PROJECT SUMMARY
Early Childhood Caries (ECC), defined as 1 or more decayed, missing, or filled teeth in a child less than 6 years of age, is the most prevalent chronic disease in children, occurring 5 times as frequently as asthma. When left untreated, dental caries is chronic and extremely painful, resulting in costly restorative services and persisting into adulthood. In 2012 alone, US emergency dental visits cost $1.6 billion. ECC is most commonly associated with infection by *S. mutans* and *S. sobrinus* bacteria, whose carbohydrate processing activity leads to excessive creation of acidic byproducts, lowered pH, and demineralization of tooth enamel. The impact of these infections is magnified by low socioeconomic status and poor access to dental care. Arizona has one of the highest ECC rates in the nation, almost double the national average (40% versus 23%). These problems persist beyond preschool, with the State's kindergarten through 3rd grade children averaging 5 affected teeth – 3 times higher than the national average. Socioeconomic factors explain only a fraction of the overall incidence. The biological components of caries, specifically phylogenetic strain identity of circulating *S. mutans* and *S. sobrinus* strains, their virulence, and transmission characteristics are likely playing an important, yet unquantified role in the State's high ECC incidence rates. *The objective* of this study is to quantify the degree to which the genetics of *S. mutans* and *S. sobrinus* bacteria are driving the elevated incidence in ECC in southern Arizona preschoolers. *Our central hypothesis* is that *S. mutans* and *S. sobrinus* phylogenetic clade membership and strain virulence are a significant contributor to the overall ECC incidence and that classroom-based transmission plays an important role. This can help predict eventual caries outcomes in preschool children. *Our specific aims* are: 1) Quantify the impact *S. mutans* and *S. sobrinus* genomic diversity (phylogenetic clade membership), virulence, and bacterial load on caries progression in children of southern Arizona; and 2) Quantify the impact of classroom-based oral microbiome and *S. mutants*/*S. sobrinus* transmission on caries rates in children of southern Arizona. *This contribution is significant* because 1) it will almost double the number of *S. sobrinus* and *S. mutans* genomes available to the scientific community worldwide; 2) it will quantify the biological component of ECC, which can guide future clinical interventions by identifying individuals with an oral microbiome that is more susceptible to ECC or individuals with particularly pathogenic strains of *S. mutans* and *S. sobrinus*; and 3) it will help agencies already actively engaged in reducing ECC incidence more effectively target resources and intervention programs by understanding the phylogeography and transmission patterns of the disease. *The proposed research is innovative* because it combines existing oral health outreach efforts and advanced molecular characterization of ECC-causing strains, with advanced phylogenetic and phylogeographic analyses to help characterize this disease.

SPECIFIC AIMS

Defining microbiological drivers of early childhood caries in preschoolers in southern Arizona

Rationale: Dental caries is the most prevalent chronic disease in children, occurring 5 times as frequently as asthma, and Arizona has one of the highest rates of Early Childhood Caries (ECC) in the United States. By age 4, a staggering 52% of Arizona's children (as opposed to 23% nationwide) have one or more teeth with untreated decay. These problems persist throughout childhood, with the State's kindergarten through 3rd grade children averaging 5 affected teeth – 3 times higher than the national average. ECC is chronic in nature, and when left untreated, results in the need for costly intervention – nationwide, $1.6 billion was spent on emergency dental care in 2012 alone. In Arizona, socio-economic factors explain only part of the elevated ECC incidence rates (no more than 31% of the overall variation, from our analyses of clinical records), with biological components of ECC likely having a strong impact. Infection by bacteria from mutans streptococci group, most commonly *S. mutans* and *S. sobrinus* species, have been implicated as the cause of dental caries. When established in the oral cavity, these mutans streptococci metabolize carbohydrates and produce highly acidic byproducts that change the pH of the oral cavity, leading to demineralization of tooth enamel. *The impact of the amount of mutans streptococci in the oral cavity and the effect of differential strain virulence on caries outcome are unknown but likely to have a significant impact on the observed ECC incidence and transmission patterns*. We intend to address these gaps in our proposed work.

Specific Aim #1: *Quantify the impact S. mutans and S. sobrinus genomic diversity (phylogenetic clade membership), virulence, and bacterial load on caries progression and outcomes in children of southern Arizona*. Rationale: The impact (or lack thereof) of genomic differences in caries-causing strains on ECC incidence is, at best, unclear. We propose to address this gap by producing high quality genome drafts for *S. mutans* and *S. sobrinus* strains circulating in children of southern Arizona. By surveying the genomic diversity and function of mutans streptococci strains we can determine if phylogenetic differences in southern Arizona strains, when comparison to strains from elsewhere in U.S., can explain the elevated ECC rates. At the same time, we propose to evaluate the predictive potential of mutans streptococci genotype and bacterial load at first screen (beginning of academic year) on ECC outcomes (number and extent of impacted teeth) measured at the end of the academic year and beyond. Results may guide the implementation and need for preventive interventions. **Hypotheses:** (1) When compared to the rest of the U.S., there will be observable and statistically significant genomic differences in *S. mutants* and *S. sobrinus* strains circulating in pre-school age children of southern Arizona; and (2) *S. mutans* and *S. sobrinus* strain virulence and bacterial load at first collection event serve as a statistically significant predictors for the speed and extent of caries progression later in the academic year (6 months after first collection event) and beyond (1 year after first collection event). **Approach**: We will collect and analyze oral microbiota (saliva and plaque) samples from up to 300 pre-school children (1-5 years old), across 3 sampling events (6 months apart), focusing on *S. mutants* and *S. sobrinus* strain genotype. Collection will occur through an existing partnership with an early childhood oral health program (operated by Smart Smiles, First Things First, and the Arizona Department of Health Services), whose dental hygienists provide bi-annual oral healthcare services to preschool age children of southern Arizona.

Specific Aim #2: *Quantify the impact of classroom-based oral microbiome and S. mutants / S. sobrinus transmission on caries rates in children of southern Arizona.* Rationale: The initial transmission of *mutans streptococci* strains to an affected child has been strongly tied to parental (typically mother's) oral cavity microbiome. At the same time, close quarters interactions common in preschool settings, likely facilitate additional classroom-based, as opposed to familial, microbiome convergence and mutans streptococci transmission. We intend to quantify the change, if any, in child's oral microbiome and *S. mutans* and *S. sobrinus* genotype upon joining a new preschool setting. **Hypothesis:** Microbiome composition and mutans streptococci genotypes from the classroom will out-compete the familial microbiome and genotype once the child enters preschool. **Approach:** We will identify a subset of children who have recently started pre-school or joined a particular classroom, and collect oral microbiota samples from both the child and the primary guardians (as in Aim 1). Oral microbiomes of the children and their guardians will be sequenced using traditional 16S RNA methods, with ECC-causing bacterial strains genotyped as in Aim 1. Microbiome and mutans streptococci genotype change over time will be assess vial Bayesian and ANCOVA techniques.

Impacts / Outcomes: We will explore important contributors to dental caries in children: phylogeny, virulence, and geographic distribution of caries-causing bacteria. Identifying differentially virulent pathogen genotypes or differentially susceptible microbiome backgrounds can lead to identification of children in need of frequent intervention. Understanding the importance of familial- versus classroom-based *S. mutans* and *S. sobrinus* transmission patterns can help local/state agencies design appropriate intervention programs.