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Microbiome Associated with Severe Caries in Canadian First Nations Children

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Abstract

Young Indigenous children in North America suffer from a higher degree of severe early childhood caries (S-ECC) than the general population, leading to speculation that the etiology and characteristics of the disease may be distinct in this population. To address this knowledge gap, we conducted the first microbiome analysis of an Indigenous population using modern molecular techniques. We investigated the caries-associated microbiome among Canadian First Nations children with S-ECC. Thirty First Nations children <72 mo of age with S-ECC and 20 caries-free children were recruited in Winnipeg, Canada. Parents or caregivers completed a questionnaire on general and dental health, diet, and demographics. The plaque microbiome was investigated by sequencing the *16S rRNA* gene. Sequences were clustered into operational taxonomic units and taxonomy assigned via the Human Oral Microbiome Database, then analyzed at the community level with alpha and beta diversity measures. Compared with those who were caries free, children with S-ECC came from households with lower income; they were more likely to live in First Nations communities and were more likely to be bottle-fed; and they were weaned from the bottle at a later age. The microbial communities of the S-ECC and caries-free groups did not differ in terms of species richness or phylogenetic diversity. Beta diversity analysis showed that the samples significantly clustered into groups based on caries status. Twenty-eight species-level operational taxonomic units were significantly different between the groups, including *Veillonella* HOT 780 and *Porphyromonas* HOT 284, which were 4.6- and 9-fold higher, respectively, in the S-ECC group, and *Streptococcus gordonii* and *Streptococcus sanguinis*, which were 5- and 2-fold higher, respectively, in the caries-free group. Extremely high levels of *Streptococcus mutans* were detected in the S-ECC group. Overall, First Nations children with S-ECC have a significantly different plaque microbiome than their caries-free counterparts, with the S-ECC group containing higher levels of known cariogenic organisms.

Keywords: oral health, dental health survey, preschool child, healthcare disparities, Indigenous population, *Streptococcus mutans*

Introduction

Early childhood caries (ECC), defined as decay involving the primary dentition in children <72 mo of age, is the most common chronic disease of childhood (American Academy of Pediatrics 2016). ECC is a critical public health concern due to its high prevalence, high treatment costs, negative effect on quality of life, and potential long-term complications (Schroth et al. 2009; Martins-Júnior et al. 2013; Schroth et al. 2016). Severe ECC (S-ECC) is an aggressive form of decay that is overrepresented among Indigenous children in North America, including Canadian First Nations, Métis and Inuit, and American Indian and Alaska Natives, and it reflects an underlying extreme oral health disparity in these populations (American Academy of Pediatrics and Canadian Paediatric Society 2011; Irvine et al. 2011). In some Canadian First Nations on-reserve communities, the prevalence of decay in the primary dentition can exceed 90% (Schroth et al. 2005). S-ECC is a major cause of hospital visits for young children (Sheller et al. 1997), and it frequently requires rehabilitative dental surgery under general anesthesia due to the extent of decay and the young age of the children affected (Schroth and Smith 2007; American Academy of Pediatrics 2016). Alarmingly, children living in communities with a high proportion of Aboriginal residents have pediatric dental surgery rates nearly 8 times higher than those living in communities with a low Aboriginal population among children 1 to 5 y old (Canadian Institute for Health Information 2013; Schroth et al. 2016)

In addition to the well-known microbial and host-related causal factors of caries, the etiology of ECC includes many

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additional factors, such as socioeconomic status, nutrition, and education (Reisine and Douglass 1998; Fisher-Owens et al. 2013). The early presentation and rapid progression in young Canadian First Nations, Métis, American Indian and Alaska Native children suggests that ECC in these populations may have distinct attributes and etiology (Schroth et al. 2009; QUEST 2015), which warrants further study.

In the current study, we utilized next-generation sequencing to analyze the plaque microbiome from Canadian First Nations and Métis children, with and without S-ECC, to investigate the role of the oral microbiome and to identify microbial characteristics that may account for the aggressive presentation. Defining the etiologic microbiota for S-ECC in these populations will potentially facilitate improvements in care and caries prevention policies, important steps for reducing the extent of S-ECC and improving overall quality of life.

Materials and Methods

Study Population and Design

The study protocol was approved by the University of Manitoba's Health Research Ethics Board and reviewed by the Assembly of Manitoba Chiefs' Health Information Research Governance Committee. Children who were <72 mo of age and identified by their parent or legal caregiver as being Canadian First Nations or Métis were included in the study. Thirty children with S-ECC had severe tooth decay involving multiple primary teeth and were recruited from the Misericordia Health Centre in Winnipeg, Canada, on the day of their scheduled dental rehabilitative surgery under general anesthesia. Twenty caries-free children were recruited from the community and assessed to ensure that there was no evidence of caries (dmft = 0, no cavitations or white spot lesions). Dental examinations and caries assessment were performed by R.J.S. Children who had taken antibiotics within the last 3 mo were excluded. All parents or caregivers of participating children provided written informed consent.

Health-Related Questionnaire

All parents and caregivers completed an interviewed questionnaire proctored by members of the study team. Information was collected on nutritional habits, oral hygiene habits, socioeconomic and demographic characteristics, and history of previous dental visits.

Sample Collection and Sequencing Analysis

Plaque samples were collected from each subject by swabbing a sterile interdental brush on all available tooth surfaces, and samples were immediately frozen at −80 °C in 15% glycerol until used for analysis. Extracted DNA was sent to the Forsyth Institute for library preparation and Illumina sequencing of the amplified V3-V4 16S region. Sequencing data were analyzed with QIIME 1.9.1 (Quantitative Insights into Microbial Ecology; Caporaso et al. 2010). Detailed DNA extraction, sequencing, and analysis methods are supplied in the Appendix.

Statistical Analysis

Questionnaire and microbiological data were linked in an Excel spreadsheet (Microsoft Office) and analyzed with Number Cruncher Statistical Software 10 and GraphPad Prism 7. Bivariate analyses, such as chi-square, Fisher's exact, and *t* tests (Aspin-Welch *t* test for unequal variance), were performed where appropriate. For sequencing data, differences in the relative abundances of taxa between the groups were determined with the Kruskal-Wallis test, controlling the false discovery rate to correct for multiple comparisons (Hochberg and Benjamini 1990). A corrected P value ≤0.05 was considered significant. Differences in weighted and unweighted Unifrac distances between the groups were analyzed with analysis of similarity. A P value ≤0.05 was considered statistically significant.

Results

Demographics and Health-Related Questionnaire Data

A total of 50 children were recruited: 30 with S-ECC and 20 caries free. The mean age of all children was 40.7 ± 11.6 mo. Results from the health-related questionnaire are presented in Table 1. A considerable proportion (56.7%) of children with S-ECC resided in First Nations communities, while all of the caries-free children lived in the Winnipeg region. We found a significant difference in household income ($P = 0.032$) between the groups, with S-ECC children coming from households with lower incomes.

There were significant differences in the proportion of children with S-ECC who were bottle-fed in comparison with caries-free children ($P = 0.021$). Children with S-ECC were also bottle-fed for a significantly longer duration ($P = 0.028$), and the age in which the child was weaned from the breast was significantly lower among S-ECC children (3.3 ± 5.4 mo vs. 12.9 ± 11.4; $P = 0.015$). Children with S-ECC were also less likely to be exclusively breastfed at any point in their infancy ($P = 0.0015$).

Plaque Microbial Community

Plaque samples were obtained from 20 caries-free subjects and 30 subjects with S-ECC. Sequencing generated a total of 3,502,879 sequences after quality filtering, with an average of 66,855 (range, 34,190 to 89,179) sequences per sample and a median length of 421.

Alpha (within-sample) diversity was calculated at a maximum depth of 30,000 sequences per sample, with the rarefaction curves shown in Figure 1A. On average, the samples from caries-free and S-ECC subjects did not differ in terms of species richness or phylogenetic diversity.

Principal coordinates analysis was used on weighted and unweighted Unifrac distances to examine clustering of samples between the S-ECC and caries-free groups (beta diversity;

Table 1. Demographics and Health Characteristics of Study Population.

Variable	Caries Status		*P* Value
	Caries Free	S-ECC	
Age, mo[a]	37.4 ± 10.3	42.8 ± 12.2	0.11
Sex[b]			
Male	9 (32.1)	19 (67.9)	0.20
Female	11 (50.0)	11 (50.0)	
Weight at birth, g[a]	3,529.9 ± 699.0	3,421.3 ± 573.2	0.57
Premature[b]			
Yes	2 (16.7)	10 (83.3)	0.073[c]
No	18 (48.7)	19 (51.4)	
Feeding habits[b]			
Child was breastfed			
Yes	13 (52.0)	12 (48.0)	0.083
No	7 (28.0)	18 (72.0)	
Child was exclusively breastfed			
Yes	12 (70.6)	5 (29.4)	**0.0015**
No	8 (24.2)	25 (75.8)	
Child was bottle-fed			
Yes	16 (34.8)	30 (65.2)	**0.021**[c]
No	4 (100.0)	0 (0.0)	
Age the child was weaned			
From the breast[a]	12.9 ± 11.4	3.3 ± 5.4	**0.014**
From the bottle[a]	17.9 ± 8.9	25.8 ± 12.0	**0.028**
Times per day the child snacks[a]	3.7 ± 1.7	3.9 ± 1.4	0.71
Oral hygiene habits[b]			
Child brushes ≥ daily	17 (51.2)	16 (48.5)	**0.032**[c]
Child brushes < daily	3 (17.7)	14 (82.4)	
Yearly household income, $[b]			
≥28,000	7 (70.0)	3 (30.0)	**0.032**[c]
<28,000	12 (32.4)	25 (67.6)	
Family size[b]			
Other children	2 (50.0)	2 (50.0)	1.00[c]
Only child	18 (39.1)	28 (60.9)	
Receives social assistance[b]			
Yes	13 (37.1)	22 (62.9)	0.41
No	7 (50.0)	7 (50.0)	
Lives in a First Nations community[b]			
Yes	0 (0.0)	17 (100.0)	**0.000010**[c]
No	20 (60.6)	13 (39.4)	
Age of child at first dental visit, mo[a]	20.8 ± 16.0	27.8 ± 14.6	0.11

Values are presented as mean ± SD or *n* (%). Bold value indicates $P \leq .05$.
S-ECC, severe early childhood caries.
[a]*T* test.
[b]Chi-square analysis.
[c]Fisher's exact test.

Fig. 1B). Weighted Unifrac distances take into account abundance of each taxon, while unweighted distances are based only on presence/absence data (Lozupone and Knight 2005). The samples significantly cluster according to caries status (caries free vs. S-ECC) for both weighted and unweighted distance measures ($P < 0.05$, analysis of similarity).

Taxonomic Identification and Relative Abundance

Taxonomy assignment revealed 10 phyla, 4 of which were differentially represented in the caries-free versus S-ECC groups: Firmicutes (39.4% vs. 47.2%, $P = 0.01$), Actinobacteria (14.4% vs. 6.8%, $P = 0.002$), Fusobacteria (16.8% vs. 11.3%, $P = 0.008$), and TM7 (0.5% vs. 0.24%, $P = 0.008$). A total of 95 genera and 290 species were detected, and those with the highest relative abundances are listed in Table 2. Twenty-eight species-level operational taxonomic units were significantly different ($P < 0.05$) in the S-ECC versus caries-free groups. Most of these species have been associated with either health or caries; for example, the caries-free group had 5- and 2-fold higher abundances of *Streptococcus gordonii* and *Streptococcus sanguinis*, respectively, than the S-ECC group, while the S-ECC group had 7- and 9-fold higher levels of an *Haemophilus* species (HOT 036) and a *Porphyromonas* species (HOT 284), respectively. In addition, a *Veillonella* species (HOT 780) was 4.6-fold higher in the S-ECC group, although the relative abundances were low.

Streptococcus mutans was detected in all samples, with a 3-fold higher amount detected in the S-ECC group as compared with the caries-free group. However, the S-ECC group contained

subjects with extremely high values. Six subjects in the S-ECC group were carrying >5% *S. mutans*, 2 subjects with >10%, and 1 subject with an extraordinarily high level of almost 23% of the total species detected. For comparison, in the caries-free group, there was only 1 subject with >5% *S. mutans* (Fig. 2). *Scardovia wiggsiae* has been recently characterized as a possible important factor in ECC (Tanner, Mathney, et al. 2011); in the current study, S-ECC children had on average 7-fold-higher levels of this organism than the caries-free children, although the relative abundances were low (0.007% vs. 0.001%).

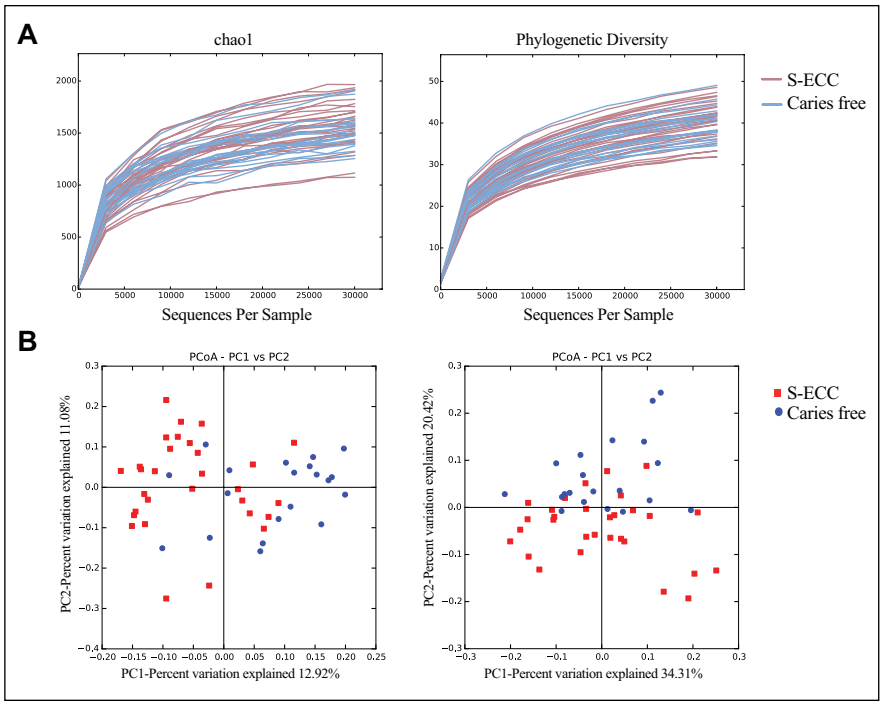

Figure 1. Diversity analyses. (**A**) Rarefaction curves of alpha diversity indices. Left: chao1 (species richness); right, Faith's phylogenetic diversity index. (**B**) Beta diversity shown by principal coordinates analysis (PCoA) of unweighted Unifrac distances (left) and weighted Unifrac distances (right). The plaque microbial communities significantly clustered by caries status ($P < 0.05$, analysis of similarity). S-ECC, severe early childhood caries.

S-ECC Subgroup Analysis

Since the majority of children with S-ECC (17 of 30) resided in First Nations communities, we further investigated the microbiome of this group according to place of residency (First Nations community vs. non–First Nations urban community). Beta diversity analysis based on weighted Unifrac distances revealed that the subgroups were significantly different (Fig. 3A). The average species richness (chao1) of the samples in each subgroup was not different (data not shown). At the species level, *Fusobacterium nucleatum* subsp. *vincentii* was significantly higher in the children who did not reside in a First Nations community versus those from First Nations communities (0.5% vs. 1.7%, $P < 0.05$). While the average relative abundance of *S. mutans* was higher in the subjects from non–First Nations communities, this was not statistically significant (4.9% vs. 1.4%, $P = 0.3$). At the phylum level, Fusobacteria was also significantly higher in the children with S-ECC who did not reside in a First Nations community (14.9% vs. 8.5%, $P < 0.05$). The top 100 species-level taxa identified in each subgroup are shown in Figure 3B.

Additionally, we investigated if species richness correlated with caries severity based on dmft (mean 10.8 ± 3.3) and dmfs (45.3 ± 19.1) scores among those with S-ECC, and we found no correlation in either instance (Pearson's $r = 0.20$ and $r = 0.23$, respectively).

Discussion

To the best of our knowledge, this study is the first to use advanced microbial analyses to investigate the oral microbiome of North American Indigenous children, specifically, Canadian First Nations and Métis children, affected by S-ECC. Despite recent advances in understanding the role of the oral microbiome in health and disease, knowledge of its importance in the etiology of ECC is lacking, especially in Indigenous populations. The literature clearly reveals that Indigenous children suffer considerable oral health disparities when compared with other children of the same age. Much of this discrepancy stems from the historical and ongoing effects of colonialism and racism that have resulted in major socioeconomic and health care inequities (American Academy of Pediatrics and Canadian Paediatric Society 2011). With rates of S-ECC in these populations drastically higher than rates in the general population and with the potential for S-ECC to negatively affect systemic health and quality of life, it is critical to investigate the underlying causes and identify any potential unique risk factors that may exist (Schroth et al. 2009; Schroth et al. 2016).

The results of the health questionnaire confirm previously reported behavioral and socioeconomic risk factors, including less frequent brushing, bottle-feeding, later age at weaning, and lower household income (Reisine and Douglass 1998; American Academy of Pediatrics and Canadian Paediatric Society 2011). It has become apparent that S-ECC is a complex, multifactorial disease and that there is a major microbiological component (Irvine et al. 2011; QUEST 2015).

We analyzed the plaque microbiome in each group by sequencing a region of the *16S rRNA* gene. The results of the beta diversity analysis revealed a statistically significant separation between the microbiomes of children with S-ECC and those caries free, indicated in Figure 1 by clustering of the samples into their groups. Overall, this result signifies that plaque microbial communities from the S-ECC subjects were more similar to others within the S-ECC group than they were

Table 2. Relative Abundance of the Top 25 Species- and Genus-Level OTUs Detected in Plaque of Caries-Free Children and Children with S-ECC.

	Median Relative Abundance, % (Range)	
OTU	Caries Free (*n* = 20)	S-ECC (*n* = 30)
Species level		
Streptococcus HOT 058	23.7 (12.3 to 42.0)	26.7 (11.1 to 42.3)
Leptotrichia shahii	3.4 (0 to 16.7)	2.1 (0.2 to 11.7)
Lautropia mirabilis	3.2 (0.2 to 11.6)	2.2 (0.05 to 10.0)
Haemophilus parainfluenzae	2.0 (0.01 to 7.2)	3.1 (0.12 to 12.6)
Veillonella dispar	2.2 (0.15 to 9.4)	3.0 (0.33 to 19.0)
Rothia aeria[a]	2.4 (0.37 to 7.6)	0.52 (0.004 to 1.7)
Corynebacterium matruchotii[a]	2.0 (0.66 to 5.4)	0.85 (0.13 to 5.4)
Actinomyces naeslundii[a]	1.8 (0.68 to 6.2)	0.67 (0.15 to 5.7)
Rothia dentocariosa	1.7 (0.12 to 24.5)	0.70 (0.16 to 5.5)
Abiotrophia defectiva	1.1 (0.07 to 6.3)	1.3 (0.003 to 5.7)
Gemella haemolysans	0.87 (0.09 to 3.8)	1.2 (0.15 to 5.3)
Granulicatella adiacens	0.82 (0.14 to 2.3)	1.1 (0.02 to 2.4)
Porphyromonas HOT 279	0.63 (0.03 to 3.5)	1.1 (0.02 to 8.4)
Granulicatella elegans[a]	0.32 (0.03 to 1.2)	1.0 (0.003 to 4.0)
Leptotrichia HOT 225	1.0 (0.03 to 4.5)	0.45 (0.008 to 5.9)
Fusobacterium nucleatum ss. vincentii	0.54 (0.04 to 3.8)	0.88 (0.09 to 4.1)
Corynebacterium durum	0.80 (0.14 to 9.4)	0.41 (0 to 4.3)
Streptococcus mutans	0.15 (0.006 to 10.4)	0.73 (0.02 to 22.9)
Prevotella melaninogenica[a]	0.10 (0.002 to 3.6)	0.71 (0.03 to 11.8)
Alloprevotella HOT 473[a]	0.04 (0 to 1.7)	0.69 (0.001 to 9.3)
Gemella morbillorum	0.58 (0.11 to 3.3)	0.69 (0.07 to 2.0)
Haemophilus HOT 036[a]	0.07 (0.003 to 0.3)	0.56 (0.001 to 3.5)
Streptococcus sanguinis[a]	0.56 (0.19 to 0.8)	0.28 (0.13 to 0.7)
Neisseria mucosa	0.44 (0.09 to 1.2)	0.34 (0.02 to 1.2)
Aggregatibacter HOT 458	0.25 (0.003 to 2.3)	0.43 (0.05 to 2.8)
Genera level		
Streptococcus	28.3 (16.8 to 49.6)	31.3 (12.8 to 50.0)
Leptotrichia[a]	10.5 (4.2 to 23.7)	5.7 (0.61 to 30.4)
Neisseria	7.5 (0.70 to 27.9)	9.0 (0.22 to 26.4)
Rothia[a]	4.8 (0.72 to 29.9)	1.7 (0.04 to 10.3)
Fusobacterium	4.8 (0.65 to 12.3)	3.7 (1.1 to 9.5)
Haemophilus	2.1 (0.01 to 7.5)	4.6 (0.13 to 12.8)
Veillonella	2.4 (0.18 to 10.1)	4.1 (0.39 to 19.8)
Corynebacterium[a]	3.3 (1.4 to 14.8)	1.6 (0.01 to 8.1)
Actinomyces[a]	3.2 (1.4 to 9.4)	1.8 (0.25 to 7.4)
Lautropia	3.2 (0.19 to 11.6)	2.2 (0.05 to 10.2)
Prevotella	0.93 (0.17 to 9.1)	2.5 (0.20 to 26.5)
Granulicatella	1.3 (0.22 to 2.6)	2.3 (0.06 to 5.7)
Gemella	1.6 (0.20 to 4.9)	2.0 (0.29 to 7.0)
Porphyromonas	1.3 (0.08 to 5.1)	1.8 (0.023 to 9.2)
Capnocytophaga	1.5 (0.48 to 5.1)	0.94 (0.19 to 2.7)
Abiotrophia	1.1 (0.07 to 6.3)	1.3 (0.003 to 5.7)
Kingella	1.2 (0.61 to 2.5)	0.80 (0.065 to 2.1)
Alloprevotella[a]	0.13 (0.003 to 1.7)	1.0 (0.006 to 9.5)
Aggregatibacter	0.88 (0.01 to 3.3)	0.99 (0.14 to 4.1)
Campylobacter	0.59 (0.14 to 2.5)	0.46 (0.08 to 3.9)
Selenomonas	0.29 (0.02 to 3.7)	0.54 (0.02 to 4.2)
Cardiobacterium[a]	0.45 (0.04 to 2.2)	0.17 (0.002 to 0.7)
Lachnoanaerobaculum	0.43 (0.16 to 2.6)	0.32 (0.03 to 1.6)
TM7 [G-1][a]	0.33 (0.004 to 1.4)	0.11 (0.005 to 1.8)
Bergeyella	0.33 (0.03 to 0.8)	0.27 (0.05 to 1.2)

HOT, Human Oral Taxon; OTU, operational taxonomic unit; S-ECC, severe early childhood caries.
[a]$P \leq 0.05$, Kruskal-Wallis test, corrected for multiple comparisons by the false discovery rate method.

to the communities from caries-free subjects. This finding demonstrates that the composition of the entire microbial community is a determining factor in S-ECC for this population.

Alpha diversity describes the number of different types of sequences within a sample. In the current study, we calculated species richness and phylogenetic diversity of each sample, and on average there was no difference between the S-ECC and caries-free groups (Fig. 1). Some studies have shown that increased alpha diversity is associated with health (Gross et al. 2012; Belstrøm et al. 2014; Xiao et al. 2016); however, other

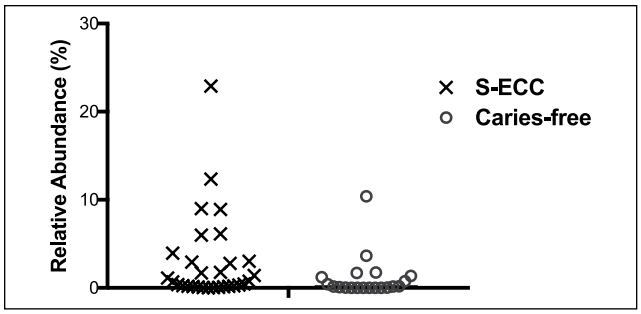

Figure 2. Relative abundance of *Streptococcus mutans* in all subjects. Percentage relative abundance of *S. mutans* is plotted for each subject. S-ECC, severe early childhood caries.

studies report the opposite (Griffen et al. 2012; Xu et al. 2014; Johansson et al. 2016). For example, in a study of young children, Xu et al. (2014) found no significant difference in the species diversity of those with caries and those without. Interestingly, Johansson et al. (2016) showed that in a population of Swedish adolescents with and without caries, the groups did not differ in terms of alpha diversity, but when compared with a caries-active population of Romanian adolescents, the Swedish subjects had much lower alpha diversity. This finding suggests that the extent to which species richness correlates to caries status is not the same in all populations, with the environment potentially playing a major role.

Four phyla were significantly differentially represented in each group. The S-ECC group had a higher abundance of Firmicutes, while Actinobacteria and Fusobacteria were higher in the caries-free group. This result supports a recent longitudinal study in young children, in which Actinobacteria decreased and Firmicutes increased as caries progression proceeded (Gross et al. 2012) and a study that reported a significantly higher relative abundance of Firmicutes in children with S-ECC versus caries-free controls (Jiang et al. 2013). Regarding the most abundant taxa, 7 of the top 25 genera detected were significantly different between the groups. *Alloprevotella* was significantly increased in the S-ECC group; this genus was also reported to be increased in a study of adult subjects with caries (Xiao et al. 2016). The genera *Leptrotrichia, Rothia, Corynebacterium, Actinomyces, Cardiobacterium*, and TM7 [G-1] were significantly higher in the caries-free group. These genera have been frequently identified in plaque and associated with health (Tanner, Kent, et al. 2011; Xu et al. 2014; Johansson et al. 2016; Xiao et al. 2016).

Of the top 25 most abundant species detected, *Granulicatella elegans, Prevotella melaninogenica*, and a *Haemophilus* species (HOT 036) were significantly more abundant in the S-ECC group. *G. elegans* and *Prevotella melaninogenica* have been reported to be increased in children with S-ECC when compared with those caries free (Kanasi et al. 2010; Ling et al. 2010; Tanner, Kent, et al. 2011). Conversely, we found that *Rothia aeria, Corynebacterium matruchotii, Actinomyces naeslundii*, and *Streptococcus sanguinis* were significantly increased in the caries-free group. Both *C. matruchotii* and *A. naeslundii* have been associated with health and caries-free status (Marchant et al. 2001; Gross et al. 2010; Tanner, Kent, et al.

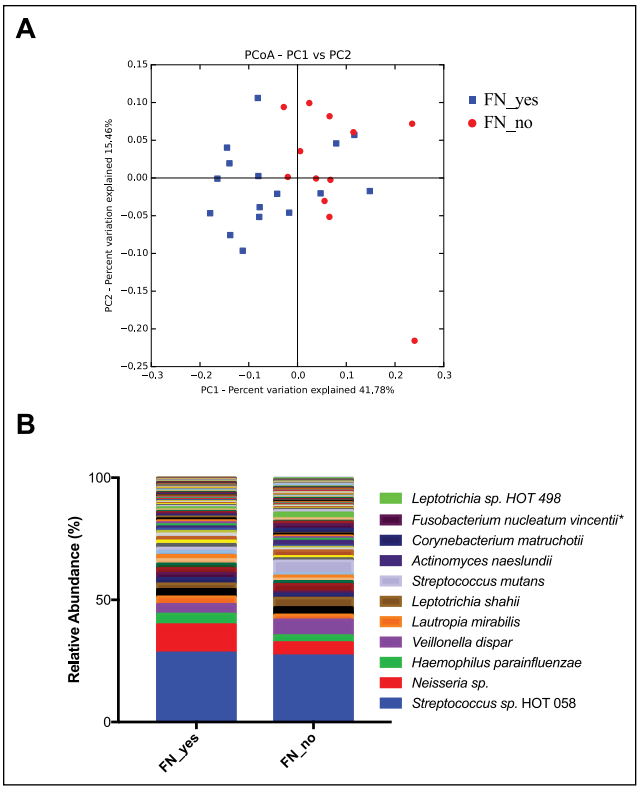

Figure 3. S-ECC subgroup analysis. Subjects from the S-ECC group were further divided per their residency in a First Nations community. (**A**) Beta diversity shown by principal coordinates analysis (PCoA) of weighted Unifrac distances. The plaque microbial communities significantly clustered by place of residency ($P < 0.05$, analysis of similarity). Blue, resides in First Nations community; red, does not reside in a First Nations community. (**B**) Average of the top 100 most abundant species identified in each group. FN_no = does not reside in a First Nations community ($n = 13$); FN_yes = resides in a First Nations community ($n = 17$). *$P < 0.05$ (Kruskal-Wallis with false discovery rate correction). S-ECC, severe early childhood caries.

2011; Ma et al. 2015). *S. sanguinis* is a known health-related species that has been shown to have an inverse and antagonistic relationship with *S. mutans* (Caufield et al. 2000; Kreth et al. 2008). *Rothia* spp. are commonly detected in plaque (Aas et al. 2008; Bik et al. 2010; Kanasi et al. 2010; Ling et al. 2010), but *R. aeria* has not been previously well associated with health. Caries-free subjects in our study had almost 5 times the amount of *R. aeria* on average as compared with the S-ECC children. Interestingly, another *Rothia* species, *R. dentocariosa*, has been associated with S-ECC (Jiang et al. 2016), but in our study, the caries-free group had >2-fold-higher levels. This discrepancy may be one example of the uniqueness of this particular population, and it reinforces the need for further study of dental health in Indigenous children.

The relative abundance of *S. mutans*, the quintessential cariogenic organism, was 3 times higher in the S-ECC group than in the caries-free group, but the average value masks the extremely high levels of some children in the S-ECC group (Fig. 2) of up to 23% of the total taxa detected. Interestingly, a recent study comparing the microbiomes of European adolescents with and without caries from 2 countries showed that the

relative importance of *S. mutans* in determining caries status was different according to where the populations resided; the role of *S. mutans* as an important etiologic factor was more pronounced in the population lacking access to caries prevention and treatment strategies, as opposed to one in which there was adequate dental care (Johansson et al. 2016). This finding supports the idea that the cariogenic etiology of certain populations may be unique on the basis of socioeconomic or geographic factors, and the high levels of *S. mutans* in some subjects in our study may be a reflection of that.

Notably, 2 subjects in the caries-free group (of 20 total) had a high relative abundance (>2%) of *S. mutans*; these 2 subjects also had high levels (>2%) of *R. aeria* and *C. matruchotii*, 2 species significantly associated with caries-free status in this study. In the S-ECC group, 10 subjects (of 30 total) had high abundances of *S. mutans*, with none exhibiting high levels of *R. aeria* and *C. matruchotii.* This result suggests that certain health-related species may protect against the risk of carrying a high abundance of *S. mutans*, and it indicates that the balance and structure of the microbial community as a whole may be the most important factor in determining caries risk. The ecologic plaque hypothesis describes plaque as a dynamic microbial community in which pathogenic and protective species exist in a delicate balance, and the development of caries is the consequence of a shift in the population toward a virulent state, as opposed to the consequence of the virulence of a single pathogen (Takahashi and Nyvad 2008, 2011). Our results fit in with this ecologic perspective.

Interestingly, subgroup analysis of the S-ECC group based on residency in a First Nations community revealed that the plaque microbiomes of the 2 subgroups are overall significantly different, with the phylum Fusobacteria significantly higher in the children who did not live in a First Nations community (Fig. 3). Previous studies have found the genus *Fusobacterium* associated with healthy tooth surfaces (Jiang et al. 2013; Xu et al. 2014). This observation generates questions regarding environment as a risk factor, and it paves the way for further investigation.

This study is not without limitations. Due to budgetary constraints, we relied on a convenience sample of children with S-ECC on the day of their dental surgery. All controls were from Winnipeg, and those with S-ECC were from different First Nations communities or off-reserve communities, including Winnipeg. Some questions were retrospective, which might have resulted in recall bias, and the potential for response bias on the part of parents and caregivers is noted. The primary goal of this pilot study was to generate data on this understudied population to provide the foundation for future larger studies.

Overall, this study yielded important information on the microbiome of First Nations and Métis children with S-ECC and those free from caries. The only previous study to investigate the microbiology of Canadian First Nations children with S-ECC was a longitudinal observation in 1985 (Milnes and Bowden 1985). Therefore, this study is the first to investigate the microbiome of this population using modern molecular techniques. We confirmed previous reports that implicate behavioral as well as microbiological factors in the development of S-ECC, with *S. mutans* as the major cariogenic factor, along with many other species. Furthermore, we found that the S-ECC and caries-free groups represent disparate plaque microbial communities, supporting the notion that there is potentially a distinct caries-causing community that can eventually be identified and used for diagnosis and prognosis. It is clear that socioeconomics, cultural factors, and microbiology all play a role in the high rates of S-ECC experienced by Canadian Indigenous populations, but the finer details are still very much unknown. Therefore, it is imperative to continue to study the underlying causes (including the microbiome) of the extreme oral health disparities that these populations face to provide prevention and treatment services that accurately reflect the underlying etiology.

Author Contributions

M. Agnello, L. Cen, contributed to data analysis and interpretation, drafted and critically revised the manuscript; J. Marques, contributed to data acquisition, analysis, interpretation, drafted and critically revised the manuscript; B. Mittermuller, A. Huang, N. Chaichanasakul Tran, contributed to data acquisition, drafted and critically revised the manuscript; W. Shi, contributed to conception, design, data analysis, and interpretation, drafted and critically revised the manuscript; X. He, contributed to design, data analysis, and interpretation, drafted and critically revised the manuscript; R.J. Schroth, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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