# THE EFFECT OF EXCLUSIVE BREASTFEEDING, EXCLUSIVE BOTTLE-FEEDING, MIXED FEEDING, AND ORAL *ROTHIA* ON EARLY CHILDHOOD CARIES

by

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**ABSTRACT:**

**Objective:** To investigate the association between feeding practices (exclusive breastfeeding, exclusive bottle-feeding, and mixed) with early childhood caries (ECC) and the association between the prevalence of oral *Rothia*, at the species and genus level, with ECC.

**Methods:** A total of 438 children (178 caries-free, 260 with ECC) were included in this case-control study. A comprehensive questionnaire that included a section on feeding history, was completed by the parent or caregiver of each participant. Based on the feeding history responses, participants were classified as either exclusively breastfed, exclusively bottle-fed, or mixed (both bottle-fed and breastfed). A supragingival plaque sample was collected from each participant and then assessed with 16S rRNA sequencing for *Rothia* genus and species' relative abundances. The association between feeding practices and *Rothia* prevalence with ECC, accounting for confounding variables, was analyzed using multivariable logistic regressions.

**Results:** A low relative abundance of *Rothia aeria* was significantly associated with ECC ($p<0.05$), whereas there were no significant associations found between the relative abundance of *Rothia dentocariosa* or *Rothia mucilaginosa* with caries status ($p>0.05$). While the odds of association between a low relative abundance of *Rothia* (genus) and ECC was 1.63, this relationship failed to reach the threshold of significance ($p=0.054$). Feeding practices were not significantly associated with ECC after adjusting for confounding variables. However, bedtime bottle use, bedtime snacking, living in a rural/remote area, being an older age, the use of fluoridated toothpaste, and having less-educated guardians were significantly associated with ECC ($p\leq0.05$)

**Conclusions:** Findings in this study suggest that low relative abundances of *R. aeria* and *Rothia* (genus) are associated with ECC. Feeding practices were not key risk factors for ECC in this

sample, as other factors related to the social environment and bedtime habits were more strongly associated. Since the current literature is limited, prospective studies examining *Rothia* and feeding practices with ECC would be beneficial to understand the causal relationship between *Rothia* and ECC and to help reach a consensus on the effect of different feeding practices on ECC risk.

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## INTRODUCTION:

Early childhood caries (ECC) is a global health concern, with 48% of preschool children affected by this chronic childhood disease. [1] The American Academy of Pediatric Dentistry (AAPD) defines ECC as one or more decayed, missing or filled surfaces (dmfs) in any primary tooth in a child less than 72 months old.[2] ECC is affected by a myriad of factors like access to dental care and oral hygiene habits and ultimately has a negative effect on a child's quality of life, affecting domains such as growth and development, sleep quality, and school attendance and performance.[3] In most Canadian hospitals, ECC treatment under general anaesthesia is the most common surgery performed for Canadian preschool children, with a rate of 12.1 per 1000 children.[4] With the high associated costs of general anaesthesia (GA) and the long waitlists for treatment, ECC is a pressing concern that can be entirely prevented.

Breastfeeding has many benefits to infants including its high nutritional content and protection against sudden infant death syndrome. [5,6] The World Health Organization (WHO) and Health Canada recommend that infants should be exclusively breastfed (only breastmilk is given; no other liquids or solids are given) for the first 6 months of life and to continue to be breastfed for up to 2 years of age or longer with appropriate complementary feeding.[5,6,7] Yet, Canada's Breastfeeding Progress Report revealed that while 91% of Canadian mother's initiated breastfeeding, 22% stopped between one and six months and only 34.5% were exclusively breastfeeding. [7] Barriers to breastfeeding include socioeconomic factors like education level, breastfeeding challenges, medical conditions, and environmental contaminants or medications or drugs in breast milk.[7] Thus, other feeding practices like bottle-feeding or mixed feeding (combination of breastfeeding and bottle-feeding) are utilized.

A systemic review of breastfeeding and ECC concluded that breastfeeding was a protective factor under 1 year of age but was unable to determine if it was a risk or protective factor beyond that age due to conflicting studies with heterogeneous confounding factors.[10] In general, studies have not shown consensus regarding the relationship of feeding practices with ECC, with most studies only focusing on ECC's relation to breastfeeding. [8,9] Thus, more research is required to understand the association between different feeding practices and ECC.

Oral cavity colonization begins in utero, with more than 700 species of bacteria and 100 species of fungi identified in the oral microbiome.[11,12] *Rothia* is a bacterium that is an early colonizer of the oral cavity, found within the first 3 months after birth, and is one of the main genera involved in the initial supragingival plaque formation.[13] Even though the relative prevalence of *Rothia* decreases with the continued eruption of the primary dentition and evolving complexity of the oral microbiota, it is still recognized as an important bacteria seen in caries-free subjects.[13,14] However, *Rothia* has been less studied than other bacteria such as *Streptococcus mutans*.[14] While studies have confirmed a decreased *Rothia* abundance in caries-free children, there are existing reports that also demonstrate an increased *Rothia* prevalence in carious patients.[15,16]

With the lack of consensus in literature regarding the relationship of feeding practices and *Rothia* with ECC, it is imperative to understand these relationships to aid in caries risk assessment and the formulation of appropriate caries management plans. Therefore, the purpose of this study was to understand the association of infant feeding practices with caries status and *Rothia* relative abundance with caries status in young children.

## METHODS

### Study design

Children included in this study were recruited into a larger multiyear case-control study, "The Role of Taste and Oral Microbiome on Caries Risk in Young Children", undertaken by the Schroth and Chelikani labs at the University of Manitoba. ECC status, *Rothia* relative abundances, and feeding histories were obtained from the primary study's data to allow for this case-control study. Caries status was the dependent variable and relative abundance of *Rothia* and feeding practices were the independent variables.

Since the larger-linked study's goal was to evaluate the relationship between taste signaling pathways and the functional plaque microbiome in caries-free and ECC children, the oral microbial diversity was analyzed using 16S rRNA amplican sequencing. This allowed bacterial identification by comparing obtained 16S rRNA sequences to available reference gene sequence databases. *Rothia* was one of the genera identified and the obtained *Rothia* data was used in this study. ECC or caries-free status were obtained for each participant after a dental examination.

### Study sample

The study size consisted of 473 participants, with ECC participants recruited from Misericordia Health Centre (MHC) on the day of their dental rehabilitation surgery and children with ECC and caries-free participants recruited from community dental clinics in Winnipeg or directly from the Children's Hospital Research Institute of Manitoba (CHRIM). Exclusion criteria included an incomplete Section D (Child's Feeding History), incomplete answers for certain questions relating to oral health and family characteristics, and any participants aged 79 months or above. Even though ECC is defined as being a disease for those <72 months of age, the 6 month wait time for dental GA was accounted for, expanding the inclusion criteria to <79 months. After the inclusion and exclusion criteria were applied, the study sample consisted of 438 participants, 260 with ECC and 178 who were caries-free.

An algorithm (Figure 1) using information from section D “Child’s Feeding History”, was used to categorize each participant into one of three feeding practices:

1. Exclusive breastfeeding: Only breastfeeding with no other solids or liquids for the first 6 months of life.
2. Mixed feeding: A combination of bottle-feeding and breast-feeding after birth, with no period of exclusive breastfeeding.
3. Exclusive bottle-feeding: No breastfeeding had occurred.

**Data collection**

Participants legal caregivers were given a cross-sectional questionnaire to be reviewed with research staff. This questionnaire, which was used for the larger linked study, had questions pertaining to the child’s general and oral health, feeding methods, parental oral health, family characteristics, and health information about the birth mother.

Caries status was already determined if the participant was recruited from MHC or a community dental clinic in Winnipeg. For participants who responded directly to study advertisements or were unable to be properly examined at a community dental clinic, they were seen at CHRIM where an experienced dentist (R.J.S.) conducted clinical examinations with artificial light and no radiographs to confirm the caries status of the child. During the dental examination, all available tooth surfaces were swabbed with a sterile interdental brush to complete supragingival plaque sample collection. The brush was placed into 1mL of RNAprotect Bacteria Reagent to allow for DNA extraction and frozen at -80C on dry ice until it could be analyzed at the McGill University-Genome Quebec Innovation Centre.

## Data Analyses

### *Questionnaire Analyses*

After questionnaire collection, data were entered into REDCap (Research Electronic Data Capture) and then exported into Microsoft Excel workbook. Participants, after being categorized into a feeding practice based on the algorithm in Figure 1, were then recoded as “1” for exclusive breastfeeding, “2” for mixed feeding, and “3” for exclusive bottle-feeding. Caries status was categorized as “1” for caries-free or “2” for ECC. Wilcoxon rank sum test and Pearson’s Chi-squared test were performed when appropriate.  The ages for when breastfeeding and bottle-feeding were stopped were characterised by Kaplan-Meier estimates of the proportion still using the feeding practice at each age. Corresponding 95% confidence intervals (95% CI) were included, at least to the extent possible due to censoring, represented with “-“ (where feeding was ongoing at the time of the study).

### *Oral Microbiome Analyses*

The extracted DNA was received at the McGill University-Genome Quebec Innovation Center for library preparation and paired-end Illumina MiSeq PE250 sequencing of the V4 region of bacterial 16S rRNA. QIIME2 2018.11 software (Quantitative Insights into Microbial Ecology) was used to analyze the provided sequencing data.

Oral microbiome data was provided for secondary analyses at both the genus and species level as a relative abundance – the percentage from total bacteria measured that is comprised of a certain taxon. For this study's purpose, microbiome analysis focused on *Rothia* at both the genus and species level, and other genera were excluded. *Rothia* abundances was categorized into low relative abundance (represented as "0") or high relative abundance (represented as "1") based on a relative median value, seen in Table 1. Wilcoxon rank sum test and Pearson's Chi-squared test were performed when appropriate, with $p$-value $\leq 0.05$ considered statistically significant.

*Logistic Regression Models*

Multivariable logistic regression models were used to estimate the association between ECC with feeding practices and the relative abundance of *Rothia*. A separate model was fitted for each *Rothia* species and genus (the "full models") and backwards elimination based on the Akaike Information criterion (AIC) was used to reduce the number of predictors. However, age, sex and urban/rural were included in each of the "reduced" models" to adjust for these demographic variables in a standardized way across the four models. Odds ratios (OR), 95% confidence intervals (95% CI), and p-values were reported, with a p-value $\leq 0.05$ considered statistically significant.

**Ethical considerations**

This study was approved by the University of Manitoba's Health Research Ethics Board (HS25961; H2023:120). It utilized data that was collected as part of a multiyear study "Role of Taste and Oral Microbiome on Caries Risk in Young Children" (HS22388/H2018:472) completed at the University of Manitoba.

Written and informed consent were obtained by from parents and caregivers of 473 children. Since this project utilized data from a larger-linked project that already received consent, no additional consent was required from participants for our data analyses.

## RESULTS

*Participant Characteristics*

Baseline demographic and behavioural characteristics of participants are presented in Table 2. A total of 438 children (50% female, 50% male) were used for secondary analysis with a mean age of 45 ± 15 months. Overall, 178 participants were caries-free and 260 had ECC. There was a statistically significant association between age and caries status, with participants in the ECC group being an older age than those who were caries-free (48 ± 14 months vs. 41 ±16, $p<0.001$). Overall, place of residence, feeding practice, bedtime bottle use, frequent bedtime snacks, the use of fluoridated toothpaste, parental education level, and receiving government assistance differed significantly by caries status ($p\leq0.05$). Children with ECC were more likely to be exclusively breastfed, go to bed with a bottle, frequently snack before bedtime, use fluoridated toothpaste, receive government assistance, and have a parent with a lower education level. A larger proportion of participants were from urban communities (71%) and were more likely to be caries-free, while participants from rural communities were more likely to have ECC.

*Feeding History*

As represented in Table 2, most participants (222/438, 51%) were exclusively breastfed, followed by exclusively bottle-fed (126/438, 29%) and lastly, mixed fed (90/459, 21%). Chi-square analysis revealed that there was a significant association between feeding practices and caries status ($p<0.001$). Children who were exclusively breastfed were more likely to have ECC and mixed feeding seemed to have the lowest association with ECC. Out of the three feeding practices, exclusively bottle-fed children were more likely to have ECC compared to caries-free children (93/126 with ECC, 33/126 caries-free).

Overall, 312 participants were breastfed. As seen in Table 3, in the caries-free group, the estimated median age (50% percentile) at which breastfeeding was stopped was at 12 months (95% CI: 12, 15). For the ECC group, the median was also at 12 months (95% CI: 9, 18). At the 75% percentile, the age when breastfeeding was stopped was higher for the ECC group (24 months; 95% CI: 24, 24) than for the caries-free group (18 months; 95% CI: 18, 24).

There were 330 participants who were bottle-fed in this study (including those in the exclusive breastfeeding group who used a bottle at a later age). Table 3 displays the estimated median age that bottle-feeding was stopped. In the caries-free group, it was 24 months (95%CI: 18, 24) and in the ECC group, it was also 24 months (95% CI: 23, 24). At the 75% percentile, the age for which bottle-feeding was stopped was older for the ECC group (36 months; 95% CI: 32, 36) than for the caries-free group (30 months; 95% CI: 24, -).

*Rothia Composition of Plaque*

Table 2 shows the relative abundances' of *Rothia* among participants, as being either high or low. *Rothia* (genus) and *R. aeria* were found to have statistically significant associations with caries status ($p \leq 0.05$) as those with ECC were more likely to have a low relative abundance of *R. aeria* and *Rothia* (genus). No statistically significant relationship was found with either *R. dentocariosa* or *R. mucilaginosa* with caries status.

Figure 2 shows the relative distribution of *Rothia* at the species level for each feeding practice and caries status. The average relative abundance of *Rothia* (genus) was higher in the caries-free group, compared to the ECC group ($p<0.002$; Table 2; Figure 2). *R. aeria* was also found to have a higher relative abundance in the caries-free group compared to the ECC group ($p<0.001$; Table 2, Figure 2).

*Multivariable analysis*

Table 4 provides the results on the association between caries status, feeding practices, and *Rothia* after adjusting for confounding variables. No statistically significant associations between feeding practices and ECC were found in the models (p>0.05).

The OR of developing ECC with a low abundance of *R. dentocariosa* was 1.30 times (95% CI: 0.78, 2.17), but the association was statistically non-significant (p>0.05). The OR of developing ECC with a low abundance of *R. mucilaginosa* was 0.92 times (95% CI: 0.55, 1.52), but the relationship was also not statistically significant (p>0.05). Neither species were retained as variables in their respective reduced models.

*Rothia* (genus) was kept as a variable in the reduced model for Table 4, with the relationship between *Rothia* and ECC approaching the threshold of significance (p=0.054). Children with a low abundance of *Rothia* (genus) were 1.63 times more likely to have ECC than children with a high abundance (95% CI: 0.99, 2.71). In this reduced model, snacks given before bedtime (OR=1.81; 95% CI: 1.10, 3.00), bedtime bottle use (OR=1.85; 95% CI: 1.85, 5.35), and the use of fluoridated toothpaste (OR=2.10; 95% CI: 1.13, 3.97) were associated with ECC. Children of parents who finished high school (OR=0.47; 95% CI: 0.17, 1.19) or completed more than a high school level education (OR=0.21; 95% CI: 0.08, 0.49) were less likely to have ECC. Living in a rural or remote community was strongly associated with ECC (OR=22.8; 95% CI: 10.1, 59.6).

Multivariate analysis revealed that only one statistically significant bacterial association with caries status remained, namely *R. aeria* (p<0.05). In the full model, children with a low abundance of *R. aeria* were 1.73 times more likely to have ECC than children with a high abundance of the bacteria (95% CI: 1.05, 2.89). In this model, there was no significant association between feeding practices and ECC, with a lower odds of ECC development in the mixed fed children (OR=0.58; 95% CI: 0.30, 1.11) and the exclusively bottle-fed children (OR=0.70; 95% CI: 0.34, 1.39). Having frequent bedtime snacks (OR=1.86; 95% CI: 1.12, 3.17), bedtime bottle use (OR=3.43; 95% CI: 1.95, 6.18), using a fluoridated toothpaste (OR=2.18, 95% CI: 1.17, 4.16), living in a rural/remote area (OR= 24.8; 95% CI: 10.9, 65.9), and age (OR=1.04, 95% CI: 1.03, 1.06) were significantly associated with ECC. Parents who completed high school (OR=0.44; 95% CI= 0.16, 1.13) or more than a high school education (OR=0.23; 95% CI= 0.09, 0.55) were significantly more likely to have a child with lower odds of having ECC. Toothbrushing at least two times a day had an OR of 1.10 (95% CI: 0.65, 1.88), but there was no statistically significant association found between frequency of toothbrushing and ECC ($p>0.5$). A nonsignificant result was also found between using government assistance and ECC ($p>0.05$; OR=1.31; 95% CI: 0.72, 2.39). Females had a lower risk of developing ECC compared to males, but the result was nonsignificant ($p>0.05$; OR=0.75; 95% CI: 0.45, 1.25). The reduced model for *R. aeria*, adjusting for the variables of sex, gender, and place of residence, identified having a low relative abundance of *R. aeria*, bedtime snacking, bedtime bottle use, the use of fluoridated toothpaste, and parental education levels as the strongest predictors of ECC. The trends seen in the reduced model were the same as in the full model, with slight deviations in ORs and 95% CIs.

A summary of the strongest risk factors for ECC from each model, with their associated ORs and 95% CIs, can be seen in Figure 3's forest plot. Feeding practices were not included in any of the models and the only bacteria included were *R. aeria* and *Rothia* (genus). Living in a rural community had the largest OR across all four models to predict EC.

## DISCUSSION

In this study, we investigated the relationship between caries status, feeding practices, and *Rothia* prevalence. A low relative abundance of *Rothia*, and more significantly, a low relative abundance of *R. aeria*, was observed in the ECC group. This is consistent with the study by Xu et al. (2018) who found that a decrease in *Rothia* abundance at the genus level, was significantly associated with having caries in the deciduous dentition for a sample of preschool children in China.[16] A study by Richards et al. (2017), examining the microbiome of 55 children in the USA, also had similar findings, detecting a higher prevalence of *R. aeria* in the caries-free group and lower amounts in the caries active group.[18] A study by Jiang et al. (2016) studying the microbial flora diversity in 40 Hong-Kong kindergarten children and another study by Viera et al. (2019) comparing major microbial species in the saliva samples of 95 children in Pittsburgh, USA, both associated a higher *Rothia* relative abundance with caries. [19,20] However, Jiang et al., only identified a higher abundance of *R. dentocariosa* with their caries-affected group, while Viera et al. identified *R. mucilaginosa* in their caries-active subjects.[19,20] We were unable to find a significant association between *R. mucilaginosa* and *R. dentocariosa* with caries status in our study, which may be attributed to the much smaller sample sizes used in the other studies, variances in patient populations used, and differences in microbial identification methods as *R. aeria* was not mentioned in their studies.[19,20]

The role of *Rothia* in caries prevention can be explained by its nitrate-reducing property.[21] Nitrate is primarily found in a vegetable-diet, where it's absorbed in the gastrointestinal system and then circulated to and secreted by the oral salivary glands. [21] Humans are unable to effectively reduce nitrate into nitrite, but some oral bacteria like *Rothia* can. [21] Nitrite is then metabolized by human enzymes and nonenzymatic pathways to nitric oxide.[21,]

Nitric oxide is a free radical that is antimicrobial and protective against anaerobic species involved in periodontal disease and caries-associated genera such as Streptococcus. [21,22] Nitrate can also buffer acidic salivary changes, potentially through its conversion into nitrite where lactic acid is consumed and during the reduction of nitrite to ammonium where proton consumption occurs, thus limiting enamel demineralization.[21] By preventing acidification, it also prevents the overgrowth of acidogenic/aciduric species and maintains the abundance of beneficial oral species. [21]

In this study, after adjusting for confounding factors, it was found that there was no statistically significant association between feeding practices and caries status. Even though it was non-significant, exclusive breastfeeding had the highest odds of being associated with ECC, followed by exclusive bottle-feeding, and lastly, mixed feeding. The only systematic review to look at breastfeeding and bottle-feeding's association with dental caries, by Avila et al. (2015), analyzed seven studies to determine that breastfeeding was more protective than bottle-feeding during early childhood for caries, reinforcing the breastfeeding recommendations by WHO and Health Canada.[8] However, that review was unable to determine if the two feeding practices were protective factors or risk factors beyond early childhood due to conflicting studies with different study designs.[8] The discrepancy of our results from Avila et al.'s, could be explained by the confounding factors that we adjusted for, which may have not been present in the studies included in the review. Also, in the current study, participants were not followed through time for caries development, and since caries risk changes with time, the importance of feeding practices could have changed with age. Therefore, after a certain period of time, the effects of feeding practices may not be as important as other variables in the development of caries. A systematic review only looking at breastfeeding and caries risk by Tham et al., (2015), concluded that breastfeeding up to 12 months of age was unassociated with caries risk and perhaps a protective factor.[23] The risks of breastfeeding beyond this 12-month duration however were associated with an increased caries risk.[23] In a study by Park and Choi (2022) who examined multiple feeding practices (bottle-feeding, breastfeeding, mixed feeding) with ECC in 2,772 Korean children aged one to three, it was found that breastfeeding increased the risk of dental caries and that the mixed-feeding group had a significantly lower caries risk than the breast-feeding group. [9] In Table 2, when confounding variables were not accounted for, our results were in line with Park and Choi and Tham et al.'s, regarding the significance risk of breastfeeding and ECC. However, when looking at Table 4, these results were non-significant, though the mixed feeding group had the lowest odds of developing ECC, similar to Park and Choi's significant finding. The difference in results between feeding practices and caries status in Table 2 and Table 4 reinforces our previous suggestion that confounding factors like socioeconomic variables over time, play a larger role as a predictor for caries status, than feeding practices do.

Multiple demographic and behavioural results (Table 1) were in line with existing evidence on ECC risk factors, such as living in a rural/remote community (a barrier in the access to care), nighttime bottle use, bedtime snacks, and parental education levels.[12,26] The oral hygiene indices however had results that were unexpected. Fluoridated toothpaste and toothbrushing at least twice daily are protective factors against ECC and are part of the first line recommendations for caries prevention by the AAPD.[25] However, in this study, fluoridated toothpaste use was significantly associated with the ECC population and toothbrushing frequency was not a significant factor in ECC development, which was a similar result in Park and Choi's study. [9] This inconsistency could be explained by an increased caregiver's knowledge on the benefits of fluoridated toothpaste and regular toothbrushing after ECC diagnosis. With increased awareness, caregivers would have been more likely to implement these strategies by the time of questionnaire completion.

A study from Hong Kong looking at the caries risk factors in 1,204 children aged three to five, found a significant association between gender and caries prevalence and a study by Cruz de Jesus et al. (2020) evaluating oral microbiota and children's gender with ECC, found that sex may be a predictor of ECC.[24,12] Our analyses did not show a significant relationship between sex and ECC, which is consistent with the results of systematic review of caries risk factors by Lam et al. (2022) that included 18 cohort or case-controlled studies[26] A possible explanation for this could be that children, regardless of their gender, are not in control of risk factors like diet and low-parental education level, which ultimately serve a larger role in caries development than any biologically-driven sex differences.

The strength of this study is that it is the first of our knowledge to specifically look at feeding practices, *Rothia*, and caries status. It adds to the limited literature assessing more than just breastfeeding or bottle-feeding alone, as three feeding practices were analyzed. There were several limitations to this study. Although the questionnaire provided was quite comprehensive, it would have been beneficial to ask specifically if the participant was exclusively bottle-fed or mixed fed. This would have reduced misclassification bias. Since questionnaires were utilized, there was a potential for response and recall bias. The confounding variables included in this study were limited and many other factors involved in caries development were not controlled for, such as sugar intake. With caries being a polymicrobial disease, another limitation was that we only focused on *Rothia*, without acknowledging any interactions it may have with other components of the microbiota that may contribute towards caries status.

## CONCLUSION

Based on the findings of our study, the following conclusions have been made:

1. There was no significant association between feeding practices and caries status.
2. There was a significant association between *Rothia aeria* prevalence and ECC, with a low relative abundance associated with ECC.
3. A low relative abundance of *Rothia* (genus) was associated with ECC.
4. Parents should still follow the WHO/Health Canada recommendation for exclusive breastfeeding in the first 6 months followed by complementary breastfeeding for up to two years of age, due to the myriad of other systemic health benefits to the infant and mother.
5. With limited studies examining the relationship between ECC with feeding practices and *Rothia*, more studies would aid in gaining a consensus on the effect and determining a causal relationship between these variables.

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**Figure 1. Feeding practices algorithm**

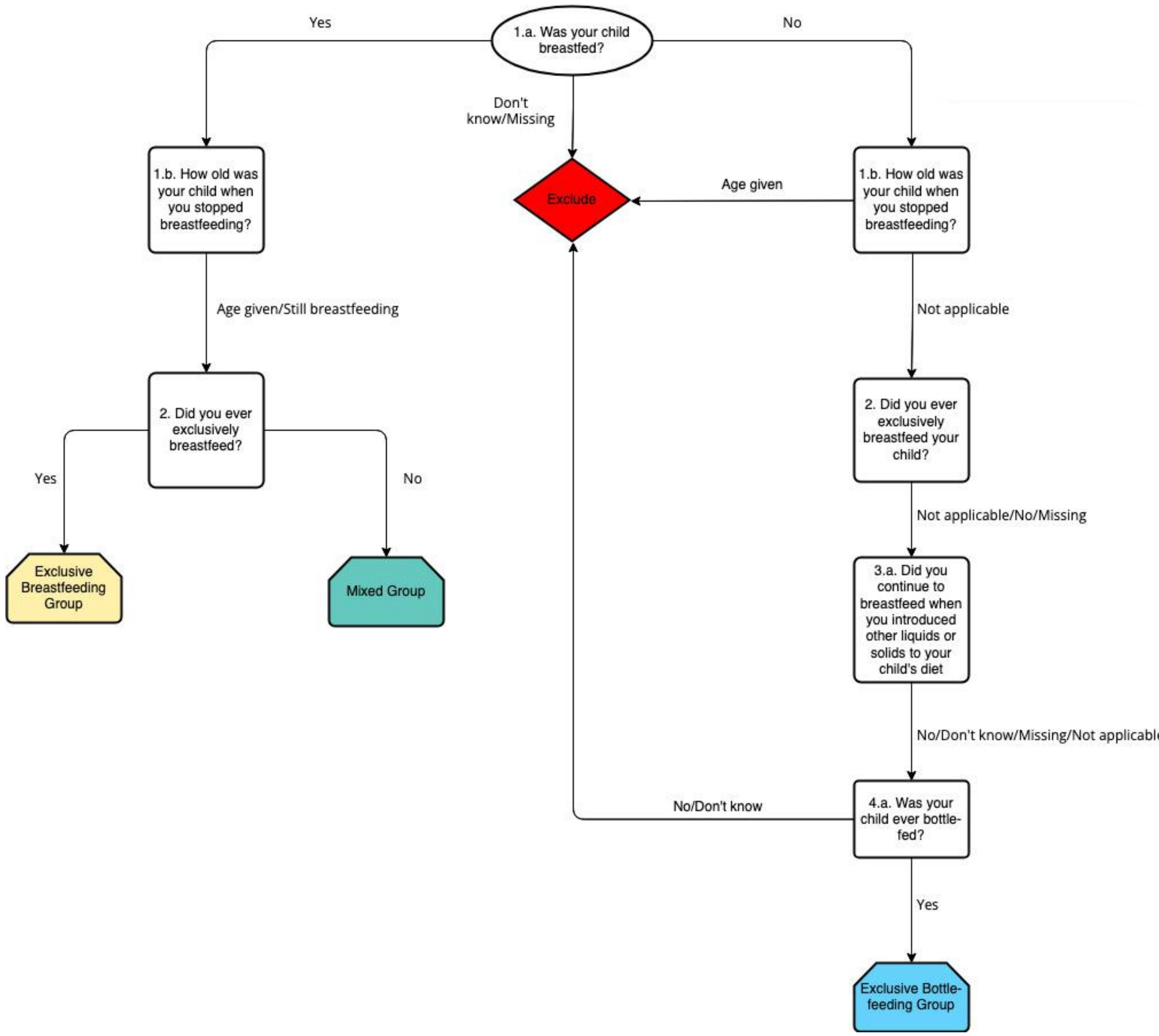

**Table 1: Median Relative Abundance**

| **Taxa** | **Median Relative Abundance (%)** |
|---|---|
| *Rothia* (genus) | 4.5341 |
| *Rothia dentocariosa* | 2.9793 |
| *Rothia aeria* | 3.2272 |
| *Rothia mucilaginosa* | 0.0506 |

**Figure 2: Taxonomic profile of children's dental plaque at the *Rothia* species level according to caries status and feeding practices**

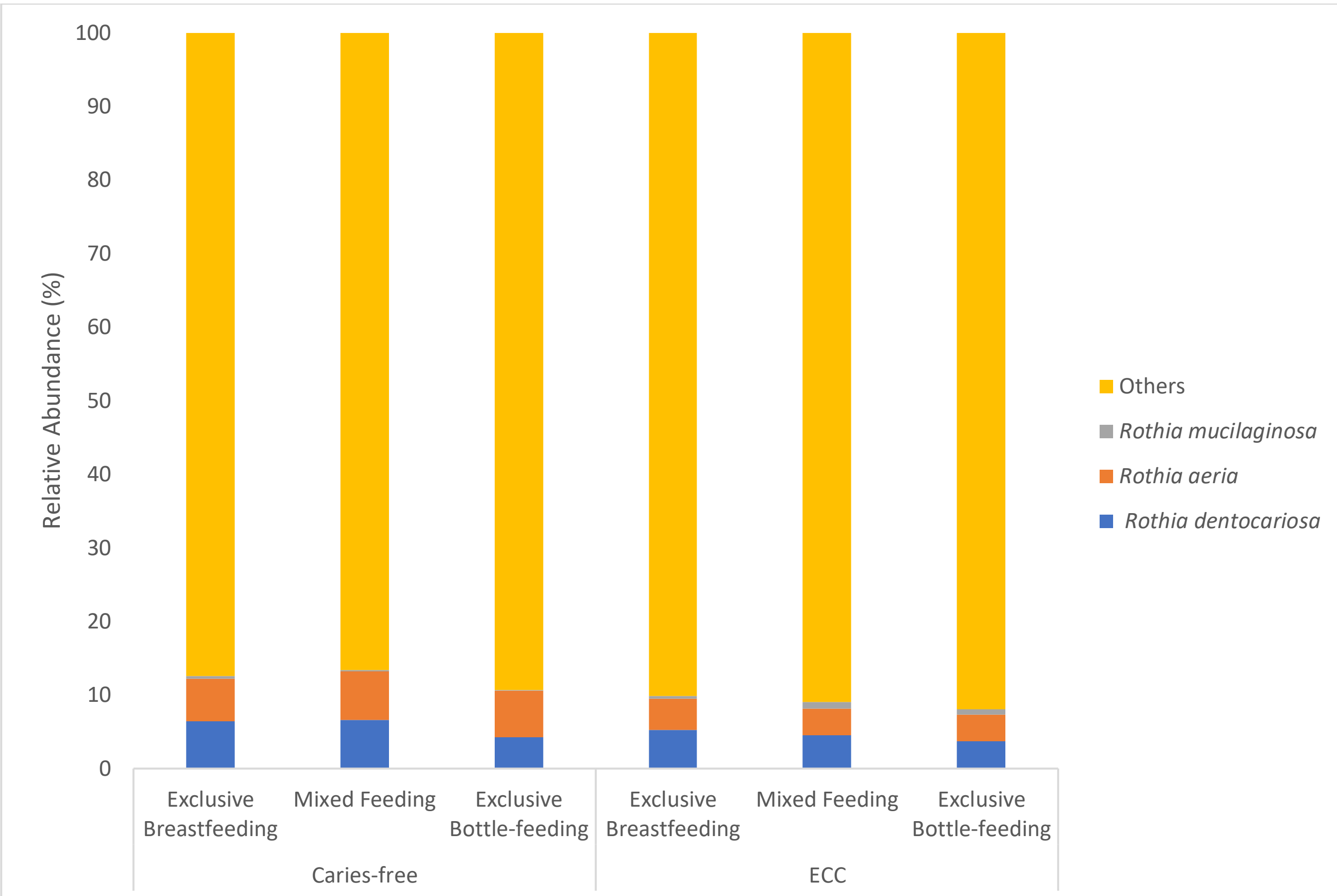

**Table 2: Demographic and behavioural characteristics and *Rothia* relative abundances of study participants**

| **Characteristic** | **Caries-free**, N = 178[1] | **ECC**, N = 260[1] | **Overall**, N = 438[1] | **p-value**[2] |
|---|---|---|---|---|
| Age, mo | 41 (16) | 48 (14) | 45 (15) | <0.001 |
| Sex | | | | 0.7 |
| Male | 87 (49%) | 132 (51%) | 219 (50%) | |
| Female | 91 (51%) | 128 (49%) | 219 (50%) | |
| Place of residence | | | | <0.001 |
| Urban | 171 (96%) | 139 (53%) | 310 (71%) | |
| Rural/remote | 7 (3.9%) | 121 (47%) | 128 (29%) | |
| Feeding practices | | | | <0.001 |
| Exclusive breastfeeding | 102 (57%) | 120 (46%) | 222 (51%) | |
| Mixed | 43 (24%) | 47 (18%) | 90 (21%) | |
| Exclusive bottle-feeding | 33 (19%) | 93 (36%) | 126 (29%) | |
| Was put to bed with a bottle | 47 (26%) | 140 (54%) | 187 (43%) | <0.001 |
| Snacks were given before bedtime | 64 (36%) | 142 (55%) | 206 (47%) | <0.001 |
| Fluoridated toothpaste used | 119 (67%) | 213 (82%) | 332 (76%) | <0.001 |
| Toothbrushing frequency | | | | 0.2 |
| Less than 2 times/day | 77 (43%) | 128 (49%) | 205 (47%) | |
| At least 2 times/day | 101 (57%) | 132 (51%) | 233 (53%) | |
| Parental/caregiver education | | | | <0.001 |
| Less than high school | 9 (5.1%) | 51 (20%) | 60 (14%) | |
| Completed high school | 34 (19%) | 103 (40%) | 137 (31%) | |
| More than high school | 135 (76%) | 106 (41%) | 241 (55%) | |
| Receives government/social assistance | 53 (30%) | 130 (50%) | 183 (42%) | <0.001 |
| *Rothia (genus)* | | | | 0.002 |
| High | 106 (60%) | 115 (44%) | 221 (50%) | |
| Low | 72 (40%) | 145 (56%) | 217 (50%) | |
| *R. dentocariosa* | | | | 0.3 |
| High | 96 (54%) | 126 (48%) | 222 (51%) | |
| Low | 82 (46%) | 134 (52%) | 216 (49%) | |
| *R. aeria* | | | | <0.001 |
| High | 107 (60%) | 112 (43%) | 219 (50%) | |
| Low | 71 (40%) | 148 (57%) | 219 (50%) | |
| *R. mucilaginosa* | | | | 0.3 |
| High | 84 (47%) | 137 (53%) | 221 (50%) | |
| Low | 94 (53%) | 123 (47%) | 217 (50%) | |

[1]Mean (SD); n (%)
[2]Wilcoxon rank sum test; Pearson's Chi-squared test

**Table 3: Ages that study participants stopped breastfeeding and bottle-feeding**

| Caries status | 25% Percentile[1] | 50% Percentile[1] | 75% Percentile[1] |
|---|---|---|---|
| **Breastfeeding** | | | |
| Caries-free | 6 (6, 8) | 12 (12, 15) | 18 (18, 24) |
| ECC | 3 (3, 6) | 12 (9, 18) | 24 (24, 24) |
| **Bottle-feeding** | | | |
| **Caries status** | **25% Percentile[1]** | **50% Percentile[1]** | **75% Percentile[1]** |
| Caries-free | 12 (12, 15) | 24 (18, 24) | 30 (24, —[2]) |
| ECC | 12 (12, 14) | 24 (23, 24) | 36 (32, 36) |

[1] Age in months (95% CI); [2]Censored because age cannot be estimated

## Table 4: Logistic regression for ECC Prediction

| | Full Model | | | Reduced Model | | |
|---|---|---|---|---|---|---|
| **Characteristic** | **OR**[1] | **95% CI**[1] | **p-value** | **OR**[1] | **95% CI**[1] | **p-value** |
| **Taxon: *Rothia* (genus)** | | | | | | |
| *Rothia* (genus) | | | 0.068 | | | 0.054 |
| High | — | — | | — | — | |
| Low | 1.60 | 0.97, 2.68 | | 1.63 | 0.99, 2.71 | |
| Feeding practices | | | 0.2 | | | |
| Exclusive breastfeeding | — | — | | | | |
| Mixed | 0.56 | 0.29, 1.08 | | | | |
| Exclusive bottle-feeding | 0.63 | 0.31, 1.26 | | | | |
| Snacks were given before bedtime | | | 0.014 | | | 0.020 |
| No | — | — | | — | — | |
| Yes | 1.91 | 1.14, 3.23 | | 1.81 | 1.10, 3.00 | |
| Was put to bed with a bottle | | | <0.001 | | | <0.001 |
| No | — | — | | — | — | |
| Yes | 3.51 | 1.98, 6.34 | | 3.12 | 1.85, 5.35 | |
| Fluoridated toothpaste used | | | 0.018 | | | 0.019 |
| No | — | — | | — | — | |
| Yes | 2.11 | 1.14, 4.01 | | 2.10 | 1.13, 3.97 | |
| Toothbrushing frequency | | | 0.9 | | | |
| Less than 2 times/day | — | — | | | | |
| At least 2 times/day | 1.05 | 0.62, 1.78 | | | | |
| Parental/caregiver education | | | 0.002 | | | <0.001 |
| Less than high school | — | — | | — | — | |
| Completed high school | 0.44 | 0.16, 1.14 | | 0.47 | 0.17, 1.19 | |
| More than high school | 0.22 | 0.08, 0.54 | | 0.21 | 0.08, 0.49 | |
| Receives government/social assistance | | | 0.4 | | | |
| No | — | — | | | | |
| Yes | 1.31 | 0.72, 2.40 | | | | |
| Age, mo | 1.04 | 1.03, 1.06 | <0.001 | 1.04 | 1.02, 1.06 | <0.001 |
| Place of residence | | | <0.001 | | | <0.001 |
| Urban | — | — | | — | — | |
| Rural/remote | 24.8 | 10.8, 65.6 | | 22.8 | 10.1, 59.6 | |
| Sex | | | 0.3 | | | 0.2 |
| Male | — | — | | — | — | |
| Female | 0.75 | 0.45, 1.24 | | 0.74 | 0.45, 1.23 | |
| **Taxon: *Rothia dentocariosa*** | | | | | | |
| *Rothia dentocariosa* | | | 0.3 | | | |
| High | — | — | | | | |
| Low | 1.30 | 0.78, 2.17 | | | | |
| Feeding practices | | | 0.2 | | | |
| Exclusive breastfeeding | — | — | | | | |
| Mixed | 0.56 | 0.29, 1.07 | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Exclusive bottle-feeding | 0.63 | 0.31, 1.26 | | | | |
| Snacks were given before bedtime | | | 0.017 | | | 0.018 |
| No | — | — | | — | — | |
| Yes | 1.87 | 1.12, 3.16 | | 1.82 | 1.11, 3.01 | |
| Was put to bed with bottle | | | <0.001 | | | <0.001 |
| No | — | — | | — | — | |
| Yes | 3.36 | 1.91, 6.05 | | 2.97 | 1.77, 5.05 | |
| Fluoridated toothpaste used | | | 0.014 | | | 0.011 |
| No | — | — | | — | — | |
| Yes | 2.16 | 1.17, 4.09 | | 2.21 | 1.20, 4.16 | |
| Toothbrushing frequency | | | >0.9 | | | |
| Less than 2 times/day | — | — | | | | |
| At least 2 times/day | 1.03 | 0.61, 1.75 | | | | |
| Parental/caregiver education | | | <0.001 | | | <0.001 |
| Less than high school | — | — | | — | — | |
| Completed high school | 0.41 | 0.15, 1.06 | | 0.43 | 0.16, 1.08 | |
| More than high school | 0.21 | 0.08, 0.50 | | 0.20 | 0.08, 0.46 | |
| Receives government/social assistance | | | 0.3 | | | |
| No | — | — | | | | |
| Yes | 1.35 | 0.74, 2.46 | | | | |
| Age, mo | 1.04 | 1.03, 1.06 | <0.001 | 1.04 | 1.02, 1.06 | <0.001 |
| Place of residence | | | <0.001 | | | <0.001 |
| Urban | — | — | | — | — | |
| Rural/remote | 25.5 | 11.2, 67.3 | | 23.5 | 10.5, 61.0 | |
| Sex | | | 0.3 | | | 0.3 |
| Male | — | — | | — | — | |
| Female | 0.77 | 0.46, 1.27 | | 0.76 | 0.46, 1.26 | |
| **Taxon: *Rothia aeria*** | | | | | | |
| *Rothia aeria* | | | 0.033 | | | 0.021 |
| High | — | — | | — | — | |
| Low | 1.73 | 1.05, 2.89 | | 1.80 | 1.09, 2.98 | |
| Feeding practices | | | 0.2 | | | |
| Exclusive breastfeeding | — | — | | | | |
| Mixed | 0.58 | 0.30, 1.11 | | | | |
| Exclusive bottle-feeding | 0.70 | 0.34, 1.39 | | | | |
| Snacks were given before bedtime | | | 0.017 | | | 0.025 |
| No | — | — | | — | — | |
| Yes | 1.87 | 1.12, 3.17 | | 1.78 | 1.08, 2.95 | |
| Was put to bed with bottle | | | <0.001 | | | <0.001 |
| No | — | — | | — | — | |
| Yes | 3.43 | 1.95, 6.18 | | 3.15 | 1.86, 5.41 | |
| Fluoridated toothpaste used | | | 0.014 | | | 0.014 |
| No | — | — | | — | — | |
| Yes | 2.18 | 1.17, 4.16 | | 2.17 | 1.17, 4.12 | |
| Toothbrushing frequency | | | 0.7 | | | |
| Less than 2 times/day | — | — | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| At least 2 times/day | 1.10 | 0.65, 1.88 | | | | |
| Parental/caregiver education | | | 0.002 | | | <0.001 |
| Less than high school | — | — | | — | — | |
| Completed high school | 0.44 | 0.16, 1.13 | | 0.47 | 0.17, 1.20 | |
| More than high school | 0.23 | 0.09, 0.55 | | 0.21 | 0.08, 0.50 | |
| Receives government/social assistance | | | 0.4 | | | |
| No | — | — | | | | |
| Yes | 1.31 | 0.72, 2.39 | | | | |
| Age, mo | 1.04 | 1.03, 1.06 | <0.001 | 1.04 | 1.02, 1.06 | <0.001 |
| Place of residence | | | <0.001 | | | <0.001 |
| Urban | — | — | | — | — | |
| Rural/remote | 24.8 | 10.9, 65.9 | | 22.8 | 10.1, 59.5 | |
| Sex | | | 0.3 | | | 0.3 |
| Male | — | — | | — | — | |
| Female | 0.75 | 0.45, 1.25 | | 0.75 | 0.45, 1.24 | |
| **Taxon: *Rothia mucilaginosa*** | | | | | | |
| *Rothia mucilaginosa* | | | 0.7 | | | |
| High | — | — | | | | |
| Low | 0.92 | 0.55, 1.52 | | | | |
| Feeding practices | | | 0.2 | | | |
| Exclusive breastfeeding | — | — | | | | |
| Mixed | 0.56 | 0.29, 1.07 | | | | |
| Exclusive bottle-feeding | 0.66 | 0.33, 1.32 | | | | |
| Was put to bed with bottle | | | <0.001 | | | <0.001 |
| No | — | — | | — | — | |
| Yes | 3.27 | 1.86, 5.86 | | 2.97 | 1.77, 5.05 | |
| Snacks were given before bedtime | | | 0.013 | | | 0.018 |
| No | — | — | | — | — | |
| Yes | 1.91 | 1.14, 3.23 | | 1.82 | 1.11, 3.01 | |
| Fluoridated toothpaste used | | | 0.011 | | | 0.011 |
| No | — | — | | — | — | |
| Yes | 2.22 | 1.20, 4.19 | | 2.21 | 1.20, 4.16 | |
| Toothbrushing frequency | | | 0.9 | | | |
| Less than 2 times/day | — | — | | | | |
| At least 2 times/day | 1.05 | 0.62, 1.77 | | | | |
| Parental/caregiver education | | | 0.002 | | | <0.001 |
| Less than high school | — | — | | — | — | |
| Completed high school | 0.40 | 0.15, 1.04 | | 0.43 | 0.16, 1.08 | |
| More than high school | 0.22 | 0.08, 0.52 | | 0.20 | 0.08, 0.46 | |
| Receives government/social assistance | | | 0.3 | | | |
| No | — | — | | | | |
| Yes | 1.39 | 0.77, 2.52 | | | | |
| Age, mo | 1.04 | 1.03, 1.06 | <0.001 | 1.04 | 1.02, 1.06 | <0.001 |
| Place of residence | | | <0.001 | | | <0.001 |
| Urban | — | — | | — | — | |
| Rural/remote | 25.4 | 11.1, 67.4 | | 23.5 | 10.5, 61.0 | |
| Sex | | | 0.3 | | | 0.3 |

| | | | | |
|---|---|---|---|---|
| Male | — | — | — | — |
| Female | 0.77 | 0.46, 1.27 | 0.76 | 0.46, 1.26 |

[1]OR = Odds Ratio, CI = Confidence Interval

**Figure 3: The odds ratios and 95% confidence intervals of predictors for ECC (from reduced models)**