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July 29, 2015

American Society of Tropical Medicine and Hygiene
60 Revere Drive, Suite 500
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Dear Sir or Madam:

I am a Fellow in the Duke Medical Center Pediatric Infectious Diseases Fellowship Program and I am writing to apply for the Burroughs Wellcome Fund/American Society of Tropical Medicine and Hygiene Postdoctoral Fellowship in Tropical Infectious Diseases for 2016. I am currently entering my 3rd year of fellowship and am applying for support for a 4th year of fellowship and first year as a faculty member.

Between 2011 and 2013, I lived in Gaborone, Botswana and worked as a pediatric hospitalist at Princess Marina Hospital. I have been conducting research in Botswana to investigate the poor health outcomes of HIV-exposed, uninfected (HIV-EU) infants. Despite the absence of HIV infection, these infants have immune abnormalities and are at higher risk of common infections and mortality during the first two years of life. The available data suggest that the excess deaths observed among HIV-EU infants occur primarily during the first six months of life and are related to lower respiratory infections. I have served as the Principal Investigator for a study of childhood pneumonia in Gaborone, Botswana since 2012. Preliminary results from this project were published in the *Journal of Pediatric Infectious Diseases* and indicated that HIV-EU infants with pneumonia have higher treatment failure and case fatality rates than the children of HIV-negative mothers (HIV-unexposed).

In this proposal, we will investigate the role of the nasopharyngeal microbiome in mediating the poor respiratory health outcomes of HIV-EU children. This study will enroll children shortly after birth and follow them for the first 12 months of life, enabling us to examine temporal associations between the nasopharyngeal microbiome and respiratory outcomes. This research is of critical importance to the health of children in sub-Saharan Africa given the rapid rising numbers of HIV-EU children in the region.

This Fellowship would help me to advance my career in academic medicine, supporting me as I make the difficult transition from fellow in training to faculty and independent clinical investigator. The proposed dates for this Fellowship would be July 1, 2016 to June 30, 2018. I would use these two years of funding to gather preliminary data and additional research experience in an international setting in preparation for submission of a National Institutes of Health Research Project Grant Program (R01). During each year of this Fellowship, I will spend at least three months in Botswana, where I will receive continued mentorship from Dr. Andrew Steenhoff and other Botswana-UPenn Partnership investigators and the necessary infrastructure to complete the proposed research.

Thank you for your consideration of my application.

Sincerely,

Matthew S. Kelly

Duke Children's
HOSPITAL & HEALTH CENTER

A. Background and Significance

The burden of childhood pneumonia is highest in low- and middle-income countries. Pneumonia is the leading killer of children beyond the neonatal period, accounting for 1.1 million child deaths each year.[1] Although substantial progress was made in reducing pneumonia-related mortality in low- and middle-income countries over the past decade, pneumonia remains a disease of the world's poorest children. More than 99% of child deaths from pneumonia occur in low- and middle-income countries, and nearly half are in sub-Saharan Africa (SSA).[1]

HIV is a major obstacle to reducing pneumonia mortality in SSA. The scaling up of programs to prevent mother-to-child transmission of HIV resulted in a 60% decline in new child HIV infections in SSA over the past decade.[2] As a result, of the approximately 1.3 million HIV-exposed infants who will be born in the region this year, 1.1 million will not acquire HIV.[2] Despite the absence of HIV infection, HIV-EU infants are at increased risk of common infections and have higher early childhood mortality (Figure 1) than the infants of HIV-negative mothers (HIV-unexposed).[3-6] Most of the excess mortality among HIV-EU infants results from pneumonia.[5, 6] However, the precise mechanisms for this disparity are unknown. Proposed contributors include infant feeding practices, exposure to infections (e.g. tuberculosis), socioeconomic factors, and immune abnormalities resulting from *in utero* exposure to HIV and antiretroviral therapy (Table 1).[7] In preliminary experiments, we observed differences in the nasopharyngeal microbiomes of HIV-EU and HIV-unexposed children. *We hypothesize that the nasopharyngeal microbiome of HIV-EU infants increases pneumonia risk by facilitating colonization and invasion by bacterial respiratory pathogens.*

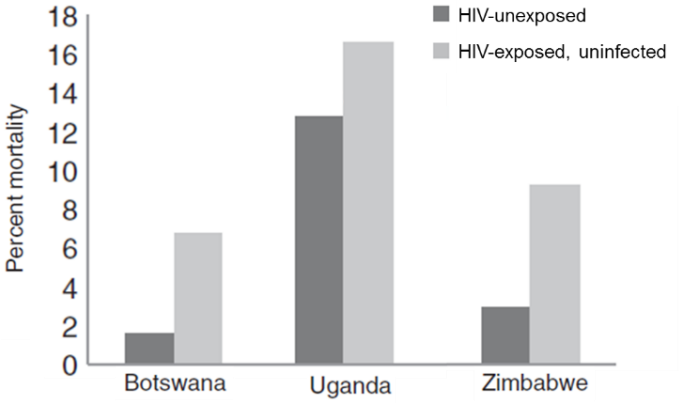


Figure 1. Mortality among HIV-exposed, uninfected and HIV-unexposed African children under 24 months of age. Figure adapted from Filteau et al., 2009.[8] Data are percentages among children in Botswana from 2002-2005,[4] Uganda from 1994-1998,[3] and Zimbabwe from 1997-2000.[6]

HIV-EU infants are at prone to infections caused by bacterial respiratory pathogens. Compared with HIV-unexposed infants, HIV-EU infants have a higher incidence of infections caused by encapsulated bacteria, particularly the respiratory pathogens *Streptococcus pneumoniae* and *Haemophilus influenzae*.[15, 16] Nasopharyngeal colonization is a prerequisite for the development of pneumonia caused by these bacteria.[17-19] However, the vast majority of colonization events do not result in symptomatic infection.[19] Once colonization is established, variations in the local environment of the nasopharynx influence whether overgrowth and invasion by these pathogens occurs.[17, 18] The specific factors that affect risk of colonization and invasion are poorly understood. *This research will contribute to a broader understanding of the dynamics of respiratory colonization and infection by bacterial respiratory pathogens among children in SSA.*

Table 1. Immune abnormalities among HIV-exposed, uninfected infants

Adaptive Immunity	
T lymphocytes	↓ naïve and total CD4+ cells[8, 9] ↓ naïve and total CD8+ cells[8, 9]
Antibodies	↓ *Streptococcus pneumoniae* IgG[10] ↓ measles IgG[11] ↓ group B streptococcus IgG[12]
Innate Immunity	
Neutrophils	↓ total neutrophil counts[9] ↑ incidence of high-grade neutropenia[13]
Cytokines	↑ serum interleukin-7[8] ↑ interferon-γ production[14]

IgG, immunoglobulin G

The nasopharyngeal microbiome may influence the risk of colonization by bacterial respiratory pathogens. Within the human nasopharynx resides a diverse community of microorganisms that have co-evolved with our species. A major function of this nasopharyngeal microbiome is to provide a barrier to colonization by bacterial pathogens through consumption of nutrients, production of inhibitory substances, or modulation of the local immune response.[20] Several previous studies observed an inverse relationship between the density of specific commensal bacteria, particularly viridans group streptococci, and *S. pneumoniae* colonization.[21, 22] Moreover, intranasal or oral administration of probiotics reduces the risk of nasopharyngeal colonization by *S. pneumoniae*, suggesting that these products may reduce pneumonia risk in children.[20, 23] *In this proposal, we will use S. pneumoniae as a model to define the role of the nasopharyngeal microbiome in colonization and invasion by bacterial respiratory pathogens in HIV-EU infants.*

B. Innovation

Novel explanation for the poor health outcomes of African HIV-EU children. Despite the poor health outcomes of HIV-EU children in SSA being recognized for more than a decade, surprisingly little is known about the underlying mechanisms.[24] *This lack of knowledge* has prevented development of interventions addressing the underlying causes of the excess mortality among HIV-EU children. The proposed research will elucidate a previously unrecognized mechanism for the increased susceptibility of HIV-EU children to pneumonia and invasive infections caused by *S. pneumoniae* and other bacterial respiratory pathogens.

Access to a repository of nasopharyngeal specimens. I will use 450 nasopharyngeal specimens collected over a >3-year period from well-characterized cohorts of children in Botswana to compare the nasopharyngeal microbiomes of HIV-EU and HIV-unexposed children. Data available from these children include demographics (e.g. age, sex), socioeconomic status (e.g. household electricity, exposure to smoke from solid fuels, food insecurity), HIV clinical data (e.g. HIV testing, antiretroviral prophylaxis or treatment), nutritional information (e.g. height, weight, current diet), and microbiological testing (e.g. extended respiratory virus testing). Use of specimens from children in a variety of states of respiratory health will enable me to contextualize observed differences in the nasopharyngeal microbiome by HIV exposure status.

Examining temporal associations between the nasopharyngeal microbiome and bacterial respiratory pathogen colonization in SSA. Most prior studies investigating associations between the nasopharyngeal microbiome and colonization by bacterial pathogens were cross-sectional.[25, 26] As a result, these studies were unable to examine the dynamic relationships that exist between bacterial pathogens and commensal bacteria, respiratory viruses, and the host mucosal immune system. Moreover, no previous studies examined the nasopharyngeal microbiomes of children with and without pneumonia in SSA. In Aim 3, I will follow a cohort of infants in Botswana from birth to 12 months of age. I will identify unique characteristics of the nasopharyngeal microbiome of African children while examining whether changes in the nasopharyngeal microbiome over time predict colonization by bacterial pathogens.

Developing a clinician-scientist with a unique skillset. This proposal will provide me with salary support to continue my research in Botswana and become an independent investigator in respiratory microbial ecology and a leader in child global health. During the fellowship, I will acquire didactic training in advanced statistical methods, computational biology, and bioinformatics (Table 2). This coursework will be complemented by practical experience performing analyses of high-throughput sequencing data. In conducting these analyses, I will work closely with Dr. Patrick Seed (Duke University), who has expertise in the analysis of high-throughput sequencing data and has successfully competed for federal, industry, and institutional funding for this research. In Aim 1, I will apply skills from introductory coursework in computational biology and bioinformatics while gaining practical experience in metagenomic data analyses. In Aim 2, I will apply my training in causal inference to perform mediation analyses while conducting metagenomic data analyses under the continued mentorship of Dr. Seed. Finally, in Aim 3, I will use the skills acquired from coursework in all areas and the practical experience from Aims 1 and 2 to perform longitudinal analyses of high-throughput sequencing data. With this skillset, *I will be uniquely positioned* to design and conduct studies using targeted manipulation of the microbiome for the prevention of respiratory diseases among children in low- and middle-income countries.

Table 2. Completed and planned coursework

Relevant coursework completed to date		**Coursework planned during the grant period**	
Harvard School of Public Health		**Duke University**	
BIO 201	Introduction to Statistical Methods	CRP 253	Research Ethics and Responsible Conduct of Research
BIO 224	Survival Methods in Clinical Research		
BIO 551	Linear and Longitudinal Regression	BIO 311	Introduction to Systems Biology
EPI 201	Introduction to Epidemiology	BIO 557L	Microbial Ecology and Evolution
EPI 202	Elements of Epidemiologic Research	BIO 723	Statistical Computing for Biologists
EPI 203	Study Design in Epidemiologic Research	MGM 720	Computational Tools in Next Generation Genomic Analysis
EPI 204	Analysis of Case-Control and Cohort Studies		
RDS 280	Decision Analysis for Medical Practices	CBB 540	Statistical Methods for Computational Biology
RDS 282	Economic Evaluation for Health Policy and and Program Management	CBB 561	Computational Sequence Biology
		CBB 720	Sequencing-Based Genomics
UNC-Chapel Hill School of Public Health		**UNC-Chapel Hill School of Public Health**	
BIOS 511	Introduction to Statistical Computing and Data Management	BIOS 776	Causal Inference in Biomedical Research
		BIOS 780	Theory and Methods of Survival Analysis

C. Specific Aims & Hypotheses

Aim 1: Examine the nasopharyngeal microbiome of children with pneumonia by HIV exposure status.
Hypothesis 1: *S. pneumoniae* nasopharyngeal domination, defined as occupation of >50% of the nasopharyngeal microbiome, will be identified more frequently in HIV-EU children.

Aim 2: Elucidate the effect of HIV exposure on the nasopharyngeal microbiome.
Hypothesis 2: HIV exposure will be associated with loss of nasopharyngeal microbial diversity. Losses of commensal bacterial taxa and gains of species that are not typical flora will be observed in HIV-EU children.

Aim 3: Determine whether the nasopharyngeal microbiome predicts *S. pneumoniae* colonization.
Hypothesis 3: A reduction in nasopharyngeal microbial diversity will be associated with a higher incidence of S. pneumoniae colonization in the following month. We will observe heterogeneity in the specific bacterial taxa associated with *S. pneumoniae* colonization according to HIV exposure status.

D. Research Strategy

D.1. Preliminary studies

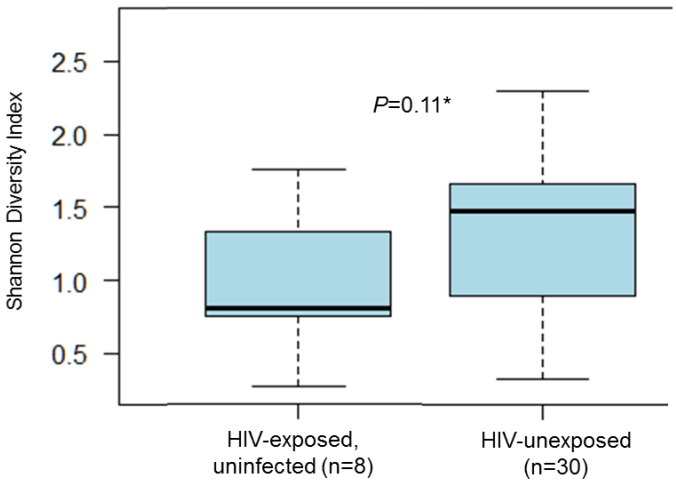
Prospective cohort study of pneumonia (n=365): Pneumonia cases were recruited between April 2011 and June 2015 at Princess Marina Hospital, a tertiary medical center in Gaborone, Botswana. Children were 1-23 months of age with pneumonia, defined by the World Health Organization (WHO) as "cough or difficult breathing with lower chest wall indrawing."[27] We excluded children with chronic medical conditions predisposing to pneumonia (other than HIV), hospitalization in the prior 14 days, asthma diagnosis, or resolution of lower chest wall indrawing with ≤2 bronchodilator treatments. Children were recruited within 6 hours of the Emergency Department triage time. Nasopharyngeal swab specimens were collected from all participants at enrollment, stored in a -80˚C freezer, and shipped on dry ice to our collaborators at McMaster University (Hamilton, Ontario). These specimens were tested for *S. pneumoniae* and respiratory viruses using polymerase chain reaction (PCR)-based assays.

Community-based controls without pneumonia (n=165): Controls were matched to pneumonia cases by primary care clinic and date and were recruited at 18 public clinics in Gaborone between August 2011 and June 2015. Controls were 1-23 months of age and did not meet WHO criteria for pneumonia. We excluded children with chronic medical conditions predisposing to pneumonia (other than HIV), hospitalization in the prior 14 days, or asthma diagnosis. A nasopharyngeal swab specimen was collected from all controls at enrollment. As for children in the pneumonia cohort, nasopharyngeal specimens were shipped to our collaborators at McMaster University and tested for *S. pneumoniae* and respiratory viruses by PCR.

Outcomes of HIV-EU children with pneumonia. Results from the prospective pneumonia cohort indicate that HIV-EU children require more days of respiratory support (3.1 vs. 1.8 days, *P*=0.02), have longer lengths of stay (4.2 vs. 3.1 days, *P*=0.02), and have higher in-hospital mortality (13.1% vs. 2.7%, *P*=0.001) than HIV-unexposed children. These outcome differences by HIV exposure status were observed only among children <6 months of age and were not accounted for by differences in the detection of respiratory viruses. Preliminary findings from these analyses were published in the *Journal of Pediatric Infectious Diseases* and *PLoS One*.[28, 29]

Nasopharyngeal microbial diversity of control children by HIV exposure status. We examined the nasopharyngeal microbiome of a small number of community-based control children (N=38: 8 HIV-EU, 30 HIV-unexposed) through next generation sequencing of the V3 region of bacterial 16S ribosomal RNA. In preliminary analyses of these data (Figure 2), HIV-EU children tended to have lower nasopharyngeal microbial diversity than HIV-unexposed children (*P*=0.11). In addition, we examined the proportion of the microbiome

Figure 2. Alpha diversity boxplots of nasopharyngeal specimens shown for children without pneumonia by HIV exposure status.



**Wilcoxon rank sum test comparing diversity by HIV exposure status*

occupied by specific operational taxonomic units by HIV exposure status. An operational taxonomic unit (OTU) is a cluster of similar 16S ribosomal RNA sequences that correspond to a species or a group of closely related species. The nasopharyngeal microbiomes of HIV-EU children were characterized by significant gains of *Comamonadaceae* (P=3x10^{-84}) and *Chyrseobacterium* (P=2x10^{-92}) and losses of *Prevotella* (P=3x10^{-92}) and *Dialister* (P=5x10^{-83}) OTUs.

D.2. Design and Methods

Setting. Botswana is the ideal setting for this research. Although the country remains severely affected by the HIV epidemic, with an HIV prevalence of ~35% among pregnant women, Botswana has implemented Africa's most successful program to prevent mother-to-child transmission of HIV.[30] Since the program's inception in 1999, the percentage of HIV-exposed infants who acquire HIV has declined from 21% to ~2%.[30] *Haemophilus influenzae* type B (Hib) conjugate vaccine was included in the country's immunization schedule in 2010, while pneumococcal conjugate vaccine (PCV-13) was introduced in July 2012. Both vaccines are given at 2, 3, and 4 months of age. Coverage estimates in 2013 for 3 doses of Hib and PCV-13 were 79% and 65%, respectively.[31]

Aim 1: Examine the nasopharyngeal microbiome of children with pneumonia by HIV exposure status.

Study population. I will use previously collected nasopharyngeal swab specimens from HIV-negative children (n=320: 100 HIV-EU, 220 HIV-unexposed) enrolled in the prospective cohort study of pneumonia. Median (interquartile range) age was 6.5 (2.8-13.3) months, and 44% were female.

Specimen processing. PCR will be used to amplify the V3 region of 16S ribosomal RNA of bacteria using broad-range primers, and amplicons will be sequenced per established protocols in the Department of Pathology and Molecular Medicine at McMaster University. All sequencing data will be transferred securely to Dr. Kelly at Duke University for data analyses.

Exposure and outcome definitions. HIV exposure status was determined from written or electronic medical records. Children of mothers with documented negative testing for HIV during pregnancy or at delivery were considered HIV-unexposed. Children whose mothers tested positive for HIV before or at delivery were considered HIV-exposed. HIV-exposed children were classified as HIV-EU if they tested negative for HIV after 6 weeks of age if exclusively formula fed or at least 6 weeks after breastfeeding cessation. Nasopharyngeal domination will be defined as occupation of >50% of the nasopharyngeal microbiome. In our preliminary data, *S. pneumoniae* nasopharyngeal domination did not occur in any healthy controls despite 47 of 119 (39%) having high-density *S. pneumoniae* colonization. This suggests, but does not prove, that *S. pneumoniae* nasopharyngeal domination is a reasonable surrogate for pneumococcal pneumonia.

Data analysis and statistical considerations. I will use multivariable logistic regression to examine whether the proportion of pneumonia episodes with *S. pneumoniae* nasopharyngeal domination differs by HIV exposure status. Models will adjust for age, sex, season, infant feeding practices, household use of solid fuels, PCV-13 doses, antibiotic treatment in the preceding 7 days, and respiratory virus detection. We estimate that *S. pneumoniae* nasopharyngeal domination will be observed among 15% of HIV-unexposed children with pneumonia. Assuming a two-sided statistical test and a significance level of 0.05, we will have >85% power to detect a RR of 2.0 for the effect of HIV exposure status on *S. pneumoniae* nasopharyngeal domination.

Aim 2: Elucidate the effect of HIV exposure on the nasopharyngeal microbiome.

Study population. We will use previously collected nasopharyngeal swab specimens from HIV-negative controls without pneumonia (n=150: 30 HIV-EU, 120 HIV-unexposed). Median (interquartile range) age of these children was 6.2 (3.2-11.2) months, and 49% were female.

Specimen processing. Sequencing of the bacterial 16S ribosomal RNA gene will be performed as described in Aim 1. Sequencing data will be transferred securely to Dr. Kelly for data analyses.

Exposure and outcome definitions. HIV exposure status was determined as described in Aim 1. Diversity of the nasopharyngeal microbiome will be quantified using the Shannon Diversity Index (SDI), a measure that incorporates both the number (richness) and relative abundances (evenness) of species in a community.[32] The SDI of the healthy nasopharyngeal microbiome typically ranges between 1 and 4, while the index takes on a theoretical minimum value of 0 when only a single bacterial species is present.[33]

Data analysis and statistical considerations. I will use multivariable linear regression to examine whether HIV exposure status is associated with the SDI of the nasopharyngeal microbiome.

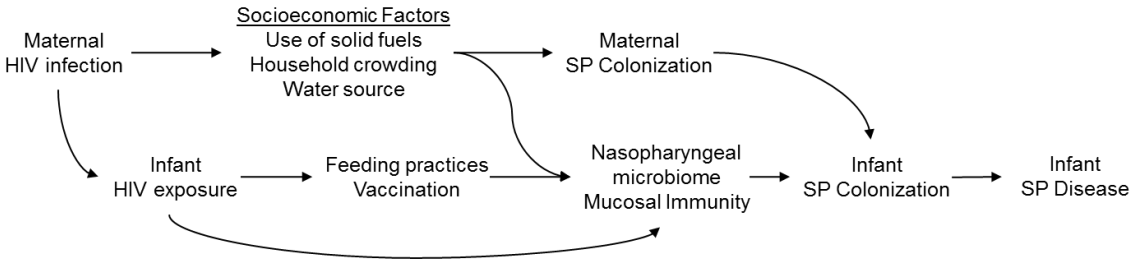


Figure 3. Causal diagram depicting mechanisms for the increased risk of disease caused by *Streptococcus pneumoniae* (SP) among HIV-EU infants.

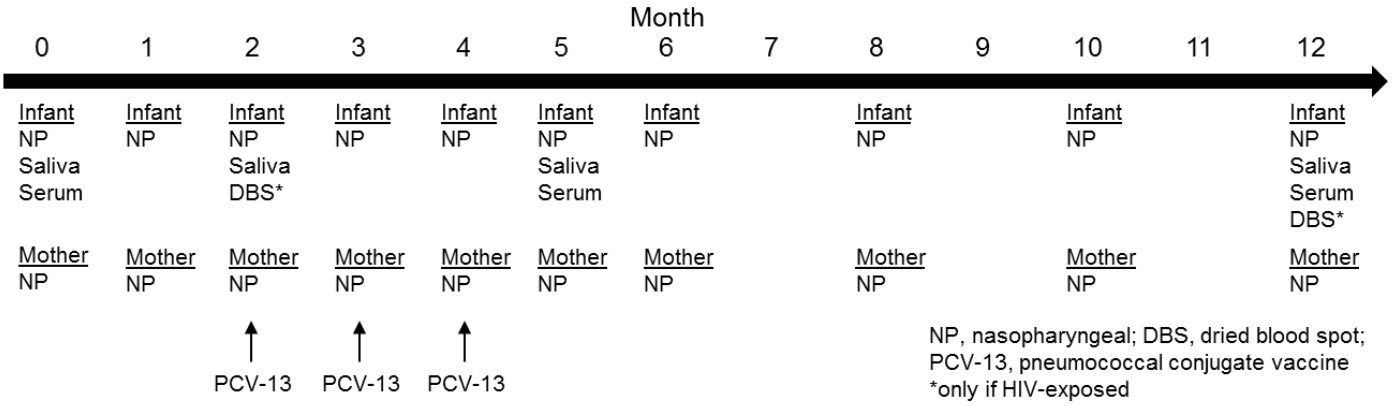
Models will adjust for the following potential confounders identified based on a literature review and construction of a causal diagram (Figure 3): age, sex, season, household use of solid fuels, municipal or private water source, antibiotic exposure in the preceding 7 days, and respiratory virus detection.[34-37] I will use inverse probability weighting to examine the role of infant feeding practices and PCV-13 doses in mediating the observed association. Based on preliminary data, we estimate that the mean (standard deviation) SDI of healthy HIV-unexposed children will be 1.5 (0.5). Assuming a two-sided statistical test and a significance level of 0.05, we will have >95% power to detect a 15% difference in the mean SDI by HIV exposure status. Exploratory analyses of the effect of HIV exposure status on specific bacterial taxa will use pairwise t-tests with post-test corrections for false discovery using the Benjamini-Hochberg procedure.[38]

Aim 3: Determine whether the nasopharyngeal microbiome predicts *S. pneumoniae* colonization.

Study Population. Mother-infant pairs (n=90; 45 HIV-infected mothers, 45 HIV-negative mothers) will be recruited within 48 hours of delivery at 3 geographically and socioeconomically diverse public clinics in Gaborone. Exclusion criteria will include maternal age <18 years, infant birth weight <2000 grams, multiple gestation pregnancy, and Caesarian delivery.

Design and Methods. After written informed consent is obtained, sociodemographic and clinical information will be collected from a detailed face-to-face questionnaire with the infant's mother, review of infant and maternal medical records, and collection of laboratory specimens (Figure 4). Data collected at enrollment will include demographics (e.g. date and time of birth, sex, race), HIV clinical data (e.g. maternal HIV status and CD4 count during pregnancy, antiretroviral treatment), infant feeding practices, socioeconomic factors (e.g. food insecurity, water supply, household income, use of solid fuels), and details of the household (e.g. number and ages of adults and children). After the enrollment visit, mothers and infants will be seen for monthly (0-6 months) or every other month (after 6 months) until the child is 12 months of age. At all follow-up visits, a brief face-to-face questionnaire will assess for recent or current infant respiratory symptoms, antibiotic exposures, and infant feeding practices. Each infant's written medical record will be reviewed for vaccination dates, pneumonia episodes, invasive pneumococcal infections, and hospitalizations.

Figure 4. Proposed timeline for the collection of specimens from study participants (Aim 3)



Laboratory Methods. All specimens will be transported to the Botswana-UPenn Centers for AIDS Research core laboratory in Gaborone for initial processing and storage in a -80˚C freezer.

Nasopharyngeal swab specimens will be collected into 1.6-mL M-swab media using flocked nasal swabs (Copan Italia). Specimens will be split into three ~0.5-mL aliquots. One 0.5-mL specimen will be shipped to the laboratory of Dr. Marek Smieja at McMaster University. Specimens from children with upper respiratory symptoms (cough, nasal discharge or congestion) will be tested for respiratory viruses by multiplex PCR. Specimens from children without respiratory symptoms will be tested for rhinovirus-enterovirus using a uniplex PCR assay. All specimens will be tested for *S. pneumoniae* using qPCR. For samples from which *S. pneumoniae* is detected at ≥6 log copies/mL, the second 0.5-mL specimen aliquot will be shipped to the laboratory of Dr. Stephen Pelton at Boston Medical Center. This aliquot will be plated on selective media for *S. pneumoniae* and isolates will be serotyped using the Quellung reaction to antisera to specific capsular antigens.[39] For specimens for which traditional serotyping fails, PCR deduction of 40 pneumococcal serotypes will be performed using primers developed by the Centers for Disease Control and Prevention. The final 0.5-mL specimen aliquot will be shipped to Dr. Seed's laboratory at Duke University. PCR will be used to amplify the V4 region of bacterial 16S ribosomal RNA.[40, 41] Barcoded amplicons will be multiplexed and co-sequenced in pools on an Illumina MiSeq instrument in the Duke Genome Sequencing and Analysis core facility.

Saliva and serum specimens will be shipped to Dr. Sallie Permar's laboratory at Duke University for measurement of immunity to *S. pneumoniae*. Antibody-mediated immunity to the polysaccharide capsule appears to be the primary host defense mechanism against *S. pneumoniae*, with secretory immunoglobulin A (sIgA) being particularly important for colonization.[18, 42, 43] A multiplex binding assay will be used to measure levels of immunoglobulin G (IgG) and secretory immunoglobulin A (sIgA) specific to 23 *S. pneumoniae* serotypes, including all PCV-13 serotypes.[44]

Dried blood spots obtained from HIV-exposed infants will be sent to Lancet Laboratories, a private laboratory accredited by the South African National Accreditation System (SANAS) for performance of HIV-1 DNA PCR. Pre-test counseling will be performed by research staff who have been trained to provide this service in Botswana. For any child with positive or indeterminate results, a legal guardian will be informed immediately so that the child may be retested for HIV and urgently referred to the Botswana-Baylor Children's Clinical Centre of Excellence in Gaborone. HIV-exposed children are also anticipated to have HIV DNA PCR performed at 4-6 weeks of age through the public health system, as per standard care in Botswana HIV treatment guidelines.[45]

Exposure and Outcome Definitions. Diversity of the nasopharyngeal microbiome will be assessed using the Shannon Diversity Index. The primary outcome will be serotype-specific nasopharyngeal colonization by *S. pneumoniae*. As there are >90 serotypes, children may experience multiple colonization events.

Statistical Analysis. I will examine whether diversity of the nasopharyngeal microbiome is associated with *S. pneumoniae* colonization events using a marginal, recurrent event, time-varying Cox proportional hazards model. Because we are modeling multiple events for each subject, there may be within-subject correlation resulting in incorrect variance calculations. To account for potentially correlated observations, we will use the 'robust' sandwich variance estimator.[46] Models will adjust for age, infant feeding, household use of solid fuels, antibiotic exposures, PCV-13 doses, serum pneumococcal IgG (proportion of serotypes with protective IgG), ratio of pneumococcus-to-total sIgA, respiratory virus detection, and maternal *S. pneumoniae* colonization. To identify preliminary microbial signatures and specific taxa as variables predictive of *S. pneumoniae* colonization, we will employ machine learning using Random Forests to develop a predictive model.[47]

Limitations and Alternative Approaches

Loss to follow-up: Given that participants for the study proposed in Aim 3 are followed for 12 months, some loss to follow-up is anticipated. To minimize this occurrence, we will collect the phone numbers of mothers and two additional contacts as well as the plot number of the family's residence. Previous Botswana-UPenn Partnership studies have used a similar approach with loss to follow-up rates that have typically been <10%.[48] If loss to follow-up is limiting our ability to assess Aim 3 in older infants, we will amend the study protocol to increase our sample size and permit staggered enrollment of infants throughout the first year of life.

Infant feeding practices: To determine the effect of HIV exposure on the nasopharyngeal microbiome independent of reduced breastfeeding, infant feeding practices will be assessed at each study visit in Aim 3 and included as a time-varying variable in statistical models. Moreover, to ensure sufficient variability in feeding practices for these analyses, we aim to enroll equal numbers of breastfed and formula fed HIV-exposed infants. If we are unable to identify sufficient participants for either of these groups of HIV-EU infants, we will amend the study protocol to expand enrollment to additional public clinics in the Gaborone area. We anticipate identifying few HIV-unexposed infants who are exclusively formula fed from birth.

Bibliography

1. United Nations Children's Fund. Committing to child survival: a promise renewed. Progress report 2013. New York: UNICEF; 2013.
2. Joint United Nations Programme on HIV/AIDS. 2014 progress report on the Global Plan. Available at: http://www.unaids.org/en/resources/documents/2014/JC2681_2014-Global-Plan-progress. Accessed June 18, 2015.
3. Kuhn L, Kasonde P, Sinkala M, Kankasa C, Semrau K, Scott N, et al. Does severity of HIV disease in HIV-infected mothers affect mortality and morbidity among their uninfected infants? Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2005;41(11):1654-61.
4. Brahmbhatt H, Kigozi G, Wabwire-Mangen F, Serwadda D, Lutalo T, Nalugoda F, et al. Mortality in HIV-infected and uninfected children of HIV-infected and uninfected mothers in rural Uganda. JAIDS Journal of Acquired Immune Deficiency Syndromes. 2006;41(4):504-8.
5. Shapiro RL, Lockman S, Kim S, Smeaton L, Rahkola JT, Thior I, et al. Infant morbidity, mortality, and breast milk immunologic profiles among breast-feeding HIV-infected and HIV-uninfected women in Botswana. The Journal of infectious diseases. 2007;196(4):562-9.
6. Marinda E, Humphrey JH, Iliff PJ, Mutasa K, Nathoo KJ, Piwoz EG, et al. Child mortality according to maternal and infant HIV status in Zimbabwe. The Pediatric infectious disease journal. 2007;26(6):519-26.
7. Filteau S. The HIV-exposed, uninfected African child. Tropical medicine & international health : TM & IH. 2009;14(3):276-87.
8. Clerici M, Saresella M, Colombo F, Fossati S, Sala N, Bricalli D, et al. T-lymphocyte maturation abnormalities in uninfected newborns and children with vertical exposure to HIV. Blood. 2000;96(12):3866-71.
9. Pacheco SE, McIntosh K, Lu M, Mofenson LM, Diaz C, Foca M, et al. Effect of perinatal antiretroviral drug exposure on hematologic values in HIV-uninfected children: An analysis of the women and infants transmission study. The Journal of infectious diseases. 2006;194(8):1089-97.
10. Jones CE, Naidoo S, De Beer C, Esser M, Kampmann B, Hesseling AC. Maternal HIV infection and antibody responses against vaccine-preventable diseases in uninfected infants. JAMA : the journal of the American Medical Association. 2011;305(6):576-84.
11. Farquhar C, Nduati R, Haigwood N, Sutton W, Mbori-Ngacha D, Richardson B, et al. High maternal HIV-1 viral load during pregnancy is associated with reduced placental transfer of measles IgG antibody. Journal of acquired immune deficiency syndromes (1999). 2005;40(4):494-7.
12. Dangor Z, Kwatra G, Izu A, Adrian P, van Niekerk N, Cutland CL, et al. HIV-1 is associated with lower Group B Streptococcus capsular and surface-protein IgG antibody levels and reduced transplacental antibody transfer in pregnant women. Journal of Infectious Diseases. 2015:jiv064.
13. Bunders M, Thorne C, Newell ML. Maternal and infant factors and lymphocyte, CD4 and CD8 cell counts in uninfected children of HIV-1-infected mothers. AIDS (London, England). 2005;19(10):1071-9.
14. Kuhn L, Coutsoudis A, Moodley D, Mngqundaniso N, Trabattoni D, Shearer GM, et al. Interferon-gamma and interleukin-10 production among HIV-1-infected and uninfected infants of HIV-1-infected mothers. Pediatric research. 2001;50(3):412-6.
15. Kinabo GD, van der Ven A, Msuya LJ, Shayo AM, Schimana W, Ndaro A, et al. Dynamics of nasopharyngeal bacterial colonisation in HIV-exposed young infants in Tanzania. Tropical medicine & international health : TM & IH. 2013;18(3):286-95.
16. von Mollendorf C, von Gottberg A, Tempia S, Meiring S, de Gouveia L, Quan V, et al. Increased risk and mortality of invasive pneumococcal disease in HIV-exposed-uninfected infants <1 year of age in South Africa, 2009-2013. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015.
17. Wolter N, Tempia S, Cohen C, Madhi SA, Venter M, Moyes J, et al. High nasopharyngeal pneumococcal density, increased by viral coinfection, is associated with invasive pneumococcal pneumonia. The Journal of infectious diseases. 2014;210(10):1649-57.
18. Garcia-Rodriguez JA, Fresnadillo Martinez MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. The Journal of antimicrobial chemotherapy. 2002;50 Suppl S2:59-73.
19. Bogaert D, de Groot R, Hermans P. Streptococcus pneumoniae colonisation: the key to pneumococcal disease. The Lancet infectious diseases. 2004;4(3):144-54.
20. Cangemi de Gutierrez R, Santos V, Nader-Macias ME. Protective effect of intranasally inoculated Lactobacillus fermentum against Streptococcus pneumoniae challenge on the mouse respiratory tract. FEMS immunology and medical microbiology. 2001;31(3):187-95.
21. Johanson WG, Jr., Blackstock R, Pierce AK, Sanford JP. The role of bacterial antagonism in pneumococcal colonization of the human pharynx. The Journal of laboratory and clinical medicine. 1970;75(6):946-52.
22. Faden H, Stanievich J, Brodsky L, Bernstein J, Ogra PL. Changes in nasopharyngeal flora during otitis media of childhood. The Pediatric infectious disease journal. 1990;9(9):623-6.
23. Gluck U, Gebbers JO. Ingested probiotics reduce nasal colonization with pathogenic bacteria (Staphylococcus aureus, Streptococcus pneumoniae, and beta-hemolytic streptococci). The American journal of clinical nutrition. 2003;77(2):517-20.

24. Newell M-L, Coovadia H, Cortina-Borja M, Rollins N, Gaillard P, Dabis F. Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. The Lancet. 2004;364(9441):1236-43.
25. Chien YW, Vidal JE, Grijalva CG, Bozio C, Edwards KM, Williams JV, et al. Density interactions among Streptococcus pneumoniae, Haemophilus influenzae and Staphylococcus aureus in the nasopharynx of young Peruvian children. The Pediatric infectious disease journal. 2013;32(1):72-7.
26. Sakwinska O, Schmid VB, Berger B, Bruttin A, Keitel K, Lepage M, et al. Nasopharyngeal microbiota in healthy children and pneumonia patients. Journal of clinical microbiology. 2014;52(5):1590-4.
27. Seaman SR, White IR. Review of inverse probability weighting for dealing with missing data. Statistical methods in medical research. 2013;22(3):278-95.
28. Kelly MS, Wirth KE, Steenhoff AP, Cunningham CK, Arscott-Mills T, Boiditswe SC, Patel MZ, Shah SS, Finalle R, Makone I, Feemster KA. Treatment failures and excess mortality among HIV-exposed, uninfected children with pneumonia. J Pediatric Infect Dis Soc. 2014; doi:10.1093/jpids/piu092.
29. Kelly MS, Smieja M, Luinstra K, Wirth KE, Goldfarb DM, Steenhoff AP, et al. Association of Respiratory Viruses with Outcomes of Severe Childhood Pneumonia in Botswana. 2015.
30. UNAIDS. Botswana 2014 progress report on the Global Plan. Available at: http://www.unaids.org/sites/default/files/media/documents/UNAIDS_GlobalplanCountryfactsheet_botswana_en.pdf. Access July 16, 2015. .
31. United Nations Children's Fund, World Health Organization. Botswana: WHO and UNICEF estimates of immunization coverage, 2013 revision. Available at: http://www.data.unicef.org/fckimages/uploads/1421188039_botswana_rev_13_FINAL.pdf. Accessed 25 June 2015. .
32. Magurran AE. Measuring biological diversity. Malden, MA: Blackwell Publishing, 2004.
33. Cremers AJ, Zomer AL, Gritzfeld JF, Ferwerda G, van Hijum SA, Ferreira DM, et al. The adult nasopharyngeal microbiome as a determinant of pneumococcal acquisition. Microbiome. 2014;2(1):44.
34. Allen EK, Koeppel AF, Hendley JO, Turner SD, Winther B, Sale MM. Characterization of the nasopharyngeal microbiota in health and during rhinovirus challenge. Microbiome. 2014;2(1):22.
35. Biesbroek G, Tsivtsivadze E, Sanders EA, Montijn R, Veenhoven RH, Keijser BJ, et al. Early respiratory microbiota composition determines bacterial succession patterns and respiratory health in children. American journal of respiratory and critical care medicine. 2014;190(11):1283-92.
36. Biesbroek G, Wang X, Keijser BJ, Eijkemans RM, Trzciński K, Rots NY, et al. Seven-valent pneumococcal conjugate vaccine and nasopharyngeal microbiota in healthy children. Emerging infectious diseases. 2014;20(2):201.
37. Biesbroek G, Bosch AA, Wang X, Keijser BJ, Veenhoven RH, Sanders EA, et al. The impact of breastfeeding on nasopharyngeal microbial communities in infants. American journal of respiratory and critical care medicine. 2014;190(3):298-308.
38. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature methods. 2013;10(10):996-8.
39. Lee GM, Kleinman K, Pelton SI, Hanage W, Huang SS, Lakoma M, et al. Impact of 13-Valent Pneumococcal Conjugate Vaccination on Carriage in Young Children in Massachusetts. Journal of the Pediatric Infectious Diseases Society. 2014;3(1):23-32.
40. LaTuga MS, Ellis JC, Cotton CM, Goldberg RN, Wynn JL, Jackson RB, et al. Beyond bacteria: a study of the enteric microbial consortium in extremely low birth weight infants. PloS one. 2011;6(12):e27858.
41. Gilbert JA, Meyer F, Antonopoulos D, Balaji P, Brown CT, Brown CT, et al. Meeting report: the terabase metagenomics workshop and the vision of an Earth microbiome project. Standards in genomic sciences. 2010;3(3):243-8.
42. Kurono Y, Shimamura K, Shigemi H, Mogi G. Inhibition of bacterial adherence by nasopharyngeal secretions. The Annals of otology, rhinology, and laryngology. 1991;100(6):455-8.
43. Fukuyama Y, King JD, Kataoka K, Kobayashi R, Gilbert RS, Oishi K, et al. Secretory-IgA antibodies play an important role in the immunity to Streptococcus pneumoniae. The Journal of Immunology. 2010;185(3):1755-62.
44. Pickering JW, Hill HR. Measurement of antibodies to pneumococcal polysaccharides with Luminex xMAP microsphere-based liquid arrays. Carbohydrate Microarrays: Springer; 2012. p. 361-75.
45. Botswana Ministry of Health. Botswana national HIV & AIDS treatment guidelines. 2012.
46. Wei L-J, Lin DY, Weissfeld L. Regression analysis of multivariate incomplete failure time data by modeling marginal distributions. Journal of the American statistical association. 1989;84(408):1065-73.
47. Breiman L. Random forests. Machine learning. 2001;45(1):5-32.
48. Ravimohan S, Tamuhla N, Steenhoff AP, Letlhogile R, Nfanyana K, Bellamy SL, et al. Immunological profiling of tuberculosis-associated immune reconstitution inflammatory syndrome and non-immune reconstitution inflammatory syndrome death in HIV-infected adults with pulmonary tuberculosis starting antiretroviral therapy: a prospective observational cohort study. The Lancet Infectious diseases. 2015.

DEPARTMENT OF PEDIATRICS
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Aug 5, 2015

Dear Committee Members:

I am delighted to serve as the primary mentor for Dr. Matthew Kelly's application for a Burroughs Wellcome Fund / American Society of Tropical Medicine and Hygiene Postdoctoral Fellowship in Tropical Infectious Diseases.

Matt completed his MD and MPH degrees at Harvard University and his pediatrics residency training in the Boston Combined Residency Program in Pediatrics. Thereafter his passion for the health of children globally motivated him to pursue training in the David N. Pincus Global Health Fellowship offered by the Children's Hospital of Philadelphia. He lived in Botswana for two years and cared for HIV-infected, HIV-exposed but uninfected, and HIV-uninfected children as a hospitalist in Gaborone. During this time, his collaborative approach ensured the success of his ongoing pneumonia studies while building relationships with local providers and laboratory specialists that will facilitate continued research in Botswana on the special health needs of HIV-exposed but uninfected children. He has published several excellent manuscripts from this research but more importantly, he has built a group of collaborators from around the globe who are helping to complete multiple important studies. After completing this Global Health fellowship in 2013, Matt joined our fellowship program to acquire sub-specialty training in pediatric infectious diseases. In our program, Matt has excelled in clinical care and in research, exceeding our (already lofty) expectations. His open and caring personality, impeccable work ethic, determination, and professionalism have allowed him to position himself for a successful academic career.

During the proposed Postdoctoral Fellowship period, Matt will gain additional clinical research experience in a low- and middle-income country as the Principal Investigator of a longitudinal study of mother-infant pairs in Botswana. In implementing this study, Matt will spend at least 3 months/year in Botswana during the fellowship period. Matt's proposal is ambitious but based on his past work, I am confident that he will complete the work outlined in the timeframe proposed. This research will be funded primarily by a Duke University Center for AIDS Research (CFAR) grant. However, to further support his work, I have additional discretionary funds to assist with this project should unanticipated expenses arise. This Postdoctoral Fellowship is extremely important to further Matt's development because it will allow him to gain additional training in clinical research while acquiring the skills needed for the analysis of high-throughput sequencing data. Matt will be taking courses in Computational Biology and Bioinformatics at Duke University, and will apply this training through the analysis of the sequencing data generated in this project. These skills in combination with Matt's training in pediatric global health and infectious diseases will present a unique combination of skillsets in our field.

My Research Career

I am devoted to a career in pediatric clinical research and to mentoring junior investigators conducting clinical research in children. My current research funding includes two clinical trials conducted in the International Maternal, Pediatric, Adolescent AIDS Clinical Trials Network - a Phase I study of an investigational respiratory syncytial virus vaccine and a Phase I study of an anti-HIV monoclonal antibody in HIV-exposed infants. Additional funded research includes a Gates Foundation-funded study to improve Ebola treatment, the Duke Center for AIDS Research clinical core (Co-Director of the Clinical Core), and several smaller projects. In addition, I am the Principal Investigator for our division's National Institute of Child Health and Human Development (NICHD)-sponsored T32 training grant.

Ability to Mentor Early Career Clinician Scientists

My teaching responsibilities include the direct mentoring of medical students, house staff, fellows, and junior faculty. Mentorship and teaching are commitments that I take very seriously, as evidenced by my awards in teaching and mentorship.

I have served as mentor or co-mentor for trainees in our program including countless junior faculty and fellows in pediatric infectious diseases. Among my trainees are individuals who have secured National Institutes of Health K awards, R01s, the Presidential Award for Research excellence and numerous other competitive, peer-reviewed grants. Most have gone on to careers as physician scientists at academic institutions throughout the United States, and many remain active in clinical care and research abroad.

Primary Mentorship for Matt

1. Matt will continue to lead biweekly meetings attended by myself, Dr. Patrick Seed (see below), and his Botswana-based research staff. During these meetings, we will discuss study progress, address any issues related to implementation of the research protocols, and develop analysis plans. Dr. Steenhoff, the Director of Research at the Botswana-UPenn Partnership, is a key contributor on these calls and can provide insight regarding the logistical and regulatory considerations in Botswana.
2. I will meet weekly with Matt during this project, in person when he is in North Carolina and via Skype when he is in Botswana. At these weekly meetings, we will review research progress, grant applications, and skills vital to success in academic medicine (e.g. negotiations, personnel management).
3. I will formally assess Matt's progress toward completion of his proposal, grant writing, and manuscript preparation.
4. I will work with Matt and Dr. Seed to interpret the results from his analyses. I will do this during my weekly meetings with him as well as with the rest of his mentorship team during our biweekly research conference calls.
5. I will help him to present his data at local and national meetings both in written and oral communication.
6. I will facilitate meetings with his other mentors and external advisory panel as necessary. I have longstanding research or academic relationships with both co-mentors and all of the investigators on his external advisory panel.

In addition to my direct mentorship, Matt's research will be supported by key faculty in the Divisions of Pediatric Infectious Diseases) at Duke University and the University of Pennsylvania. **Matt will work closely with Dr. Patrick Seed to process and analyze the large amount of bacterial sequencing data that will be generated by this project**. Dr. Seed is an Associate Professor in the Department of Pediatrics at Duke University and directs a research program that is examining the microbiome during infancy. He will provide Matt with practical training in the analyses of next generation sequencing data as well as the laboratory infrastructure needed for this project. Matt will also have access to scientific colleagues and facilities within the Pediatric Infectious Diseases division at Duke and the larger university community. Additional institutional resources are available to support Matt's career development and research efforts through the Duke Global Health Institute, the Duke University Center for AIDS Research (CFAR), and the Duke Center for Genomics of Microbial Systems (GeMS).

Matt will be in regular communication with Dr. Andrew Steenhoff, his overseas institution mentor, who will assist in the oversight of this project. Dr. Steenhoff has >10 years of pediatric clinical research experience in Botswana and is a fantastic resource for any logistical, regulatory, or ethical issues that might arise in implementing this proposal**.** Matt will receive additional guidance from Dr. Tonya Arscott-Mills, the Research Director for the Botswana-UPenn Partnership, as well other clinical investigators from this organization who are based full-time in Botswana.

In summary, Dr. Matthew Kelly is an outstanding young clinical investigator who is an exceptional candidate for this Postdoctoral Fellowship. This program will provide an optimal path for Matt to launch his career in academic medicine and global pediatrics. He is dedicated to improving the health of vulnerable children worldwide and is a rising start in pediatric infectious diseases and global health. He is an extremely talented young physician who will develop into a leading investigator in pediatric medicine, and I therefore recommend him for consideration in the highest terms possible.

Sincerely,

Coleen K. Cunningham, M.D.
Chief, Divisions of Pediatric Infectious Diseases and Global Health
Vice Chair for Research, Department of Pediatrics, Duke University

BOTSWANA-UPENN PARTNERSHIP
Building a Healthy Future Together

Andrew Steenhoff, MBBCH
Botswana-UPenn Partnership &
Medical Director of Global Health,
The Children's Hospital of Philadelphia & Assistant Professor of Pediatrics
Perelman School of Medicine, University of Pennsylvania
Tel: 215 590 2017 Email: steenhoff@email.chop.edu
July 29, 2015

Review Committee
Burroughs Wellcome Fund / American Society of Tropical Medicine and Hygiene

Dear Committee Members:

I would like to express my strongest support for Dr. Matthew Kelly's grant application entitled "Investigating the Role of the Nasopharyngeal Microbiome in Mediating Pneumonia Risk Among HIV-Exposed, Uninfected Infants in Botswana." In my prior role as the Research Director at the Botswana-UPenn Partnership (BUP), I have known Matt since 2011. He is an exceptional rising physician-scientist who has developed a high-impact, innovative, and exciting line of research combined with a thoughtful plan for developing his career.

Matt is currently the Principal Investigator for two BUP studies of severe childhood pneumonia in Botswana. As the In-Country Principal Investigator for these studies, I work closely with Matt and have been impressed by his knowledge in epidemiologic methods and dedication to performing strong research. Moreover, he has shown the ability to effectively lead an international research team that includes clinicians, researchers, laboratory scientists, and research nurses. Matt's current studies have already helped us to learn more about the causes and outcomes of severe pneumonia in our patient population, particularly in a vulnerable group of children – HIV-exposed, uninfected (HIV-EU) infants. Based on current figures, 30% of infants born in Botswana are HIV-EU, so improving our care of these children will be critical to obtaining further reductions in infant and child mortality in Botswana.

Matt's current International AIDS Society-funded research has done an excellent job of building local capacity in Botswana. Two University of Botswana pediatrics residents are involved with these projects and will be using study data to complete their Masters of Medicine (MMed) thesis proposals. In addition, Matt's research funding is sponsoring tuition for a Masters student at the University of Botswana who is completing her laboratory-based thesis research using specimens collected from study participants. Matt also has developed local capacity for rapid testing for respiratory viruses. This is the first testing for these viruses that has been performed in the public sector in Botswana, and it has been useful for clinical care and vastly improves the country's ability for surveillance.

This Postdoctoral Fellowship will be critical to Matt's career development, providing him with the needed protected time to focus on his research efforts in Botswana and deepen his knowledge in the burgeoning field of microbiome research. Through this Fellowship, he will advance his skills in developing analytical methods and develop new fusion approaches between clinical epidemiology, global health, and microbiomics while paving the way to his independence as a researcher. One of the major barriers to tackling this problem will be

BOTSWANA-UPENN PARTNERSHIP
Building a Healthy Future Together

the assimilation and processing of large, often disparate datasets and digesting those data into meaningful measurements, concepts, and ideas. With this research support, Matt will be able to work closely with Dr. Patrick Seed at Duke University to gain experience in conducting analyses of microbiome data. This Fellowship will be the first step for Matt toward a productive career as an independent researcher pushing boundaries in our understanding of the microbiome and its role in changing health trajectories of children in developing countries.

BUP has an extensive clinical and research infrastructure to support Matt's research efforts. We have nearly 15 years of successful partnership experience in Botswana with both the Ministry of Health and the University of Botswana School of Medicine, and 90 full-time staff members in Botswana including a PhD laboratory scientist and experienced clinical researchers. Since 2001, BUP has attracted significant research funding from the NIH, CDC, Grand Challenges Canada, Foundations (Doris Duke, International AIDS Society, Thrasher, Ronald McDonald) and Industry and these ground-breaking projects have resulted in more than 200 peer-reviewed manuscripts to date. Additionally Matt has the benefit of having an experienced study coordinator, Ms. Charity Boiditswe. Charity is one of BUP's most skilled and competent research nurses, and she has been conducting Matt's research projects in Botswana since 2012. Together they make an incredibly productive team that is passionate about using research to improve the health of the country's children.

I have a longstanding history of mentorship of medical students, pediatric residents, and fellows in Botswana. However, in addition to my direct mentorship, Matt will also work closely with Dr. Tonya Arscott-Mills, the current Research Director at BUP. Like myself, Tonya has nearly eight years of clinical and research experience in Botswana and has been directly involved with Matt's pneumonia projects for the past 4 years. Matt will also benefit from the many other BUP investigators who are conducting clinical research studies in Botswana.

During his time in Botswana, Matt will attend monthly BUP meetings for physicians and laboratory scientists conducting research throughout Botswana. In these sessions, young investigators present their proposed research or study findings and receive feedback from experienced clinical researchers and laboratory scientists in Botswana. Matt will be expected to present the cohort study that he has proposed in this application during this fellowship. Matt will also attend (and be expected to present at) a weekly pediatric HIV journal club at Princess Marina Hospital, as well as a monthly academic lecture/seminar series organized by the University of Botswana Center for the Study of HIV/AIDS. Lastly, he will attend the monthly University of Botswana Department of Pediatrics journal club, where influential pediatric research articles are discussed and critiqued.

In summary, I believe that Matt's academic background, dedication, and scientific curiosity make him an ideal candidate for this fellowship program. With his prior clinical and research experience in Botswana, and with the assistance of his established research team, I am confident that the proposed work is feasible during this timeframe. Please do not hesitate to contact me with any questions.

Sincerely,

Andrew Steenhoff, MBBCH

DEPARTMENT OF PEDIATRICS
Division of Pediatric Infectious Diseases

WILLIAM J. STEINBACH, MD
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August 10, 2015

Dear Review Committee:

I am writing this letter in enthusiastic support of Dr. Matthew Kelly in his application for a Burroughs Wellcome Fund / American Society of Tropical Medicine and Hygiene fellowship. Matt is currently a 3rd-year fellow in our Pediatric Infectious Diseases Fellowship Training Program, and is applying for partial salary support and funds for capacity building in Botswana as he prepares to make the critical transition from fellow to faculty member. The time period that would be funded by this award, July 1 2016 to June 30 2018, will not count toward Matt's board eligibility.

I first met Matt when he was interviewing for a position in our pediatric infectious diseases fellowship. Since that time, I have had the opportunity to get to know Matt very well over the past 3 years. We offered Matt a position immediately, and unanimously, from our Division. In my 7 years as fellowship director, we have never before come to such a unanimous consensus decision. This should exemplify the strength of this candidate.

Matt has simply stellar academic credentials. He graduated *summa cum laude* from Washington University, where he won a Howard Hughes Medical Institute Summer Research Award as well as a Traveling Scholar award. He then continued on to Harvard Medical School where he completed both his MD and MPH degrees. While at Harvard, he was awarded both the Paul Dudley White Traveling Fellowship as well as the Presidential Scholars Public Service Award. **Notably, every one of his USMLE step scores are in the 99th percentile.**

Following Harvard Medical School, Matt completed his pediatrics residency in the Boston Combined Residency Program in Pediatrics. There he won the Von L. Meyer Travel Award and received rave reviews from his attending physicians, including absolutely phenomenal letters of recommendation (and personal phone calls made by myself) that we saw when he applied to our fellowship program.

His research contributions are years ahead of his peers. Matt is dedicated to pediatric global health, with clinical and research experiences in sub-Saharan Africa for almost a decade now. This culminated most recently in his Pediatric Global Health Fellowship from the Children's Hospital of Philadelphia from 2011-2013, finishing just before Matt arrived at Duke to start his infectious diseases fellowship. While this global health fellowship delayed the start of his anticipated pediatric infectious diseases fellowship, I am sure you can see why we held the spot for him (something else our Division had never done). These experiences led to a collection of first-author original research manuscripts, platform presentations and conferences at major academic conferences, and a book chapter. What struck me as particularly impressive was that, during a two-week period last year when he was not on clinical service, Matt completed the analyses of one dataset from his research in Botswana and submitted that manuscript, and nearly finished writing a second manuscript. Our department has faculty that do not move that fast.

Matt appears to possess a rare maturity and understanding of his planned trajectory that is uncommon for someone so junior. In my experience, it is generally quite readily apparent when meeting someone who is determined to succeed. **He has a great deal of personal initiative, as evidenced by successfully competing for <u>four</u> different research grants *during* his fellowships**. This is a feat largely unparalleled in graduate medical education. In the hospital, he is devoted to his patients and has a wonderful calming sense about him in his clinical interactions, always willing to stay the extra time to guarantee his patients get all the help they need.

Matt is a very unique fellow and I enthusiastically recommend him for this Postdoctoral Fellowship. I believe his natural abilities as a clinician and a researcher will propel him in academic medicine. His passion for research and medicine are a beautiful blend and his combination of perseverance, ability, and personality will carry him very far. I see in Matt all the fine qualities of an outstanding young medical doctor and clinical researcher; I have high expectations and hopes for him. There are few young gentlemen that I have come across in my career that possess the fine qualities of a sharp analytical mind, dedication to their work and coworkers, an ability to effectively communicate, and a sincere warm personality like Matt.

Sincerely,

William J. Steinbach, MD
Associate Professor of Pediatrics (Tenured), Molecular Genetics & Microbiology
Fellowship Program Training Director, Pediatric Infectious Diseases
Duke University Medical Center

Duke Children's
HOSPITAL & HEALTH CENTER

BUDGET JUSTIFICATION

Funding for the microbiological testing proposed in Aims 1 and 2 will be provided by my Collaborative Initiative for Pediatric HIV Education and Research (CIPHER) grant from the International AIDS Society. The longitudinal study of n=90 mother-infant pairs proposed in Aim 3 is funded by a Duke University Center for AIDS Research (CFAR) Small Grant. I will use preliminary data from these projects to apply for funding from the National Institutes of Health, Thrasher Research Fund, and other sponsors to enroll additional mother-infant pairs (goal of n=300 mother-infant pairs) in the longitudinal study proposed in Aim 3.

PERSONNEL

Matthew Kelly, MD, MPH, is a 3rd-year fellow in Pediatric Infectious Diseases at Duke University.

Personnel	Year 1	Year 2
Salary	$41,607	$41,607
Fringe benefits	$10,943	$10,943
TOTAL	**$52,550**	**$52,550**

TRAVEL

Funds are requested for round-trip airline tickets (RDU to GBE) and lodging for biannual, 1.5-month site visits by Dr. Kelly. In addition, we have budgeted for flight, registration, and lodging for Dr. Kelly to travel to national research conferences in Years 1 and 2 to present study findings.

Travel	Year 1	Year 2
Site visits	$8,000	$8,000
National conference	$1,000	$1,000
TOTAL	**$9,000**	**$9,000**

PUBLICATION COSTS

Funds are requested for publication costs to support dissemination of findings in open access journals. This will enable the results of our research to be accessible to health care providers in low-resource settings.

Publications	Year 1	Year 2
Open access fees	$2,000	$2,000
TOTAL	**$2,000**	**$2,000**

CAPACITY DEVELOPMENT

In order to support career development for our study coordinator (Ms. Charity Boiditswe), I have budgeted for her to attend and present study findings at the annual Botswana HIV/AIDS Conference in Years 1 and 2. In addition, we request funds for Ms. Letang Gaofiwe, a University of Botswana Masters student, to present her findings at regional research conferences. Ms. Gaofiwe's tuition is supported by funding for these projects and she is completing her laboratory-based thesis using nasopharyngeal specimens obtained from study subjects. Finally, we request funds for travel (round-trip ticket from GBE to the United States) and registration to enable Dr. Zaakir Patel to attend and present at the Annual Meeting of the Pediatric Academic Societies in Year 2. Dr. Patel is a Pediatrics resident at the University of Botswana; his thesis project is examining the effect of nutritional status on outcomes in the context of our ongoing pneumonia cohort.

DIRECT EXPENSES	Year 1	Year 2
Study coordinator conference	$450	$450
MPhil student conference	$1,000	$1,000
Pediatrics resident conference	$0	$2,550
TOTAL	**$1,450**	**$4,000**