thailand, ⁴Dental Department, Health
Promoting Hospital, Chiang Mai, Thailand,

⁵Department of Basic Science and
Craniofacial Biology, College of Dentistry,
New York University, New York, NY, USA

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Abstract – Objective: To investigate whether mode of delivery is associated with mutans streptococci (MS) colonization and early childhood caries (ECC) in preschool Thai children. **Methods:** Three hundred and fifty mothers and their 3- to 5-year-old children (184 born vaginally and 166 born by Caesarean section) participated in the study. Data included a dental examination, MS colonization assessed by the Dentocult[®] SM Strip Mutans method, and a questionnaire survey of family socio-demographic information, as well as children's birth history, dietary habits, and oral health practices. **Results:** Overall, ECC prevalence was 56% in 3-year-old and 78% in 5-year-old Thai children. Compared to children delivered by C-section, vaginally born children experienced increased ECC prevalence (73.8% versus 59.6%; $P = 0.009$) and were more likely to have higher MS scores (OR = 1.8, 95% CI = 1.1–2.9), adjusting for mother's gestational age, MS score, feeding practice habits; child's age and tooth brushing habits. Children's MS scores were highly correlated with their mothers' MS scores ($P < 0.001$). Additionally, children's age, MS colonization, and mothers' prechewing feeding habits were the most significant risk indicators for ECC in Thai children. **Conclusion:** Our findings suggest that mode of delivery is significantly correlated with MS colonization and caries outcomes in young Thai children. Future studies are needed to further understand the possible biological mechanisms linking mode of child delivery to the colonization of cariogenic microbiota and development of ECC.

Key words: early childhood caries; maternal factors; mode of delivery; mutans streptococci colonization; preschool children; Thailand

Yihong Li, Department of Basic Science and Craniofacial Biology, New York University College of Dentistry, 345 E. 24th Street, New York, NY 10010, USA
Tel: 212 998 9607; Fax: 212 995 4087
e-mail: yihong.li@nyu.edu

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Early childhood caries (ECC) is etiologically associated with bacterial colonization in deciduous dentition, and it significantly affects the oral health-related quality of life of preschool children (1–3). Since the 1980s in Thailand, the development and implementation of a community-based preventive program has been aimed at decreasing dental caries among Thai school children (4). However, the prevalence of ECC remains high among preschool children (5, 6). Caries in primary teeth has become one of the most prevalent chronic

childhood diseases impacting the overall health of Thai children (6–8).

Mutans streptococci (MS), especially *Streptococcus mutans* and *S. sobrinus*, have been associated with dental caries based on well-defined cariogenicity (9–12). Consistently, *S. mutans* and *S. sobrinus* have been commonly isolated from the saliva and dental plaque of caries-active individuals, including children with ECC. The natural history of *S. mutans* colonization from newborns through childhood has been previously studied (13–16),

suggesting that children acquire *S. mutans* primarily from their mothers (13, 15, 17, 18), and possibly other sources (19, 20). Meanwhile, Li and others reported that the mode of delivery significantly affected the time of acquisition of *S. mutans* in infants (16). A high degree of similarity of bacterial populations was also observed between the mothers and their children (21), leading to the hypothesis that mothers might transmit not only a particular cariogenic microorganism, for example *S. mutans*, but also the cariogenic microbiota to their children, thus playing a major role in the establishment of the oral microbiota in children.

In Thailand, 15.2% of infants, on average, were born by Caesarean section (C-section) in 1990. The percentage was 22.4% in 2001 (22, 23). In Chiang Mai Health Promoting Hospital, the C-section rate was as high as 29.8% in 2009 among all births (24). In addition to increased postoperative health risks for the mothers, a body of evidence has suggested that C-section delivery could lead to differences in the initial establishment of microbiota in the gut and, subsequently, contribute to variations in child health (25–27). Whether the mode of child delivery can alter the infant's oral microbiota establishment and later the development of ECC still remains unclear.

Therefore, the main objectives of the present study were to determine the correlation between the mode of delivery (vaginal versus C-section), MS colonization, and the development of caries in a 3- to 5-year-old Thai population. The null hypothesis was the absence of differences between the two delivery groups in the colonization of MS and ECC experience. Social, demographic, and potential behavioral risk factors for ECC development were also evaluated.

Materials and methods

The protocol for this study was approved by the Ethical Committee of the Faculty of Dentistry, Chiang Mai University, Thailand (No. 12/2008). A written informed consent was obtained from all mothers or responsible caregivers at the time of children's hospital visits.

Recruitment of participants

This report represents a cross-sectional study of 350 children who were 3–5 years old. One hundred and sixty-six children were born by C-section delivery; and 184 children were born by vaginal

delivery. It is a routine practice at the Health Promoting Hospital in Chiang Mai, Thailand, to provide all required immunizations for each child from birth up to the age of 5 years. On a daily basis, the hospital administration office generates a list of children who are scheduled for their immunization recall visits. The 350 participants of the study were selected from the lists generated during July to December of 2009. The families were invited and agreed to participate in the study. All of them were healthy children born at the hospital, without major congenital anomalies or chronic illness, and had not taken any antibiotics within the 6 weeks prior to examination. The C-section delivery group consisted of 85 3-year-old (C3) and 80 5-year-old children (C5). The vaginal delivery group consisted of 97 3-year-old (V3) and 88 5-year-old children (V5).

Questionnaire survey

According to the appointment schedule, mothers of the selected children were contacted by the study coordinator. They were asked to complete a structured questionnaire survey on the day when their children received a regular immunization check-up at the hospital. The survey included inquiries about family socio-demographics (age, sex, primary care-provider, and maternal/family background), maternal pregnancy and past medical history (mode of delivery, gestational age, birth weight), as well as child's feeding practice (breast feeding, bottle feeding, and mother prechewing food), dietary habits (consumption of fruit juice, snacks, gum, lollipop candy, dried fruit, soft drink, child sleeping with bottle), and oral health practices. Information about delivery methods, gestational age, and child's birth weight was also obtained from the hospital medical records.

Dental examination

Two standardized dentists performed a comprehensive dental examination for all 350 children and their mothers using the WHO criteria defined for field studies (28). The presence of caries was recorded as detectable white-spot lesion or cavity. Assessments of dental health included three measurements: (i) prevalence of caries (caries present versus caries absent) without x-ray assessment; (ii) severity of caries experience of children measured using dmft/dmfs index, the number of decayed, missing, and filled teeth/tooth surfaces for deciduous dentition; and (iii) caries experience of the mothers measured using DMFT/DMFS index, the

number of decayed, missing and filled teeth/tooth surfaces for permanent dentition.

Evaluation of mutans streptococci

The level of MS colonization in the saliva was assessed using the Dentocult Strip mutans test (Orion Diagnostica, Espoo, Finland). Specifically, the mothers and children were asked to chew a piece of paraffin wax for 1 min under the close supervision of a medical professional. Their saliva was then sampled as described by Jensen and Bratthall (29). The 'Strip mutans strips' were incubated in a selective medium containing bacitracin for 48 hours at 37°C and dried at room temperature. The levels of MS colonization were then defined based on the colony-forming units (CFU) on the strips and compared with a standard chart containing four categories: SM Strip score of '0' corresponding to $<10^4$ of MS CFU, 'I' corresponding to 10^4 – 10^5 of MS CFU, 'II' corresponding to 10^5 – 10^6 of MS CFU, and 'III' corresponding to $>10^6$ of MS CFU *per ml* of saliva. The score for each participant was read and recorded independently by three investigators. The inter-examiner agreement was 88%, indicating a high degree of inter-examiner agreement among the readers' SM Strip results.

Statistical analysis

In total, the study selected 20 variables that potentially influence Thai children's risk of ECC. Descriptive analyses were first performed to compare children with different modes of delivery in terms of socio-demographic, maternal pregnancy, and child medical histories, MS score, oral examination, and child's dietary risk factors. Logistic regression was then employed to examine associations of joint effects of multiple predictors and to estimate odds ratio (OR) to MS colonization and ECC outcome in relation to socio-demographic variables, mode of delivery, feeding practice, dietary risk factors, and oral health behaviors. A 95% confidence interval was used for comparison of MS colonization and ECC outcome between the two delivery groups. All data management and statistical analyses were performed using IBM®SPSS®Statistics 19.0 (SPSS, Inc., IBM Corp., Armonk, NY, USA). All *P*-values < 0.05 based on two-tailed were considered significant.

Results

Three hundred and fifty children were recruited in the study; 184 born by vaginal delivery and 166

born by Caesarean section. The distribution of gender, age, and other main characteristics was similar between the two groups (Table 1). Overall, two-thirds of the children were normal-term newborns (gestation age ≥ 37 weeks). Only 3.7% of the children were born with a birth weight < 2500 g. The C-section born children appeared to be slightly heavier than the vaginal born children. Most children (97.4%) were breast fed, and about 38% of children were breast fed for more than a year. Mothers who gave birth vaginally were more likely to be the primary caregivers (58% versus 42%) for their children ($P = 0.04$). Those mothers were also more likely to prechew food to feed their children than those mothers who gave birth by C-section (3-year-old group, $P = 0.015$).

The results of dental examination showed that ECC was prevalent in 66.6% of the children, 56% among 3-year-old children, and 78% among 5-year-old children ($P < 0.001$) (Table 2). Although there was no difference in ECC distribution between boys (69.1%) and girls (63.5%), boys did present more severe ECC as measured by dmft ($P = 0.027$) and dmfs ($P = 0.013$), compared with girls. Vaginally delivered children experienced higher ECC prevalence compared with children in the C-section group (72.8% versus 59.6%; $P = 0.009$; Fig. 1). Significant differences in caries experience were observed in 3-year-old children born by vaginal delivery in terms of prevalence (64.9% versus 45.9; $P = 0.010$; Fig. 1) and severity (mean dmft = 3.2 versus 2.3; $P = 0.023$ and mean dmfs = 6.8 versus 4.3; $P = 0.022$) compared with that of children in the C-section group. The ECC status was associated with the level of MS colonization (Table 2). The practice of mother prechewing was also related to the increased ECC in their children ($P = 0.003$). Among the socio-demographic and dietary risk indicators, consumption frequency of soft drinks, snacks, gum, and lollipop candy were related to higher prevalence of ECC (Table S1). In addition, the prevalence of caries was 92.6% for the mothers; the mean DMFT $\pm$ SD and DMFS $\pm$ SD was 6.0 ± 4.3 and 10.4 ± 10.7 , respectively.

Mutans streptococci colonization was evaluated for all 350 children and their mothers, except missing for one child and two mothers. The results were evaluated and scored by three independent calibrated investigators. The distribution of children's SM Strip scores in relation to age, gender, mode of delivery, and other feeding and dietary factors was showed in Table 3. Significant increases in categories II and III were observed among 5-year-old children compared to 3-year-old children ($P < 0.001$).

Table 1. A summary of the study population characteristics

Study variables	N (%)	Vaginal		Caesarean		P value
		3 years old (%)	5 years old (%)	3 years old (%)	5 years old (%)	
Number of children in the study	350	97	87	85	81	
Gender						
Boys	191 (54.6)	55 (56.7)	43 (49.4)	53 (62.4)	40 (49.4)	N.S.
Girls	159 (45.4)	42 (43.3)	44 (50.6)	32 (37.6)	41 (50.6)	
Group						
Children, mean $\pm$ SD, (years)	350	2.9 $\pm$ 0.4	5.0 $\pm$ 0.2	2.8 $\pm$ 0.4	5.0 $\pm$ 0.4	N.S.
Mother, mean $\pm$ SD, (years)	350	30.7 $\pm$ 6.0	32.6 $\pm$ 6.2	32.1 $\pm$ 4.1	33.8 $\pm$ 6.0	N.S.
Gestational age						
Mean $\pm$ SD, gestational age (weeks)		37.6 $\pm$ 2.1	37.5 $\pm$ 2.2	38.0 $\pm$ 2.2	38.3 $\pm$ 2.5	N.S.
<37 week	121 (34.6)	24 (19.8)	18 (14.9)	32 (26.5)	47 (38.8)	N.S.
$\geq$ 37 week	229 (65.4)	58 (25.4)	34 (14.9)	56 (24.6)	80 (35.1)	
Birth weight						
Mean $\pm$ SD, birth weight (g)		3108.2 $\pm$ 485.3	3094.6 $\pm$ 387.7	3248.1 $\pm$ 467.1	3299.4 $\pm$ 461.7	0.005
<2500 g	13 (3.7)	5 (5.2)	4 (4.5)	2 (2.4)	2 (2.5)	N.S.
$\geq$ 2500 g	337 (96.3)	92 (94.8)	83 (95.4)	83 (97.6)	79 (97.5)	
Primary caregiver						
Mother	176 (50.3)	60 (61.9)	42 (48.3)	35 (41.2)	39 (48.1)	0.040
All others ^a	174 (49.7)	37 (38.1)	45 (51.7)	50 (58.8)	42 (51.9)	
Child's age when weaned						
$\leq$ 6 months	131 (37.4)	29 (30.9)	33 (39.8)	35 (41.2)	34 (43.0)	N.S.
7–12 months	77 (22.0)	21 (22.3)	19 (22.9)	22 (25.9)	15 (19.0)	
>12 months	133 (38.0)	44 (46.8)	31 (37.3)	28 (32.9)	30 (38.0)	
Not breast fed	9 (2.6)	3 (3.1)	4 (4.6)	0 (0)	2 (2.5)	
Mothers prechewing food, yes	111 (31.7)	38 (39.2) ^b	30 (34.9)	19 (22.4)	24 (29.6)	N.S.
Children brush teeth, yes	328 (93.7)	90 (94.7)	85 (97.7)	75 (88.2)	78 (97.5)	0.023

^aAll others include grandmother (2.9%), grandfather (1.1%), babysitter (10.3%), and other family relatives (35.6%).

^bAmong the 3-year-old group, mothers were more likely to prechew food in the vaginal group compared to the C-section group. The difference was significant (OR = 2.2; 95% CI, 1.2–4.3; P = 0.015).

More vaginally born children were colonized with higher levels of MS compared with C-section born children (P = 0.024); and the differences in MS Strip score distribution were consistent for both age groups (Fig. 2). Bivariate analyses showed that children who slept with a bottle and consumed soft drinks and lollipops and whose mothers were regular alcohol drinkers were significantly associated with higher levels of MS colonization (Table 3). However, not all dietary risk indicators obtained from the questionnaire survey were significantly associated with and the SM Strip scores (Table S2).

Using logistic regression modeling, we first tested 20 predictors into the stepwise logistic regression model to examine their joint effort to the two outcome variables, namely MS colonization and ECC. Of the 20 predictors tested, six of them were significant in the regression model for predicting MS colonization (Fig. 3a) and two in the model for ECC

(Fig. 3b). When the logistic regression analyses were further restricted to those clinically and biologically significant factors, we found that children were likely to have a higher MS score if they were 5 years old (OR = 1.97, 95% CI = 1.2–3.2), vaginally born (OR = 1.81, 95% CI = 1.1–2.9), did not brush their teeth (OR = 7.25, 95% CI = 1.9–27.5), had mothers with higher MS score (OR = 2.15, 95% CI = 1.3–3.6), practiced prechewing feeding habits (OR = 2.02, 95% CI = 1.2–3.4), and used alcohol regularly (OR = 1.67, 95% CI = 1.0–2.8) (Table 4. For age specific analysis, please see Table S3). Logistic regression analysis also revealed that the child's age (OR = 3.02, 95% CI = 1.9–5.0) and vaginal delivery (OR = 1.7, 95% CI = 1.1–2.8), along with the mother's practice of prechewing (OR = 1.95, 95% CI = 1.1–3.4), were among the significant risk indicators for increased ECC experience (Table 5. For age specific analysis, please see Table S4).

Table 2. Children's caries experience in relation to their age, gender, mode of delivery, and mutans streptococci colonization

Variables	N	Caries prevalence (%)	OR (95% CI)	P value	dmft (mean $\pm$ SD)	P value	dmfs (mean $\pm$ SD)	P value
Total	350	233 (66.6)			4.0 $\pm$ 4.5		8.8 $\pm$ 13.3	
Gender								
Boy	191	132 (69.1)	1.3 (0.8–2.0)	0.270	4.5 $\pm$ 0.4	0.027	10.4 $\pm$ 15.5	0.013
Girl	159	101 (63.5)			3.4 $\pm$ 3.9		6.8 $\pm$ 9.8	
Age								
3 years old	182	102 (56.0)	0.4 (0.2–0.6)	<0.001	2.8 $\pm$ 3.7	<0.001	5.7 $\pm$ 9.2	<0.001
5 years old	168	131 (78.0)			5.3 $\pm$ 5.0		12.2 $\pm$ 16.0	
Delivery mode								
Vaginal	184	134 (72.8)	1.8 (1.2–2.8)	0.009	4.4 $\pm$ 4.5	0.148	9.4 $\pm$ 13.4	N.S.
C-section	166	99 (59.6)			3.7 $\pm$ 4.6		8.1 $\pm$ 13.2	
3 years old								
Vaginal	97	63 (64.9)	2.2 (1.2–4.0)	0.010	3.2 $\pm$ 3.8	0.023	6.8 $\pm$ 10.3	0.022
C-section	85	39 (45.9)			2.3 $\pm$ 3.7		4.3 $\pm$ 7.6	
5 years old								
Vaginal	87	71 (81.6)	1.6 (0.7–3.2)	0.239	5.6 $\pm$ 4.9	0.365	12.1 $\pm$ 15.7	N.S.
C-section	81	60 (74.1)			5.1 $\pm$ 5.1		12.2 $\pm$ 16.5	
SM Strip score								
0, CFU <10 ⁴	82	20 (24.4)	Chi-Sq = 116.3	<0.001	$\pm$ 2.4	<0.001	2.0 $\pm$ 7.7	<0.001
I, CFU 10 ⁴ –10 ⁵	52	25 (48.1)			1.9 $\pm$ 3.1		2.9 $\pm$ 0.8	
II, CFU 10 ⁵ –10 ⁶	88	71 (80.7)			4.0 $\pm$ 3.9		7.6 $\pm$ 9.9	
III, CFU >10 ⁶	127	116 (91.3)			6.9 $\pm$ 4.8		16.5 $\pm$ 16.3	
Mother prechewing food								
Yes	111	86 (77.5)	1.3 (1.1–1.5)	0.003	4.9 $\pm$ 4.7	0.015	11.2 $\pm$ 13.4	0.022
No	238	146 (61.3)			3.6 $\pm$ 4.5		7.7 $\pm$ 13.2	

CFU, colony-forming units.

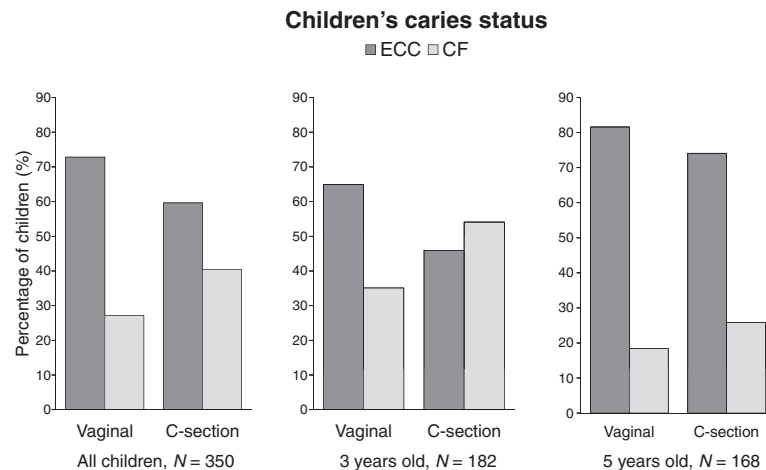


Fig. 1. Comparison of early childhood caries (ECC) prevalence in children born by different delivery methods. Overall, vaginally delivered children experienced higher ECC prevalence compared with children in the C-section group (Pearson Chi-square test; $P = 0.009$). The difference in caries experience was particularly significant among 3-year-old children born by vaginal delivery compared with children born by C-section delivery (Pearson Chi-square test; $P = 0.010$).

Discussion

In this study, we examined a set of risk factors, including cariogenic bacterial factors, oral health factors, past medical and birth factors, maternal social behavior factors, and children's dietary behavior factors, in relationship with ECC in Thai

children. First of all, among the 350 examined children, 77% were colonized by MS (CFU > 10⁴). The colonization increased with age, which was consistent with previously reported data (30–32). Compared with other studies using the same evaluation method for MS colonization, the prevalence of colonization in the present study was similar to

Table 3. Children's mutans streptococci score and the correlation with their age, gender, mode of delivery, and other feeding and dietary risk factors

Variables	N	SM Strip score, number of children (%)				Chi-Square	P value
		0, CFU <10 ⁴	I, CFU 10 ⁴ –10 ⁵	II, CFU 10 ⁵ –10 ⁶	III, CFU >10 ⁶		
Total	349	82 (23.5)	52 (14.9)	88 (25.2)	127 (36.4)		
Age							
3 years old	181	59 (32.6)	20 (11.1)	35 (19.3)	67 (37.0)	22.188	<0.001
5 years old	168	23 (13.7)	32 (19.0)	53 (31.5)	60 (35.7)		
Gender							
Boy	190	46 (24.2)	29 (15.3)	46 (24.2)	69 (36.3)	0.295	N.S.
Girl	159	36 (22.6)	23 (14.5)	42 (26.4)	58 (36.5)		
Delivery mode							
Vaginal born	184	39 (21.2)	19 (10.3)	53 (28.8)	73 (39.7)	9.482	0.024
C-section born	165	43 (26.1)	33 (20.0)	35 (21.2)	54 (32.7)		
Child slept with bottle							
Yes	162	28 (17.3)	25 (15.4)	37 (22.8)	72 (44.4)	10.54	0.015
No	181	53 (29.3)	27 (14.9)	47 (26.0)	54 (29.8)		
Soft drink							
Never/Rarely	80	30 (47.5)	8 (10.0)	15 (18.8)	19 (23.8)	39.97	<0.001
Sometimes	81	6 (7.4)	13 (16.0)	27 (33.3)	35 (43.2)		
≥ 1 per day	187	37 (19.8)	31 (16.9)	46 (24.6)	73 (39.0)		
Lollipop candy							
Never/Rarely	192	57 (29.7)	27 (14.1)	48 (25.0)	60 (31.3)	11.02	0.012
≥ 1 per day	155	24 (15.5)	25 (16.1)	39 (25.2)	67 (43.2)		
Mother prechewing food							
Yes	110	19 (17.3)	12 (10.9)	34 (30.9)	45 (40.9)	7.33	N.S.
No	237	62 (26.2)	40 (16.9)	53 (22.4)	82 (34.6)		
Mother regular alcohol use							
Yes	102	21 (20.6)	23 (22.5)	15 (14.7)	43 (42.2)	13.97	0.003
No	246	61 (24.8)	29 (11.8)	73 (29.7)	83 (33.7)		

CFU, colony-forming units.

Swedish children aged 4–5 years (74%) (33), but it was higher than Japanese children aged 2–5 years (51%) or Finnish children aged 2–3 years (25%) or 5–6 years (51%) (34), and lower than in Chinese children aged 3–4 years (95%) (35). Our data suggest that children may be at increased risk for high levels of MS colonization if their mothers experienced high levels of MS or if they were vaginally delivered. Additionally, child's age, failure to brush daily, as well as mother's prechewing of food were significant predictors for MS colonization in the children after adjusting for other risk indicators, including family socio-demographics, maternal pregnancy and pediatric medical histories, and childhood feeding practice and dietary habits. Interestingly, mother regular alcohol use remained in the final logistic analysis as a significant predictor for children's MS, particularly for 5-year-old group (Table S3). The mechanisms by which alcohol may increase MS colonization are not clear, in spite of a tendency of increasing the colonization by *S. mutans* and dental caries on smooth surfaces has been reported in an animal model (36). We hypothesize that the alcohol use could be a potential confounding factor contribut-

ing to the positive correlation, as alcohol consumption has been reported closely associated with cigarette smoking (37), poor oral hygiene behaviors (38), and higher number of surfaces with caries (39, 40). In contrast, bottle feeding and frequency of sugar consumption were not among the significant determinants predicting MS colonization in the children. The results suggest that consumption of sugar-sweetened snacks or beverages may not be the primary factor responsible for MS early colonization in the oral cavity of Thai children. Evidently, maternal influence plays more significant role in determining children's oral health.

The present study found that a significantly higher proportion of vaginal born children were colonized with high levels of MS when compared to C-section born children. Results from a previous study suggested that children born by C-section might acquire *S. mutans* earlier than children born vaginally (16). It is important to note that the two studies used different study designs and investigated the associations from two different perspectives. The earlier study was a prospective follow-up study. *Streptococcus mutans* colonization was systematically documented by repeatedly

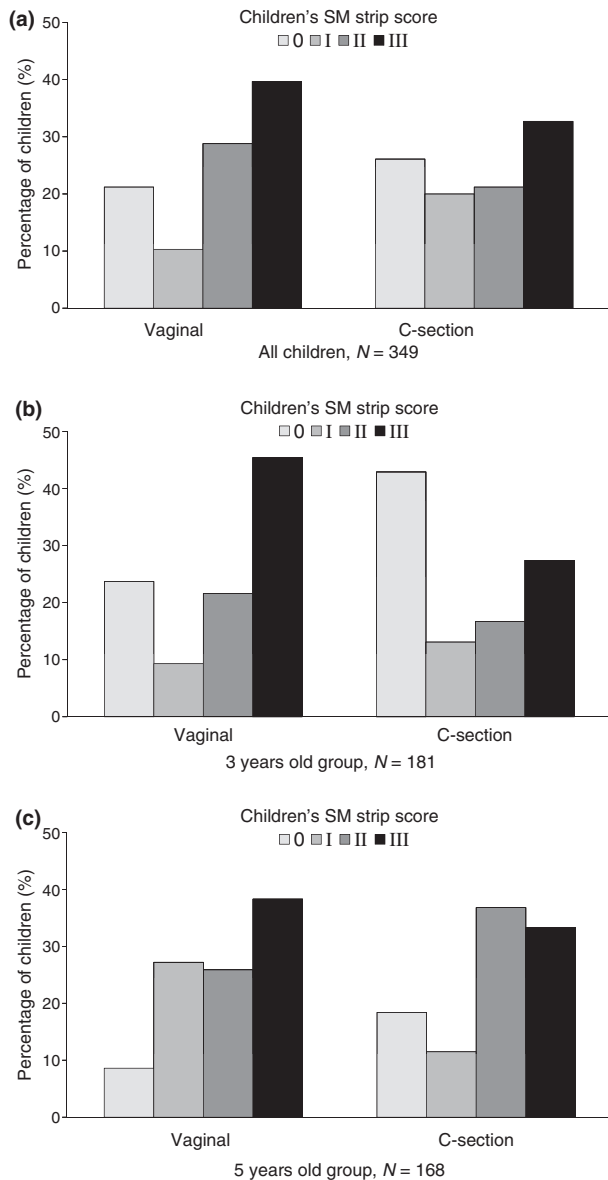


Fig. 2. Comparison of mutans streptococci (MS) colonization in children born by different delivery methods. (a) The MS Strip score distributions were significantly different in vaginally born children compared with C-section born children (Pearson Chi-square test; $P = 0.024$). (b) The differences were consistent for the 3-year-old group (Pearson chi-square test; $P = 0.010$); and (c) 5-year-old group (Pearson chi-square test; $P = 0.010$). The four categories represent MS colony-forming units (CFU) per ml of saliva of: '0' for $<10^4$, 'I' for 10^4 – 10^5 , 'II' for 10^5 – 10^6 , and 'III' for $>10^6$.

sampling the children's saliva samples from birth to 4 years of age. *Streptococcus mutans* identification was based on the conventional culture method. One main limitation of the study was its imbalanced sample size between the C-section group ($n = 29$) and the vaginally born group ($n = 127$). The present report, on the other hand, was a cross-sectional study with a balanced number of

participants for each study group; the ages of enrolled children were between 3 and 5 years, and most of them were MS-positive at the time the study was conducted; and MS including *S. mutans* measurement was based on Strep SM Strip estimation. The initial acquisition of *S. mutans* among the study population could not be determined and compared with previous reports by others. Therefore, the results of the two studies are not directly comparable. Instead, they provide two different insights into the correlation between the mode of delivery and the colonization of MS in young children.

Despite the differences in study design, both studies demonstrated that the colonization of MS in young children was affected by the mode of delivery, either at the initial acquisition of colonization or at the later level of colonization. A study carried out by Dominguez-Bello and others demonstrated that vaginally delivered newborns, as they go through the birth canal, naturally acquire a variety of maternal indigenous bacteria that are different from newborns delivered by C-section (27). Using the 16S rRNA gene pyrosequencing method, they concluded that the delivery mode might affect the direct transmission of initial bacteria from mother to her newborn. More recently, Lif Holgersson et al. (41) reported that vaginally delivered infants had distinct oral microbial colonization patterns compared with C-section born infants. Barfod et al. (42) reported that a significantly higher prevalence of previously known health-related streptococci and lactobacilli were found in vaginally delivered infants compared with infants delivered by C-section. However, it remains unclear whether vaginal delivery can facilitate health-related microorganism colonization and, if so, how such early colonized microbes might establish microbial succession in the oral cavity of vaginally born children.

Although a number of studies have related the mode of delivery and oral microbiota composition in children, information on the effect of delivery mode on caries outcome is scant. If it is true that mode of delivery can influence *S. mutans* colonization (16) and alter oral microbial composition (30), then, based on available evidence, it should be expected that children born by different methods ought to have a different risk for dental caries. Outside of the field of dentistry, a large population-based cohort study has shown that children delivered by C-section had a significantly increased risk of asthma compared with vaginally

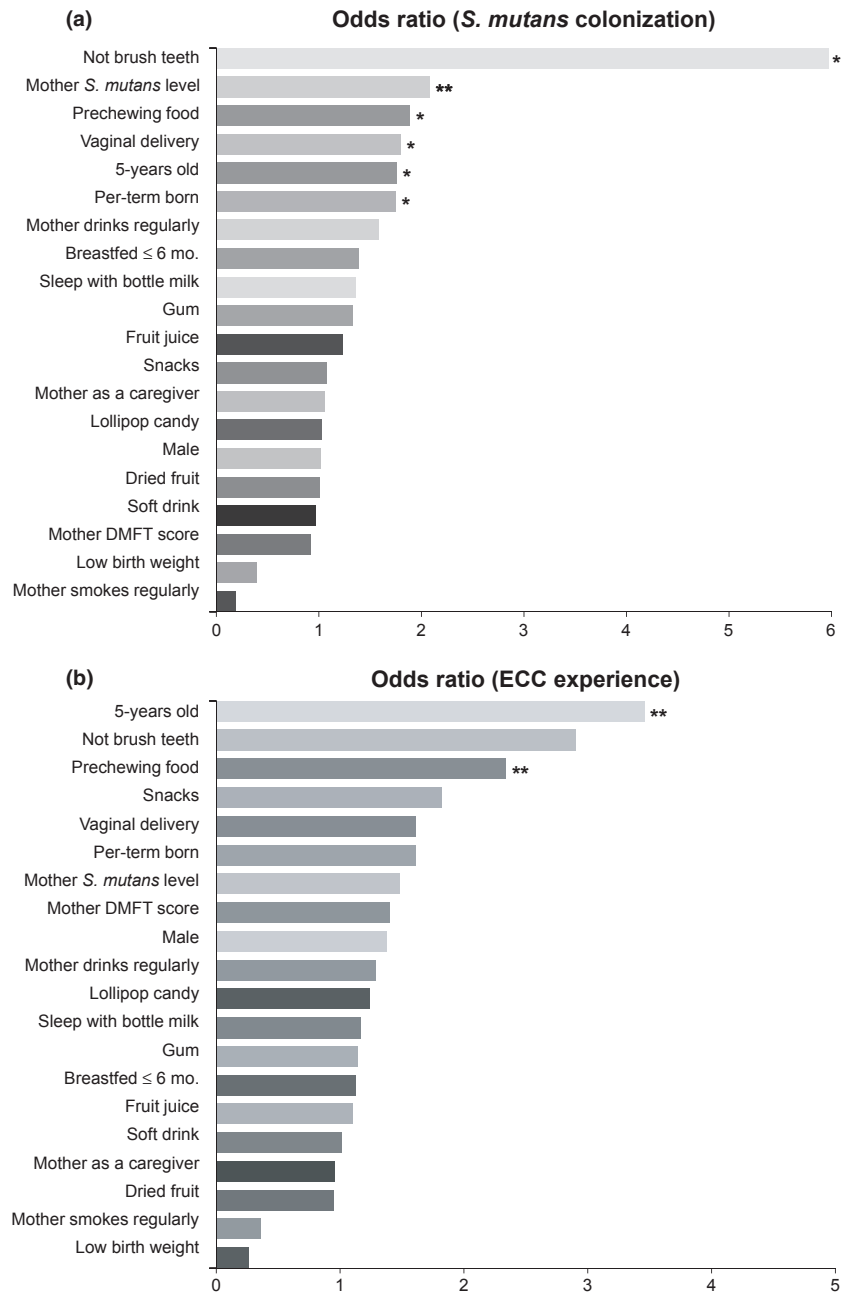


Fig. 3. Summary of odds ratios for all the independent factors tested in the initial logistic regression model. Odds ratios from initial logistic regression model predicting: (a) the level of mutans streptococci colonization and (b) early childhood caries (ECC) outcome in Thai children. Coefficient was statistically significant at the $*P < 0.05$ or $**P < 0.01$ level. The frequency of consumption was '≥ once per day' for gum, fruit juice, snacks, lollipop candy, dried fruit, and soft drinks.

delivered children (26). It was speculated that the lack of early exposure to maternal microbiota and possible initial colonization with the 'wrong' microbes might significantly impact stimulation and maturation of the infant's immune system resulting in increased frequency of severe asthma in children.

In the present study, one of the key findings was the significant association between the mode of delivery and caries outcome in a Thai children population, particularly among 3-year-old children, suggesting that children born by vaginal delivery were more likely to have an early onset of ECC after adjusting for socio-demographic,

dietary, and other oral health risk indicators. The possible explanation may be illustrated by the fact that a majority (66%) of the mothers or the primary caregivers in the study had high levels of MS in their saliva; almost all of them (93%) experienced dental caries; and more vaginal delivery mothers had practiced prechewing. Currently, there are no definitive studies that correlate mode of delivery, mother's nurturing behavior, and their influence on ECC. It has been hypothesized, however, that mothers who give birth vaginally establish a closer relationship with their new-born babies, spend more time with their babies, and engaged in increased breast feeding compared with those

Table 4. Summary of the final logistic regression model for prediction of mutans streptococci colonization in the Thai children

Independent variables	P value	Odds Ratio	95% CI
N = 348			
Mother's SM Strip score (II or III)	0.003	2.15	1.30–3.57
Children brush teeth (no)	0.004	7.25	1.92–27.46
Child's age (5 years old)	0.005	1.97	1.23–3.16
Prechewing food (yes)	0.008	2.02	1.20–3.41
Mode of delivery (vaginal)	0.012	1.81	1.14–2.89
Mother regular alcohol use (yes)	0.050	1.67	1.00–2.80
Pre-term (<37 weeks)	0.077	1.57	0.95–2.59

Model summary: -2Log likelihood = 418.91; $R^2 = 0.159$; $\chi^2 = 43.01$; $P < 0.001$.

Table 5. Summary of the final logistic regression model for prediction of early childhood caries outcome in the Thai children

Independent variables	P value	Odds Ratio	95% CI
N = 348			
Age (5 years old)	0.000	3.02	1.85–4.95
Prechewing food (yes)	0.017	1.95	1.13–3.37
Mode of delivery (vaginal)	0.023	1.74	1.08–2.82
Snack ≥ 1 time per day (yes)	0.074	1.78	0.95–3.35
Children brush teeth (no)	0.119	2.39	0.80–7.12
Mother SM Strip score (II or III)	0.135	1.48	0.89–2.47

Model summary: -2Log likelihood = 401.54; $R^2 = 0.156$; $\chi^2 = 41.74$; $P < 0.001$.

mothers who give birth by C-section (43). Studies also suggested that C-section could significantly delay the first postnatal contact and interaction between a mother and her newborn baby and exert adverse psychosocial outcomes (43–46). The findings from our study support the hypothesis that the mothers who delivered vaginally were more likely to chew food for their children and have a higher possibility to transmit cariogenic microbiota, such as MS, to their children compared with the mothers of those delivered by C-section. As MS colonization significantly associated with the prevalence and severity of ECC has been well documented (47–51), we can conclude that the vaginally delivered children in this Thai population were at increased risk for ECC.

In addition to the difference in ECC between the two delivery groups, we also found that boys experienced significantly more severe ECC in terms of mean dmft/dmfs scores than the girls. Other studies have reported similar results in various preschool children populations (52–57). The differences in eruption and exfoliation of the deciduous teeth, feeding practice, or eating habits may be associated with gender differences. In a previous *S. mutans* mother-to-infant transmission study, it was suggested that male children were 13 times more likely to experience caries than female children if they acquired *S. mutans* strains from their biological mother (15). As the present study did not include *S. mutans* identification and genotype comparisons between males and females, the possible mechanism related to the increased risk for ECC in male children could not be identified. Interestingly, frequent sugar consumption and other dietary habits were neither significant indicators for predicting MS colonization nor ECC experience in the children, although a positive correlation has been suggested by Thitasomakul et al. (57) in a Thai children population. In addition, the study showed that the failure to routinely brush teeth was highly correlated with high levels of MS colonization in children, but not a significant predictive factor for ECC after adjusting for all other risk factors in either the initial or the final regression analysis. The unexpected findings could due to the high prevalence of children (93.7%) who claimed to brush their teeth at least once a day, resulting in a limited comparison. In spite of missing detailed assessment for brushing habits in the study, it is well evidenced that teaching young children to properly brush their teeth is crucial to reducing the risk for ECC and promoting good oral health. Our study further suggests that if children do not brush their teeth regularly, they are seven times more likely to be colonized with a higher level of MS at an early age and have increased risk for ECC.

In summary, the present study demonstrated that vaginally born children experienced higher levels of MS, suggesting that mode of delivery might alter the risk for dental caries, particularly for children at a young age. The association, however, does not imply causation, because the present study was a retrospective observational study by design, which does not allow establishing a time course correlation between the two events of interest, namely the mode of delivery and colonization with MS. Despite a recent report showing that parents' oral health perception and social behaviors significantly affect the oral health-related quality of life of their children (58), our study demonstrated significant

connections between maternal influences on children's cariogenic bacterial colonization and caries status. As Kohler and others have previously demonstrated that reduction MS in the mother during the tooth emergence period of young children could have long-term influence on colonization by these bacteria in children and a reduction in childhood caries experience (59), evidence from this study further supports the notion that early interventions to control MS levels improve the oral health of mothers and primary caregivers, transform maternal attitude toward good oral health, and promote healthy behaviors and practices, is essential to reduce the risk of MS infection and ECC in young children. Meanwhile, future studies are needed to explore and advance our understanding of the possible biological mechanism underlying the mode of delivery in relation to the colonization of cariogenic microbiota and development of ECC.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The correlation of ECC experience, mode of delivery, and child's dietary and eating habits in 3-to 5-year-old Thai children.

Table S2. The correlation of mutans streptococci colonization with child's birth history, dietary and eating habits in 3- to 5-year-old Thai children.

Table S3. Prediction of mutans streptococci colonization in the Thai children analyzed by age.

Table S4. Prediction of ECC outcome in the Thai children analyzed by age.

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