Defense Threat Reduction Agency
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TECHNICAL REPORT

Characterize Respiratory Pathogens Endemic to Pakistan

October 2017

HDTRA1-10-1-0082

William A Petri, et al.

Prepared by:
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14. ABSTRACT

The objective of this study was to determine the incidence of respiratory viruses associated with severe pneumonia among children in the rural district of Matiari in Sindh, Pakistan. The study was a community-based prospective cohort of active surveillance for two years, and a total of 817 participants were followed. Of the nasopharyngeal swabs collected, 77.8% (179/230) were positive for one or more respiratory viruses. The incidence of viral-associated pneumonia was 11.9 per 100 child-years of follow up. Enterovirus/Rhinovirus was detected in 51.7% patients, followed by parainfluenza virus type III (8.3%), and RSV (5.7%). Of the blood cultures collected, 1.4% (5/356) were positive for a bacterial pathogen.

15. SUBJECT TERMS

respiratory virus; pneumonia; children; Pakistan; Enterovirus; Rhinovirus; parainfluenza virus type III; RSV.

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UNIT CONVERSION TABLE

U.S. customary units to and from international units of measurement*

U.S. Customary Units	Multiply by → / ← Divide by†		International Units
Length/Area/Volume			
inch (in)	2.54	$\times 10^{-2}$	meter (m)
foot (ft)	3.048	$\times 10^{-1}$	meter (m)
yard (yd)	9.144	$\times 10^{-1}$	meter (m)
mile (mi, international)	1.609 344	$\times 10^{3}$	meter (m)
mile (nmi, nautical, U.S.)	1.852	$\times 10^{3}$	meter (m)
barn (b)	1	$\times 10^{-28}$	square meter (m^2)
gallon (gal, U.S. liquid)	3.785 412	$\times 10^{-3}$	cubic meter (m^3)
cubic foot (ft^3)	2.831 685	$\times 10^{-2}$	cubic meter (m^3)
Mass/Density			
pound (lb)	4.535 924	$\times 10^{-1}$	kilogram (kg)
unified atomic mass unit (amu)	1.660 539	$\times 10^{-27}$	kilogram (kg)
pound-mass per cubic foot (lb ft^{-3})	1.601 846	$\times 10^{1}$	kilogram per cubic meter (kg m^{-3})
pound-force (lbf avoirdupois)	4.448 222		newton (N)
Energy/Work/Power			
electron volt (eV)	1.602 177	$\times 10^{-19}$	joule (J)
erg	1	$\times 10^{-7}$	joule (J)
kiloton (kt) (TNT equivalent)	4.184	$\times 10^{12}$	joule (J)
British thermal unit (Btu) (thermochemical)	1.054 350	$\times 10^{3}$	joule (J)
foot-pound-force (ft lbf)	1.355 818		joule (J)
calorie (cal) (thermochemical)	4.184		joule (J)
Pressure			
atmosphere (atm)	1.013 250	$\times 10^{5}$	pascal (Pa)
pound force per square inch (psi)	6.984 757	$\times 10^{3}$	pascal (Pa)
Temperature			
degree Fahrenheit (^oF)	$[T(^oF) - 32]/1.8$		degree Celsius (^oC)
degree Fahrenheit (^oF)	$[T(^oF) + 459.67]/1.8$		kelvin (K)
Radiation			
curie (Ci) [activity of radionuclides]	3.7	$\times 10^{10}$	per second (s^{-1}) [becquerel (Bq)]
roentgen (R) [air exposure]	2.579 760	$\times 10^{-4}$	coulomb per kilogram (C kg^{-1})
rad [absorbed dose]	1	$\times 10^{-2}$	joule per kilogram (J kg^{-1}) [gray (Gy)]
rem [equivalent and effective dose]	1	$\times 10^{-2}$	joule per kilogram (J kg^{-1}) [sievert (Sv)]

*Specific details regarding the implementation of SI units may be viewed at http://www.bipm.org/en/si/.

†Multiply the U.S. customary unit by the factor to get the international unit. Divide the international unit by the factor to get the U.S. customary unit.

Please answer all sections of the document. You are welcome to use figures and tables to complement or enhance the text. For annual reports, please only describe work for the period of performance (July 1, 2013 - June 30, 2014). For final reports, please describe the comprehensive effort.

Grant/Award #: HDTRA1-10-1-0082
PI Name: William A. Petri, Jr., M.D. Ph.D.
Organization/Institution: University of Virginia School of Medicine
Project Title: Characterize Respiratory Pathogens Endemic To Pakistan

What are the major goals of the project?
List the major goals of the project as stated in the approved application or as approved by the agency. If the application lists milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion. Generally, the goals will not change from one reporting period to the next. However, if the awarding agency approved changes to the goals during the reporting period, list the revised goals and objectives. Also explain any significant changes in approach or methods from the agency approved application or plan.

I. MAJOR GOALS OF THE PROJECT

This project involves a strong collaboration between Dr. William Petri and Dr. Molly Hughes at the University of Virginia with Dr. Zulfi Bhutta, Dr. Anita Zaidi, and Dr. Asad Ali at the Aga Khan University (AKU) in Karachi, Pakistan.

1. **We present here a review of the description and schedule of tasks for project HDTRA1-10-1-0082 as originally outlined in our project proposal:**

   Description and schedule of tasks:

   Year # 1 (Year 2010-2011) - *completed*

   Task 1: Complete the local IRB and DTRA Human Research Oversight Board protocol approval process before any human subject research begins - c*ompleted*

   Task 2: Organize cohort study sites (one rural site and one urban site), organize infrastructure and personnel, train personnel for initiation of study - *completed*

   Task 3: Recruitment of subjects to be initiated during the last 6 months of Year 1 in order to begin study cohort surveillance in Year 2 – *completed*

   Year # 2 (Year 2011-2012) and Year # 3 (Year 2012 – 2013) - *completed*

   Task 4: Surveillance for acute respiratory illness (ARI) and febrile illness episodes

   Task 5: Sample collection/data measurements

   Task 6: Microbiology characterization

   Task 7: Data analyses

<u>Year # 4</u> (Year 2013-2014) – *completed*

Task 4: Surveillance for ARI and febrile illness episodes

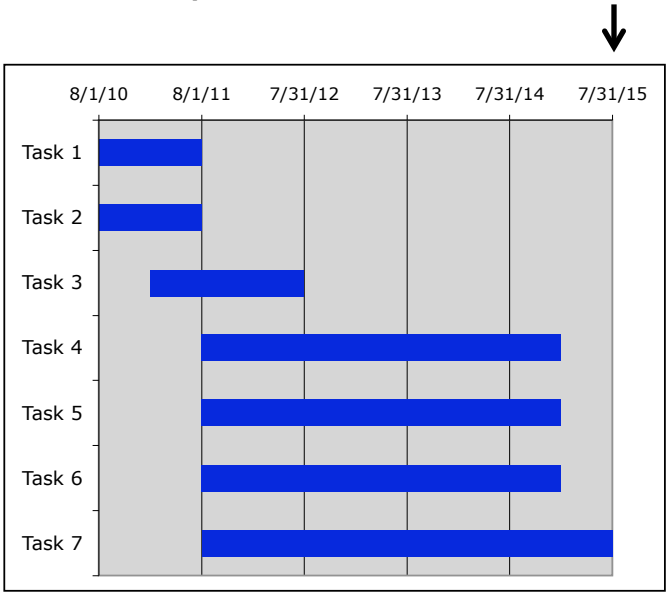
Task 5: Sample collection/measurements

Task 6: Microbiology characterization

Task 7: Data analyses

Schedule or timeline for performance of Tasks #1-7 during Years 1-4 of this project:

Submission of final report (7/31/2015 for No Cost Extension Year 5, 2014-2015)



2. **Summary of progress and adherence to timeline for performance of proposed tasks in this project:**

   a. At the time of the submission of this Final Progress Report on July 31, 2015, we are pleased to report that we remained essentially on target with the above schedule and timeline as originally proposed.

   b. We completed enrollment of subjects at our two study sites, Bilal Colony and the Matiari District, in 2012 with ongoing surveillance until end of project.

   c. Ongoing surveillance was brought to a close given that the project officially ended as of June 30, 2014, which was the end of Optional Year 4.

3. *** DETAILED RESULTS FROM ONGOING SURVEILLANCE ARE PROVIDED FROM THE MATIARI DISTRICT (PAGES 18-43) AND FROM BILAL COLONY (PAGES 44-58) AT THE END OF THIS REPORT.**

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results, including major findings, developments, or conclusions (both positive and negative); and 4) key outcomes or other achievements. Include a discussion of stated goals not met. As the project progresses, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

II. ACCOMPLISHMENTS UNDER THESE GOALS

In **YEAR 5 (No Cost Extension)** of the DTRA Grant Award HDTRA1-10-1-0082, there has been the following progress in our project entitled *"Characterize Respiratory Pathogens Endemic to Pakistan".*

Brief Statement of Progress (with detailed data reports provided on pages 18-58 of this report).

1. **Visit of UVA investigators to Pakistan in October 2013 and February 2014:** Dr. Petri and Dr. Hughes traveled to Karachi, Pakistan in November 2011 (11/14/11-11/19/11), and Dr. Hughes traveled again to Karachi in February 2012 (2/9/12-2/23/12), October 2012 (10/5/12-10/21/12), February 2013 (2/17/13-3/6/13), October 2013 (10/16/13-10/30/13), February 2014 (2/6/14-2/19/14), and October 2014 (10/9/14-10/18/14) to meet with Drs. Ali, Zaidi, and Bhutta to discuss and visit the cohort study locations in Bilal Colony (urban site in Karachi) and Matiari District (rural site about 3 hours north of Karachi in the Sindh Province).

2. **Communication between investigators:** In addition to many e-mail communications between Drs. Petri and Hughes at the University of Virginia with Drs. Ali, Zaidi, and Bhutta at the Aga Khan University, we have had at least 8-12 teleconferences per year to discuss various aspects of the project or to plan capacity building aspects of our collaborative effort, and we have met in person at least 2-3 times per year (2-3 visits to Pakistan by Dr. Hughes per year and at least 1 meeting in the United States by Drs. Petri, Hughes, Zaidi, Bhutta, and/or Ali each year at the American Society for Tropical Medicine & Hygiene Conference).

3. **Attendance at national/international meetings**:

   Drs. Petri, Hughes, Zaidi, Bhutta, and Ali attended the **2013 American Society for Tropical Medicine & Hygiene Conference in Washington, DC on November 13-17, 2013**. Dr. Ali and Dr. Hughes presented 2 posters at this meeting, as follows:

   • **Poster #1 for 2013 ASTMH international conference:**

   Patterns of growth failure in infants in a rural district of Pakistan

   Asad Ali[1], Tauseef Akhund[1], Najeeb Ur Rehman[1], Fayaz Ahmed[1], William Petri[2], Zulfiqar Bhutta[1], Anita Zaidi[1], Molly Hughes[2]

   [1]Aga Khan University, Karachi, Pakistan, [2]University of Virginia, Charlottesville, VA, United States

Abstract: Malnutrition is major underlying factor in childhood morbidity and mortality in developing countries. Prevalence of chronic malnutrition is high in Pakistan, with a recent study showing 50% of the children being stunted at 18 months of age in a rural district. There is little data regarding patterns of growth failure in children in Pakistan. This pattern is important to study so the highest risk age for growth failure can be identified and appropriate interventions can be planned accordingly. We are conducting a longitudinal observational study of childhood growth monitoring in Matiari, a rural district in Sindh province of Pakistan. We have enrolled 817 infants between 0-1 months of age and are recording their weight and height at monthly intervals. Morbidity data regarding history of upper respiratory tract infections, diarrhea and fever is also being recorded on fortnightly basis. We found that the average weight for age z-score (WAZ) at 1 month was -1.68 for girls and - 1.84 for boys, which decreased further to -1.82 in girls and -2.06 in boys at 6 months of age. At 12 months the mean WAZ increased to -1.75 for girls and -1.99 for boys. Average height for age z score (HAZ) at 1 month was -1.64 in girls and - 1.75 in boys, which decreased further to -1.76 in girls and -2.07 in boys at 6 months of age. At 12 months the mean HAZ decreased further -2.3 for girls and -2.65 for boys. This detailed analysis of growth faltering pattern in children will help identify the ages when the children are most at risk for growth failure so appropriate interventions can be planned accordingly.

• **Poster #2 for 2013 ASTMH international conference:**

Impact of respiratory illnesses during pregnancy on newborn's weight - A community based longitudinal study at an urban slum in Pakistan.

Asad Ali[1], Umber Zaman[1], Samana Zaidi[1], William Petri[2], Zulfiqar Bhutta[1], Anita Zaidi[1], Molly Hughes[2]

[1]Aga Khan University, Karachi, Pakistan, [2]University of Virginia, Charlottesville, VA, United States

Abstract: Birth weight is a powerful determinant of an infant's long term growth and survival. Although maternal health is widely believed to impact the birth weight of the baby, the exact factors during pregnancy that influence the birth weight are not clearly known. We are conducting a longitudinal observational study at Bilal Colony, a semi urban area of Karachi, Pakistan to assess the effect of maternal morbidities on the weight of the newborn. We are following 400 pregnant women from the first trimester onwards until their delivery. The pregnant women are visited weekly to record any fever or respiratory symptoms during the past seven days, and are referred to the study site clinic for treatment of observed illnesses. Each symptom episode is defined as one or more days of a self-reported symptom (fever, cough, difficulty breathing, runny nose, sore throat, head ache, chills or myalgia) in a pregnant woman who was symptom free for three days before. So far, 288 pregnancies have concluded as live deliveries, 12 as still births and 31 as spontaneous abortions. We analyzed the data of 243 pregnant

women whose newborns were weighed within 14 days of birth. The average age of pregnant women in our study was 24.1years and average weight of the pregnant woman was 56.1kg at the time of enrollment. Only 31% of the mothers had primary education or above whereas 38.3% had antenatal visits during their pregnancy. There were 51(21%) newborns with low birth weight (< 2.5 kg), whereas 192 (79%) had normal birth weight (>= 2.5kg). In pregnant women who had a low birth weight baby, the average episodes of fever, cough, headache and myalgia were 1.7, 2.3, 4.3, and 4.3 per women respectively. In pregnant women who had a normal birth weight baby, the average episodes of fever, cough, headache and myalgia were 1.7, 2.1, 5.2 and 4.6 per women respectively. The results of this study will help identify the degree to which maternal respiratory illnesses during pregnancy are a risk factor for infant's low birth weight.

Drs. Petri, Hughes, Zaidi, Bhutta, and Ali attended the **2012 American Society for Tropical Medicine & Hygiene Conference in Atlanta, Georgia on November 11-15, 2012**. Dr. Ali and Dr. Hughes presented two posters at this meeting, and the posters/abstracts were as follows:

• **Poster #1 presented at 2012 ASTMH conference:**

Characterization of respiratory pathogens endemic to Pakistan in newborns, children, and adults in a rural area of Pakistan

[1]Ali A., [1]Akhund T., [1]Bhutta Z., [2]Petri W.A., [1]Zaidi A., and [2]Hughes, M.A.

[1]Department of Pediatrics, Aga Khan University, Karachi, Pakistan, and [2]Department of Medicine, University of Virginia, Charlottesville, Virginia, United States

Background: Acute respiratory infections, especially those associated with pneumonia account for almost 24% of all under-5 child deaths in Pakistan, which lead to an estimated 90,000 deaths annually. There have been no comprehensive studies of the etiology of severe pneumonia in Pakistan since the US-sponsored Board on Science and Technology in International Development (BOSTID) studies conducted in urban Rawalpindi in the 1980s. At the recent WHO study on Global AGEing and Adult Health meeting, the complete lack of information on endemic influenza strains and H1N1 was highlighted as a major barrier to informed decision making for vaccination strategies. The goal of our current study is to determine the etiology of acute respiratory infection (ARI) and febrile illness in different age groups in Pakistan, using several diagnostic modalities, including real-time PCR and automated culture methods.

Hypothesis: We hypothesize that ARI and febrile illnesses in children and adults in Pakistan will differ in etiology, frequency, and antimicrobial resistance patterns from those of children and adults in western countries.

Study design: This is a longitudinal cohort observational study of newborns, young children, and their adult household members with fortnightly surveillance throughout the duration of this three-year study. Sample size is 1000 participants for newborns and 2000 participants each for older children and adult household members. A WHO case definition of severe pneumonia is employed in this study, and cases of febrile illness are

identified using temperature ≥ 101.3^{o}F (for newborns) or ≥ 100.4 ^{o}F (for young children and adults). Participants are evaluated by mobile medical teams for evaluation, collection of appropriate clinical samples, and delivery of medical treatment of the observed illnesses. Samples include nasopharyngeal swabs for workup of ARI by PCR identification of bacterial and viral etiologies (*Chlamydophila pneumoniae, Mycoplasma pneumoniae, Bordetella pertussis,* Influenza A and B, H1N1 influenza, parainfluenza viruses 1-4, adenovirus, respiratory syncytial viruses A and B, human metapneumovirus, rhinovirus, Coxsackie virus/echovirus family) and blood cultures and malaria immunochromatography (ICT) testing for workup of febrile illnesses.

Results: In the initial 6-month period of this study (October 2011 – March 2012), 94 cases of severe pneumonia in newborns with or without fever and 8 cases of newborns having fever ≥ 101.3^{o}F only have been identified, evaluated, and treated. In children 5 to 14 years of age, there have been 5 cases of fever ≥ 100.4 ^{o}F only and 7 cases of ARI without pneumonia. In adults, there have been 8 cases of fever ≥ 100.4 ^{o}F with influenza-like symptoms and 7 cases of ARI without fever.

To date, 35 nasopharyngeal swab samples collected from newborns have been analyzed using Luminex RVP Fast kit multiplex assay. Of these samples, 16 samples were positive for enterovirus/rhinovirus, 2 samples were positive for RSV, 5 samples were positive for human parainfluenza virus type III/IV positive, and 12 cases were negative for the pathogens tested. Blood cultures were positive in 2 cases, with one case of *Campylobacter* and one case of *E. coli* bacteremia. Malaria ICT with confirmatory peripheral smear evaluation was positive in 2 cases, one of which was positive for *Plasmodium falciparum* and one of which was positive for combined *Plasmodium falciparum* and *Plasmodim vivax*.

Conclusions: In the initial period of our ongoing cohort, we have identified a number of cases of ARI or febrile illness. The number of cases of rhinovirus/ enterovirus responsible for severe ARI/pneumonia compared to other viral etiologies was higher than anticipated given the prior BOSTID study results. Unexpectedly, two cases of malaria were identified in febrile newborns using our study protocol; otherwise, these children may not have been evaluated for malaria as an etiology given the age group (newborns) and the time of year, which is a lower prevalence period for malaria in Pakistan. Of the two cases of bacteremia in newborns, one etiology was less common (*Campylobacter*) compared to the other (*E. coli*). Additional study data for enrolled participants will continue to be analyzed over a three-year period, which includes seasonal factors as well. The results of this study should promote establishment of empiric treatment algorithms for ARI and febrile illness in Pakistan and provide a basis for future fundamental research in this area.

• **Poster #2 presented at 2012 ASTMH conference**:

Characterization of respiratory pathogens endemic to Pakistan in newborns, children, and adults in a semi-urban area of Karachi, Pakistan

[1]Ali A., [1]Zaman U., [1]Bhutta Z., [2]Petri W.A., [1]Zaidi A., and [2]Hughes, M.A.

[1]Department of Pediatrics, Aga Khan University, Karachi, Pakistan, and [2]Department of Medicine, University of Virginia, Charlottesville, Virginia, United States

Introduction: Approximately 90,000 of all under-5 deaths in a year are associated with acute respiratory infections, especially pneumonia in Pakistan. There have been no comprehensive studies of the etiology of severe pneumonia in Pakistan since the US-sponsored Board on Science and Technology in International Development (BOSTID) studies conducted in urban Rawalpindi in the 1980s. Understanding this will provide valuable information on pathogens responsible for ARI in a region of the world where there are large gaps in surveillance for respiratory infections.

Hypothesis: We hypothesize that acute respiratory infection ARI and febrile illnesses in children and adults in Pakistan will differ in etiology, frequency, and antimicrobial resistance patterns from those of children and adults in Western countries.

Objective: The objective of this study is to determine the etiologies of ARI and febrile illness in different age groups in Pakistan, using several diagnostic modalities, including PCR and automated culture methods.

Methods: We are conducting a three-year longitudinal cohort observational study in Bilal Colony, which is a semi-urban area of Karachi, where we are following a cohort of 350 pregnant women from the first trimester and their newborns from birth through the duration of the study period. The participants are visited at home once every week to record any fever or respiratory symptoms and are then referred to an established medical facility in Bilal Colony for evaluation and treatment of the observed illnesses. Each episode of fever (100.4^0F/38^0C), influenza-like illness (in pregnant women) and severe pneumonia (in children) is eligible for laboratory testing of blood, and if any respiratory symptoms are present, then a nasopharyngeal swab is also obtained. Blood samples are collected for blood cultures and malaria immunochromatography test (ICT) is performed for the workup of febrile illnesses. Nasopharyngeal swabs are processed and analyzed by PCR for detection of bacterial and viral etiologies: *Chlamydophila pneumoniae, Mycoplasma pneumoniae, Bordetella pertussis,* Influenza A and B, H1N1 influenza, parainfluenza viruses 1-4, adenovirus, respiratory syncytial viruses A and B, human metapneumovirus, rhinovirus, and Coxsackie virus/echovirus family.

Results and Discussion: For the initial 9-month period of this study (August 2011 – April 2012), enrollment of pregnant women has been completed. To date, 123 women have delivered 59 newborns as live births. Seven deliveries were stillbirths and 30 spontaneous abortions were recorded, with the latter being commensurate with current

national rates of 1 in 7 pregnancies resulting in spontaneous abortions. Surveillance is underway for episodes of ARI and febrile illness, and identification of the infectious etiologies of ARE and febrile illness is yet to be determined with work-up underway. The results of this study will help establishment of empiric treatment algorithms for ARI and febrile illness in Pakistan and will provide the basis for future fundamental research.

4. **Capacity building and opportunities for professional development:**

    a. **Workshops:** Our group has developed a series of workshops centered around the timing of the visits of Dr. Hughes and Dr. Petri to Aga Khan University. Specifically, we have held ta total of 12 workshops (listed below on pages 9-11) during the project period of this project from 2011-2014.

5. **Opportunities for individual professional development - Student and faculty training:**

    a. **Student training:** As a direct result of our current U.S.-Pakistan collaboration, Dr. Hughes has **sponsored an Aga Khan University medical student (Sobia Nizami)** at the University of Virginia to participate in research for one month and a clinical infectious diseases rotation for one month (of note, no funding was requested or used from the DTRA award for Dr. Hughes to sponsor this student). Dr. Nizami has now completed her medical school training at the Aga Khan University and is doing her Medicine residency in the United States.

    b. **Faculty training: Najeeha Talat Iqbal, PhD is** a Senior Instructor faculty member in the Department of Pediatrics and Department of Pathology & Microbiology at the Aga Khan University spent 1 month (August 11th - September 11th, 2013) working in Dr. Hughes's laboratory at the University of Virginia to learn new immunological techniques. Dr. Iqbal was supported by a Fogarty International Training Grant awarded to the Aga Khan University, but this opportunity arose as a direct result of the collaborations that have been established because of this DTRA project.

6. **Impact from this project – Newly awarded grants as a direct result of our U.S.-Pakistan collaboration:**

    Grant award under grant type "Bill & Melinda Gates Foundation Grand Challenges" was submitted jointly by Drs. Ali, Zaidi, Bhutta, Petri, and Hughes in April 2012, and it was **awarded as of January 1, 2013**. The grant is entitled "*Identification of Novel Biomarkers for Environmental Enteropathy in Children using an Evidence-Based Approach".* This **two-year grant proposal synergistically built upon and required the cohort and study infrastructure established by our HDTRA1-10-1-0082 project.**

What opportunities for training and professional development has the project provided?

If the research is not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report." Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

III. OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT PROVIDED BY THE PROJECT:

1. **New and expanding collaborations developed during this DTRA project:** During the course of the DTRA project period, we have had the opportunity to expand and/or develop new collaborations with other Pakistani investigators in different departments and disciplines at the Aga Khan University. Specifically, these collaborations are with the following faculty at Aga Khan University:

    a. **Expanding collaborations:**

    Dr. Naveed Khan, Ph.D. (Professor and Chair, Biological and Biomedical Sciences, Aga Khan University) and **Dr. Ruqaiyyah Siddiqui, Ph.D. (**Assistant Professor, Biological and Biomedical Sciences, Aga Khan University). The collaboration between Drs. Khan, Siddiqui, Ali, Zaidi, and Hughes continues to expand. Within the past three years, we jointly planned multiple workshops during Dr. Hughes's visit to Pakistan (please see list of workshops below).

    Dr. Erum Khan (Associate Professor, Department of Pathology and Microbiology, and former President of the Pakistan Biosafety Association) has become a key collaborator for educational efforts in the form of symposia/ workshops on topics related to biosafety, biorisk management, as well as antimicrobial resistance, emerging pathogens, and multi-drug resistant pathogens. Dr. Khan and Dr. Hughes organized and conducted the following symposia/workshops during this past three years, including a set of workshops with a new collaborator, **Dr. Khalil Ahmed**, Chair of the Department of Microbiology at the Karakoram International University in Gilgit, Pakistan.

2. **Capacity building and opportunities for professional development:**

    a. **Workshops:** Our group has developed a series of workshops centered around the timing of the visits of Dr. Hughes and Dr. Petri to Aga Khan University. **There have been a total of 12 workshops & conferences held in Pakistan in the period of 2011-2014 as the direct result of the collaboration established through our DTRA grant:**

1. Infection, Immunity, & Inflammation (October 15-16, 2014) – This two-day workshop held at Aga Khan University focused on integrating concepts of microbiology, immunology, pathology, and pharmacology. Organisms discussed included viral (Ebola virus), bacterial (M*ycobacterium tuberculosis)*, fungal (*Aspergillus fumigatus)*, and protozoal (*Naegleria fowleri)* pathogens. There were 25 attendees and 6 trainers. Attendees were from various institutions in Karachi as well as Islamabad and Peshawar in Pakistan.

2. War on Terror Cells: Emerging Trends in Infectious Diseases (February 13, 2014) – This one-day workshop focused on discussing in depth the modern aspects of infectious diseases including examination of pathogens using state-of-the-art technologies, antimicrobial discovery and challenges of antimicrobial resistance in combating infections. There were 45 attendees and 6 trainers. Attendees were from various institutions throughout Pakistan.

3. Research Publications in Health Sciences (February 11, 2014) – This one-day workshop was designed to provide strategies for development of research papers publishable in journals of international repute. There **were 30 attendees and** 4 trainers. Attendees were from various institutions, primarily located in Karachi.

4. Biosafety Training Workshop 2 (October 23-26, 2013)
- This four-day workshop was held at the Karakoram International University in Gilgit, Pakistan. The purpose of the workshop was to provide more advanced biosafety training and hands-on practical session for laboratory personnel and healthcare workers from various institutions in northern Pakistan. There were 45 attendees and 10 trainers.

5. Biosafety Training Workshop 1 (June 11-15, 2013)
- This five-day workshop was held at the Karakoram International University in Gilgit, Pakistan. The purpose of the workshop was to provide basic biosafety training for laboratory personnel and healthcare workers from various institutions in northern Pakistan. There were 42 attendees and 8 trainers.

6. Emerging Superbugs Symposium and Workshop (February 25-27, 2013) – This three-day symposium + hands-on workshop focused on multi-drug resistant bacterial pathogens and antimicrobial susceptibility testing & reporting and was held at the Aga Khan University in Karachi, Pakistan. There were **52 attendees** and 8 trainers. Attendees were from institutions throughout Pakistan, representing all of the provinces and various cities including Karachi, Lahore, Islamabad, Rawalpindi, Peshawar, Quetta, Sukkur, Hyderabad, Faisalabad, and Abbottabad.

7. Scientific Writing Workshop (February 20, 2013) – This was a one-day workshop focused on scientific writing held at the Aga Khan

University in Karachi, Pakistan. There were 40 attendees. Attendees were from various institutions in Pakistan and Saudi Arabia.

8. Applied Molecular Microbiology Workshop (October 8-12, 2012) – This was a one-week workshop held at Aga Khan University in Karachi, Pakistan. There were 32 attendees. Attendees were from various institutions in Pakistan.

9. Developing Biorisk Management Systems in Research and Health Care Facilities in Pakistan (October 6, 2012) – This was a one-day symposium and workshop held at Aga Khan University with approximately 60 attendees from various institutions throughout Pakistan.

10. Grant Writing Workshop (February 14-18, 2012) – This was a 4-day workshop held at the Embassy Inn Hotel in Karachi, Pakistan. There were 36 attendees. Attendees were from various institutions in Pakistan.

11. Scientific Writing Workshop (February 13, 2012) - This was a one-day workshop held at Dow University Medical School in Karachi, Pakistan. Attendees were from various institutions throughout Pakistan.

12. Grant Writing Workshop (November 18, 2011) – This was a one-day workshop held at Aga Khan University with a focus on grant writing. There were 40 attendees from various institutions in Karachi.

c. Opportunities for individual professional development - Student and faculty training:

Student training: As a direct result of our current U.S.-Pakistan collaboration, Dr. Hughes has **sponsored an Aga Khan University medical student (Sobia Nizami)** at the University of Virginia to participate in research for one month and a clinical infectious diseases rotation for one month (of note, no funding was requested or used from the DTRA award for Dr. Hughes to sponsor this student). Dr. Nizami has now completed her medical school training at the Aga Khan University. As a direct result of her experience her at the University of Virginia School of Medicine, she has successfully matched to a U.S. Internal Medicine Residency training program, and she plans to pursue additional research opportunities during her training.

Faculty training: Najeeha Talat Iqbal, PhD is a Senior Instructor faculty member in the Department of Pediatrics and Department of Pathology & Microbiology at the Aga Khan University who will be spending 1 month (August 11th - September 11th, 2013) working in Dr. Hughes's laboratory at the University of Virginia to learn new immunological techniques. Dr. Iqbal was supported by a Fogarty International Training Grant awarded to the Aga Khan University, but this opportunity arose as a direct result of the collaborations that have been established because of this DTRA project.

How have the results been disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe how the results have been disseminated to communities of interest. Include any outreach activities that have been undertaken to reach members of communities who are not usually aware of these research activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

IV. DISSEMINATION OF RESULTS TO COMMUNITIES OF INTEREST

1. **Attendance at national/international meetings**:

   a. At the **2013 American Society for Tropical Medicine & Hygiene Conference (ASTMH) held in Washington, DC on November 13-17, 2013, two posters were presented by Dr. Hughes and Dr. Ali** with data directly generated from this project. The two abstracts were:

• **Abstract for poster #1 at the 2013 ASTMH conference in November 2013:**

Patterns of growth failure in infants in a rural district of Pakistan

Asad Ali[1], Tauseef Akhund[1], Najeeb Ur Rehman[1], Fayaz Ahmed[1], William Petri[2], Zulfiqar Bhutta[1], Anita Zaidi[1], Molly Hughes[2]

[1]Aga Khan University, Karachi, Pakistan, [2]University of Virginia, Charlottesville, VA, United States

Abstract: Malnutrition is major underlying factor in childhood morbidity and mortality in developing countries. Prevalence of chronic malnutrition is high in Pakistan, with a recent study showing 50% of the children being stunted at 18 months of age in a rural district. There is little data regarding patterns of growth failure in children in Pakistan. This pattern is important to study so the highest risk age for growth failure can be identified and appropriate interventions can be planned accordingly. We are conducting a longitudinal observational study of childhood growth monitoring in Matiari, a rural district in Sindh province of Pakistan. We have enrolled 817 infants between 0-1 months of age and are recording their weight and height at monthly intervals. Morbidity data regarding history of upper respiratory tract infections, diarrhea and fever is also being recorded on fortnightly basis. We found that the average weight for age z-score (WAZ) at 1 month was -1.68 for girls and - 1.84 for boys, which decreased further to -1.82 in girls and -2.06 in boys at 6 months of age. At 12 months the mean WAZ increased to -1.75 for girls and -1.99 for boys. Average height for age z score (HAZ) at 1 month was -1.64 in girls and - 1.75 in boys, which decreased further to -1.76 in girls and -2.07 in boys at 6 months of age. At 12 months the mean HAZ decreased further -2.3 for girls and -2.65 for boys. This detailed analysis of growth faltering pattern in children will help identify the ages when the children are most at risk for growth failure so appropriate interventions can be planned accordingly.

• **Abstract for poster #2 at the 2013 ASTMH conference in November 2013:**

Impact of respiratory illnesses during pregnancy on newborn's weight - A community based longitudinal study at an urban slum in Pakistan.

Asad Ali[1], Umber Zaman[1], Samana Zaidi[1], William Petri[2], Zulfiqar Bhutta[1], Anita Zaidi[1], Molly Hughes[2]

[1]Aga Khan University, Karachi, Pakistan, [2]University of Virginia, Charlottesville, VA, United States

Abstract: Birth weight is a powerful determinant of an infant's long term growth and survival. Although maternal health is widely believed to impact the birth weight of the baby, the exact factors during pregnancy that influence the birth weight are not clearly known. We are conducting a longitudinal observational study at Bilal Colony, a semi urban area of Karachi, Pakistan to assess the effect of maternal morbidities on the weight of the newborn. We are following 400 pregnant women from the first trimester onwards until their delivery. The pregnant women are visited weekly to record any fever or respiratory symptoms during the past seven days, and are referred to the study site clinic for treatment of observed illnesses. Each symptom episode is defined as one or more days of a self-reported symptom (fever, cough, difficulty breathing, runny nose, sore throat, head ache, chills or myalgia) in a pregnant woman who was symptom free for three days before. So far, 288 pregnancies have concluded as live deliveries, 12 as still births and 31 as spontaneous abortions. We analyzed the data of 243 pregnant women whose newborns were weighed within 14 days of birth. The average age of pregnant women in our study was 24.1years and average weight of the pregnant woman was 56.1kg at the time of enrollment. Only 31% of the mothers had primary education or above whereas 38.3% had antenatal visits during their pregnancy. There were 51(21%) newborns with low birth weight (< 2.5 kg), whereas 192 (79%) had normal birth weight (>= 2.5kg). In pregnant women who had a low birth weight baby, the average episodes of fever, cough, headache and myalgia were 1.7, 2.3, 4.3, and 4.3 per women respectively. In pregnant women who had a normal birth weight baby, the average episodes of fever, cough, headache and myalgia were 1.7, 2.1, 5.2 and 4.6 per women respectively. The results of this study will help identify the degree to which maternal respiratory illnesses during pregnancy are a risk factor for infant's low birth weight.

Drs. Petri, Hughes, Zaidi, Bhutta, and Ali attended the **2012 American Society for Tropical Medicine & Hygiene Conference in Atlanta, Georgia on November 11-15, 2012**. Dr. Ali and Dr. Hughes presented two posters at this meeting, and the posters/abstracts were as follows:

• **Poster #1 presented at 2012 ASTMH conference:**

Characterization of respiratory pathogens endemic to Pakistan in newborns, children, and adults in a rural area of Pakistan

[1]Ali A., [1]Akhund T., [1]Bhutta Z., [2]Petri W.A., [1]Zaidi A., and [2]Hughes, M.A.

[1]Department of Pediatrics, Aga Khan University, Karachi, Pakistan, and [2]Department of Medicine, University of Virginia, Charlottesville, Virginia, United States

Background: Acute respiratory infections, especially those associated with pneumonia account for almost 24% of all under-5 child deaths in Pakistan, which lead to an estimated 90,000 deaths annually. There have been no comprehensive studies of the etiology of severe pneumonia in Pakistan since the US-sponsored Board on Science and Technology in International Development (BOSTID) studies conducted in urban Rawalpindi in the 1980s. At the recent WHO study on Global AGEing and Adult Health meeting, the complete lack of information on endemic influenza strains and H1N1 was highlighted as a major barrier to informed decision making for vaccination strategies. The goal of our current study is to determine the etiology of acute respiratory infection (ARI) and febrile illness in different age groups in Pakistan, using several diagnostic modalities, including real-time PCR and automated culture methods.

Hypothesis: We hypothesize that ARI and febrile illnesses in children and adults in Pakistan will differ in etiology, frequency, and antimicrobial resistance patterns from those of children and adults in western countries.

Study design: This is a longitudinal cohort observational study of newborns, young children, and their adult household members with fortnightly surveillance throughout the duration of this three-year study. Sample size is 1000 participants for newborns and 2000 participants each for older children and adult household members. A WHO case definition of severe pneumonia is employed in this study, and cases of febrile illness are identified using temperature ≥ 101.3^{o}F (for newborns) or ≥ 100.4 ^{o}F (for young children and adults). Participants are evaluated by mobile medical teams for evaluation, collection of appropriate clinical samples, and delivery of medical treatment of the observed illnesses. Samples include nasopharyngeal swabs for workup of ARI by PCR identification of bacterial and viral etiologies (*Chlamydophila pneumoniae, Mycoplasma pneumoniae, Bordetella pertussis,* Influenza A and B, H1N1 influenza, parainfluenza viruses 1-4, adenovirus, respiratory syncytial viruses A and B, human metapneumovirus, rhinovirus, Coxsackie virus/echovirus family) and blood cultures and malaria immunochromatography (ICT) testing for workup of febrile illnesses.

Results: In the initial 6-month period of this study (October 2011 – March 2012), 94 cases of severe pneumonia in newborns with or without fever and 8 cases of newborns having fever ≥ 101.3^{o}F only have been identified, evaluated, and treated. In children 5 to 14 years of age, there have been 5 cases of fever ≥ 100.4 ^{o}F only and 7 cases of ARI

without pneumonia. In adults, there have been 8 cases of fever ≥ 100.4 ^{o}F with influenza-like symptoms and 7 cases of ARI without fever.

To date, 35 nasopharyngeal swab samples collected from newborns have been analyzed using Luminex RVP Fast kit multiplex assay. Of these samples, 16 samples were positive for enterovirus/rhinovirus, 2 samples were positive for RSV, 5 samples were positive for human parainfluenza virus type III/IV positive, and 12 cases were negative for the pathogens tested. Blood cultures were positive in 2 cases, with one case of *Campylobacter* and one case of *E. coli* bacteremia. Malaria ICT with confirmatory peripheral smear evaluation was positive in 2 cases, one of which was positive for *Plasmodium falciparum* and one of which was positive for combined *Plasmodium falciparum* and *Plasmodim vivax*.

Conclusions: In the initial period of our ongoing cohort, we have identified a number of cases of ARI or febrile illness. The number of cases of rhinovirus/ enterovirus responsible for severe ARI/pneumonia compared to other viral etiologies was higher than anticipated given the prior BOSTID study results. Unexpectedly, two cases of malaria were identified in febrile newborns using our study protocol; otherwise, these children may not have been evaluated for malaria as an etiology given the age group (newborns) and the time of year, which is a lower prevalence period for malaria in Pakistan. Of the two cases of bacteremia in newborns, one etiology was less common (*Campylobacter*) compared to the other (*E. coli*). Additional study data for enrolled participants will continue to be analyzed over a three-year period, which includes seasonal factors as well. The results of this study should promote establishment of empiric treatment algorithms for ARI and febrile illness in Pakistan and provide a basis for future fundamental research in this area.

• **Poster #2 presented at 2012 ASTMH conference**:

Characterization of respiratory pathogens endemic to Pakistan in newborns, children, and adults in a semi-urban area of Karachi, Pakistan

[1]Ali A., [1]Zaman U., [1]Bhutta Z., [2]Petri W.A., [1]Zaidi A., and [2]Hughes, M.A.

[1]Department of Pediatrics, Aga Khan University, Karachi, Pakistan, and [2]Department of Medicine, University of Virginia, Charlottesville, Virginia, United States

Introduction: Approximately 90,000 of all under-5 deaths in a year are associated with acute respiratory infections, especially pneumonia in Pakistan. There have been no comprehensive studies of the etiology of severe pneumonia in Pakistan since the US-sponsored Board on Science and Technology in International Development (BOSTID) studies conducted in urban Rawalpindi in the 1980s. Understanding this will provide valuable information on pathogens responsible for ARI in a region of the world where there are large gaps in surveillance for respiratory infections.

Hypothesis: We hypothesize that acute respiratory infection ARI and febrile illnesses in children and adults in Pakistan will differ in etiology, frequency, and antimicrobial resistance patterns from those of children and adults in Western countries.

Objective: The objective of this study is to determine the etiologies of ARI and febrile illness in different age groups in Pakistan, using several diagnostic modalities, including PCR and automated culture methods.

Methods: We are conducting a three-year longitudinal cohort observational study in Bilal Colony, which is a semi-urban area of Karachi, where we are following a cohort of 350 pregnant women from the first trimester and their newborns from birth through the duration of the study period. The participants are visited at home once every week to record any fever or respiratory symptoms and are then referred to an established medical facility in Bilal Colony for evaluation and treatment of the observed illnesses. Each episode of fever (100.4^0F/38^0C), influenza-like illness (in pregnant women) and severe pneumonia (in children) is eligible for laboratory testing of blood, and if any respiratory symptoms are present, then a nasopharyngeal swab is also obtained. Blood samples are collected for blood cultures and malaria immunochromatography test (ICT) is performed for the workup of febrile illnesses. Nasopharyngeal swabs are processed and analyzed by PCR for detection of bacterial and viral etiologies: *Chlamydophila pneumoniae, Mycoplasma pneumoniae, Bordetella pertussis,* Influenza A and B, H1N1 influenza, parainfluenza viruses 1-4, adenovirus, respiratory syncytial viruses A and B, human metapneumovirus, rhinovirus, and Coxsackie virus/echovirus family.

Results and Discussion: For the initial 9-month period of this study (August 2011 – April 2012), enrollment of pregnant women has been completed. To date, 123 women have delivered 59 newborns as live births. Seven deliveries were stillbirths and 30 spontaneous abortions were recorded, with the latter being commensurate with current national rates of 1 in 7 pregnancies resulting in spontaneous abortions. Surveillance is underway for episodes of ARI and febrile illness, and identification of the infectious etiologies of ARE and febrile illness is yet to be determined with work-up underway. The results of this study will help establishment of empiric treatment algorithms for ARI and febrile illness in Pakistan and will provide the basis for future fundamental research.

What do you plan to do during the next reporting period to accomplish the goals?
If there are no changes to the agency-approved application or plan for this effort, state "No Change." Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

V. PLANS FOR NEXT REPORTING PERIOD TO ACCOMPLISH GOALS

Plans for the next reporting period to accomplish our goals: Project has now ended but dissemination of information continues.

1. **Dissemination of information** - Continue to disseminate our findings through publications.

    - **There are 2 manuscripts submitted & under review and 1 additional manuscript in preparation from the DTRA research project:**

    1. Ali S.A., Zaman U., Mahmud S., Zahid G.S., Kazi M., Petri W.A., Bhutta AZ., Zaidi A., and Hughes M.A. Impact of maternal respiratory infections on newborn weight - A community based longitudinal study in a semi-urban site in Pakistan. *Manuscript submitted, 2015*.

    2. Ali S.A., Zahid G.S., Petri W.A., Bhutta AZ., Zaidi A., and Hughes M.A. Respiratory viruses associated with severe pneumonia in children under two years old in a rural community in Pakistan. *Manuscript submitted*, 2015

    3. Ali S.A., Zahid G.S., Petri W.A., Bhutta AZ., Zaidi A., and Hughes M.A. Malaria in children under five in rural Sindh, Pakistan – A two-year prospective study. *Manuscript in preparation.*

Characterize Respiratory Pathogens Endemic to Pakistan

Matiari, Pakistan

Final Report from 2013-2014

Sub-Award Principal Investigator

Dr. Syed Asad Ali

Assistant Professor
Department of Pediatrics & Child Health
Aga Khan University, Pakistan

Contents

1. BACKGROUND

Acute respiratory infections, especially those associated with pneumonia account for almost 24% of all under-5 child deaths in Pakistan, which lead to an estimated 90,000 deaths annually. There have been no comprehensive studies of the etiology of severe pneumonia in Pakistan since the US-sponsored BOSTID studies conducted in urban Rawalpindi in the 1980s. At the recent World Health Organization Study on Global AGEing and Adult Health meeting held October 26-29, 2009, the complete lack of information on endemic influenza strains and H1N1 was highlighted as a major barrier to informed decision making for vaccination strategies. The goals of this proposal are to determine the etiology of acute respiratory infection (ARI) and febrile illness in different age groups in Pakistan, using cutting edge diagnostic modalities including real-time PCR and automated culture methods.

2. Introduction

This is a longitudinal cohort observational study of newborns, young children, and their adult household members with fortnightly surveillance throughout the duration of this three year study. A WHO case definition of severe pneumonia is employed, and cases of febrile illness are identified using temperature $\geq 101.3^{o}$F. Participants are evaluated by mobile medical teams to obtain appropriate samples, and for medical treatment of the observed illnesses. Samples include nasopharyngeal swabs for workup of ARI by PCR identification of bacterial and viral etiologies and blood cultures and malaria ICT for workup of febrile illnesses.
The study objectives are:

- Understanding of endemic and epidemic ARI and etiologies of febrile illness in Pakistan as this will provide valuable information on pathogens responsible for ARI (including both severe and non-severe causes of pneumonia) in a region of the world where there are huge gaps in surveillance for respiratory infections.

- Establishment of empiric treatment algorithms for ARI and febrile illness in Pakistan.

- Training and technology transfer to Pakistani scientists to promote a sustainable capability for infectious diseases fundamental research.

Following case definitions are used:

a) Severe pneumonia: Fast breathing (> 60/minute in children < 2 months of age) along with severe chest in-drawing.
b) Fever in children: Fever > then 101.3 ^{o}F.
c) Fever in adult: Fever > then 101.3 ^{o}F with influenza like symptoms.
d) Diarrhea: 3 or more Loose stools in last 24 hours.

3. Site description

The study site is a rural district of Sindh, Matiari, which is located 200 km north of Karachi. It comprises of two towns and 1400 villages with an official population of 0.6 million. The area is largely agrarian with mostly contracted farmers. The health infrastructure in most districts consists of three levels: basic health units (BHUs), rural health centers (RHCs) and a referral hospital. Approximately 35% of the deliveries are at home by unskilled birth attendants and most neonatal deaths occur at home and not in health care facilities. The total population of study area is approximately 60,674 with total number of household as 9,437 (Hala perinatal and newborn care study 2005). Our research data shows that the birth rate in this population is around 25 per 1000 population (conservative estimate).

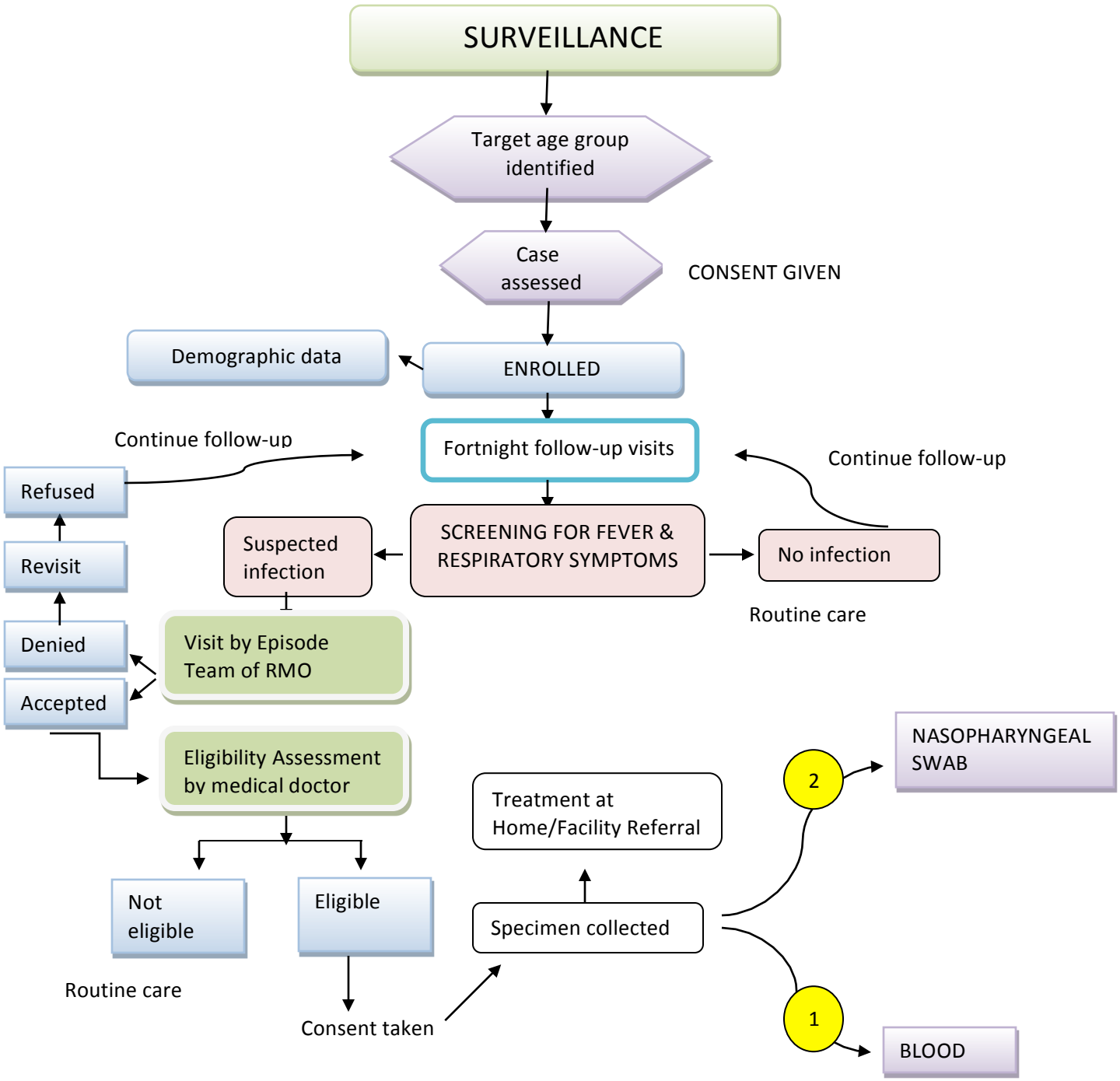
We have selected union councils Shah Alam Shah Ji Wasi and Sekhat/Khyber for the RESPAK study.

Table 1 below shows the demographic features of the selected Union councils.

Table 1: Study Site Characteristics

CHARACTERISTICS	DISTRICT PROFILE	STUDY SELECTED AREA
Total Talukas	3	0
Union Councils	18	2
District Hospital	0	0
Taluka Hospitals	3	0
RHCs	5	1
BHUs	20	1
Total villages	1,408	259
Total House Holds	74,925	9437
Population	5,04,956	60674
HH density	6.7	6.5
Child 0-59 months	94,555	12085
Live births in one year	17048	2212

4. Surveillance Methodology



- BLOOD CULTURE
- MALARIAL ICT
- BIO-BANKING

Newborns and their older household (HH) members identified by project community field workers are consented, enrolled, and followed fortnightly for the study period of 3 years.

Active surveillance for infectious disease/general child health includes frequent household visits. Participants were benefited from this active surveillance as field workers referred sick children to mobile medical teams of project / local care providers for the treatment of observed illnesses according to the local standard of care. Once a case is confirmed by a physician, samples were taken and transported to laboratory for further procedure.

This document is the fourth in the series of annual progress reports submitted to DTRA and the University of Virginia for the study "*Characterize Respiratory Pathogens Endemic to Pakistan*".

This report covers the progress made from July 2013 to June 2014. In this reporting period, the project study surveillance as well as personnel hiring & initial training plus refresher training of study staff, follow up of study subjects and monitoring and evaluation of RESPAK/DTRA study areas (2 Union Councils) were major milestones achieved.

5. Project Study Staff

In addition to staff hired last year, replacement staff was hired for those who resigned from job. The following staff is key personnel in this RESPAK study being conducted in Matiari district.

a. Research Manager

The Research Manager is responsible for monitoring of overall field activities (area mapping, baseline surveys, subject recruitments, episode enrolment, data management, quality control measures, project liaison and networking, etc.), and to ensure the completeness, proper filing and archiving of the CRFs. The Research Manager reports directly to the Principal Investigator on a regular basis.

b. Research Medical Officers (RMOs)

There are three Research Medical Officers (RMOs) in this study, and they have extensive experience in Pediatrics and General Medicine. They are responsible for validation of the suspected cases identified by the field surveillance teams or by parents or any other source and in the event of case enrollment. The RMOs take the consent from parents for the blood and respiratory samples and treat/refer the child to a health facility. They also fill in the clinical episode form, collect respiratory samples, and monitor phlebotomist performance during blood sampling.

c. Research Officer (RO)

The Research Officer (RO) is responsible for conducting quality control measures, for example, conducting re-interviews, observing interviews, performing spot checks on team activities in the field, reviewing forms, editing and checking forms as needed). The RO supervises the CHWs in order to ensure that data collection is done according to the stated instructions. The RO reports to the Research Manager on a daily basis about field activities, operational or administrative problems, and issues faced at a field site. The RO also prepares weekly reports on the field activities.

d. Research Assistants/Social Scientists (RAs/SSs)

Two Research Assistants validate randomly selected forms filled in by the CHWs, conduct follow up of refused cases, and counsel families.

e. Team Leaders (TLs)

Each Team Leader (TL) supervises a team of data collectors to ensure the data quality and timely completion of surveillance & follow ups. During surveillance, the TL meets with the community leaders to inform them about the RESPAK project, the estimated completion time of the study surveillance, and the subsequent visits. The TL is also responsible for 20% of the on-site validation of the work and reporting of the CHWs/FWs.

f. Phlebotomists

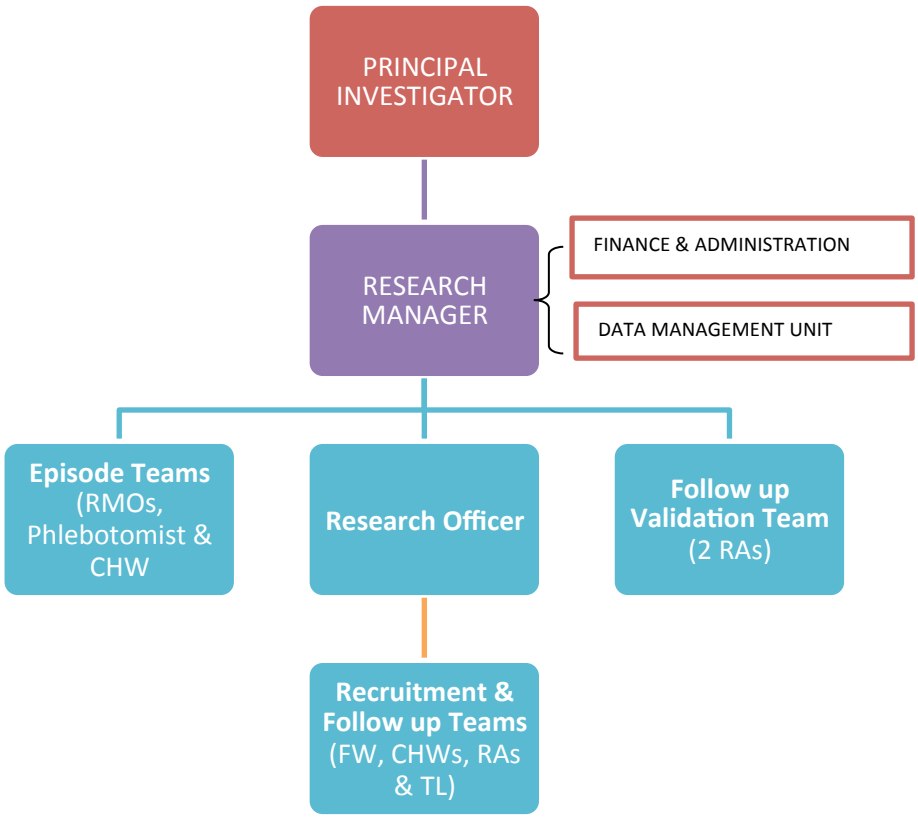
Three phlebotomists are involved in the RESPAK study to take the blood samples, label the samples, and fill the laboratory request forms. They direcrtly coordinate with laboratory technicians at the field office, maintain the records & registers, and transport samples to field laboratory under a strictly defined protocol.

g. Data collectors (Community Health Workers, Field Workers & Research Assistants (CHWs, FWs & RAs)

Data collectors were selected after a number of interviews done at the Matiari site. The CHWs & FWs were given the responsibility of 1) conducting house-to-house visits to identify pregnant women and target age group participants; 2) recruitment; 3) informing the community about the project aims and objectives; and 4) discussing the study aims with pregnant women and discuss whether they would be interested in inclusion of their newborn into the study. The data collectors are primarily responsible for the fortnightly follow-up visits, anthropometric measurements, and reporting cases of ARI/severe pneumonia and fever to the RMOs. In addition, they fill out verbal autopsy forms if a child expired during study period.

Fig 2: Team Organization Chart

Staff in the organization chart shown below has been trained for their specific roles in the RESPAK Study.



RMO= Research Medical Officer, FW= Field Worker, CHW= Community Health Worker, TL= Team Leader, RA=Research Assistant

6. Refresher Training

Training dates*:* Monday, October 07, 2013 and ongoing feedback as required.

Training participants: The team included community health workers/field workers, phlebotomists, team leaders, research assistants, research medical officers and research officer.

Training was conducted by: Dr. Tauseef Akhund (Facilitator), Dr. Fayaz Ahmed & Mr. Khadim Hussain (Co Facilitators).

Course Syllabus: Please refer to Appendix E.

Additional & replacement staff were trained regarding project aims & objectives, danger signs in children, and case report forms to be filled out for study. After completing the one day training session, new staff were accompanied by senior staff in the field for one

week before independent assignments were given to the new hires. All queries and questions were answered in detail after the end of each session/topic. In addition to this training session, participants were also briefed in detail about the consent forms to be used in the study & maintenance of records and registers of study, which include:

Logs / Registers:

- Newborn Recruitment & Follow up Register
- Recruitment & Follow up Register for Children of age 5 to 14 years
- Recruitment & Follow up Register for study participants of age 5 to 14 years
- Newborn Episode Enrollment Register
- Episode Enrollment register for Children of age 5 to 14 years
- Episode Enrollment Register for study participants of age 5 to 14 years
- Daily Activity register
- Patient referral slip
- Quality Control Register for equipment used
- Handing over sheet of forms to data management unit

Reports:

- Fortnight report format of the research officer
- Fortnight Report of the study

In addition to this training session, separate refresher training sessions were conducted for phlebotomists & RMOs for blood sample drawing and nasopharyngeal swab techniques by the Aga Khan University (Karachi) laboratory staff. Special emphasis was provided on how to avoid contamination while drawing samples during this training. QC/QA methods were reviewed in detail. The mechanism of sample transport was discussed and reviewed in detail.

7. Field Activities (data collection procedures & teams structures)

During the study activities, project reporting formats, infant and adult weighing machines, infant and adult height scales, stop watches, thermometers, BP apparatus and other equipment required for study were ordered regularly. Broken or nonfunctional equipment was replaced immediately.

a. Follow-up team

This team was responsible for fortnightly follow up of recruited study participants for any illness. CHWs enrolled 817 newborns along with children of 5 to 14 years of age and their adult household members (maximum of 2 household members per age group). Data collectors visited the enrolled study participants for the period of up to 3 years. During their follow ups, if cases of ARI and fever in the enrolled children/participants were detected, the data collectors informed the RMOs of the suspected case, and an RMO then evaluated and confirmed the case as soon as possible. This team was also responsible for monthly recording of anthropometric details of the recruited children.

b. Episode enrollment team

The team comprised of an RMO, phlebotomist, and CHW validated the suspected cases identified by the surveillance team or by parents or any other source. In the event of case enrollment, the RMO obtained consent from the parents to draw blood and collect respiratory samples, and treated the child or study participant according to the IMCI protocol or referred the child or study participant to the nearest health facility. The RMO also filled in the episode form.

8. Blood and respiratory specimens collection and processing

Phlebotomists collected the blood specimens from the field under strict aseptic conditions. Up to 4.0 ml of blood was collected from each infant by a trained phlebotomist. A maximum of two venipuncture attempts was allowed in a young infant. The specimens were then divided into 2 aliquots as indicated below in Table 2:

Table 2

BLOOD VOLUME OBTAINED	BLOOD IN ANTICOAGULANT TUBE	IN BACTEC BLOOD CULTURE BOTTLE
0.5 ml – 1.0 ml	Nil	Full volume
>1.0 ml to 2 ml	0.5 ml (only for molecular testing)	0.5-1.5 mL
>2 ml	1.0 ml (testing and bio-banking)	>1.0 mL

Respiratory samples were collected by the study physician (i.e., the RMO), and the samples were then transported to the Matiari field laboratory within 1-2 hours. At the field laboratory, blood samples were directly inoculated into a BACTEC pediatric blood culture bottle (with blood volume estimated by comparing the baseline, pre-inoculation bottle weight to the post-blood inoculation bottle weight, in grams). Blood cultures identified as positive for growth on the BACTEC instrument were sub-cultured on agar plates and sent to the Aga Khan University (Karachi) main laboratory where the organism was subsequently identified and tested for antibiotic susceptibility. Study personnel reviewed documentation of each case

of contaminated blood culture to identify possible reasons for contamination as an ongoing quality check.

a. Sample tracking system:

Specimen tracking was done manually and electronically. Phlebotomists filled the lab request form with 2 carbon copies. Original lab request form was kept at field lab and one copy was sent to the Aga Khan University (Karachi) central lab. Lab technicians at the field lab maintained the log of specimen collected in MS Excel sheets. Central lab staff also kept the records of all specimens received from the field lab. The specimen details were linked with the de-identified case numbers as well.

9. Monitoring / quality control

a. Community Health Workers (CHW's)

The performance of CHWs in field was monitored by study supervisors (research assistants, physicians, field supervisors and site supervisor). Study physicians and field supervisors assessed the counseling and infant examination skills of CHWs. All of the case report forms (100%) filled by community health workers were checked for completeness, and an additional 5% of the filled forms were verified and validated in the field for correctness of documented information by Research Assistants. In addition to this protocol, team leaders performed 20% onsite validations of CHW visits on the same day (See Appendix B).

b. Phlebotomists.

The study physicians observed 100% of blood specimen collection procedure by the phlebotomists for the correctness of aseptic techniques through a checklist (Appendix A), and another 5-10% of the blood draws were observed by field and site supervisors, respectively.

c. Field / site supervisors

The performance of the field/site supervisors was checked by the study investigators through fortnightly research meetings.

d. Research Medical officers

All of the validation of episodes (fever & severe pneumonia) reported by CHWs was conducted by the RMOs.

e. Equipment

Daily checks of equipment used for anthropometry for any errors were performed by the team leaders (Appendix C).

10. Data Entry

A data management plan was established at the beginning of the project. Data was collected by data collectors using standardized forms. Double data entry was performed locally, and discrepancies were resolved by the local site data manager. An additional error and logic checking program was run when the data was compiled in the local database, and problems were sent regularly to the site data manager for clarification. The data manager while keeping the original dataset intact made corrections or clarifications in a new field, including rationale for the changes.

For privacy purposes, participants in the study were assigned a number that is common to all of their associated data. Their names and other personal identifying data (e.g. address) were removed from the data before uploading to the local database. Data from our local laboratory assays were also entered into a standardized database that included the assay results, the date the sample was taken, and the child identification number. Samples were labeled with the barcode and child identification number and then stored in freezers at the appropriate temperatures.

11. Recruitment Status

In total 2833 cases have been recruited in all age groups (age group distribution information is provided in Table 3 below). Recruitment in the 5 to 14 years age group was less than expected. Children in this age group were absent from their homes during the project activity timings mainly due to out-of-home activities such as school, play, work, etc.

Table 3: Recruitment Status

S #	Age Category	Recruitment Status	Episodes
01	Newborns	817	450
02	5 to 14 years	655	97
03	15 to 45 years	1361	67

An additional 58 new episodes in children, 23 episodes in older children and 08 episodes in adults were identified and reported this year.

a. Drop outs

378 study participants dropped out of the study since the beginning of the project due to various reasons. Table 4 below lists the numbers and reasons for drop out from the study.

Table 4: Drop out status

S #	Reasons of drop out	Newborns	5 to 14 years	15 to 45 years
01	Refused	33	32	56
02	Migrated	63	55	95
03	Excluded	05	00	02
04	Expired*	32	03	02

*No deaths were related to this study.

12. Laboratory sample status

The age groups for whom laboratory samples were collected and the tests performed are listed in Table 5 below. In a few cases, the blood sampling and/or nasopharyngeal swab collections were refused/declined.

Table 5: Laboratory sample status

S #	Laboratory Tests	Newborns	5 to 14 years	15 to 45 years
01	Blood Culture	436	97	----
02	Malaria ICT	426	-----	----
03	Nasopharyngeal Swab	240	-----	67
04	Storage for future tests	193	97	67

13. Results

So far, as listed in Table 6 below, 262 cases of severe pneumonia in newborns with or without fever, and 188 cases of newborns having fever $\geq 101.3^{o}$F only, have been identified. In children 5 to 14 years of age, 97 cases of fever $\geq 101.3\ ^{o}$F only have been identified. In adults, 67 cases of fever $\geq 101.3\ ^{o}$F with influenza-like symptoms have been identified.

Table 6

S #	Age Category	Severe Pneumonia	Fever
01	0 to 5 years of age	262	188
03	5 to 14 years of age	----	97
04	15 to 45 years of age	----	67

To date, 230 nasopharyngeal swab samples out of 240 collected from newborns have been analyzed using Luminex assay respiratory viral panel (RVP) Fast kit. Of these samples, 175 samples were positive for viruses, 48 cases were negative, 7 cases showed inconclusive results and 10 samples are in process. As shown in **Table 7**, Enterovirus/rhinovirus, Parainfluenza 3 and Parainfluenza 4 are the main isolates. Different species of coronavirus and adenovirus were also responsible for infection in a few cases. As shown in **Table 8**, certain co-infection results were obtained whereby more than one potential etiologic organism was isolated (e.g., Coronavirus OC43 and Enterovirus/Rhinovirus were isolated from a single sample).

Table 7: Nasopharyngeal swab test results with 1 potential pathogen identified in newborns with ARI

S #	NUMBER OF INFECTIONS	TYPE OF INFECTIONS (SINGLE)
1	96	Enterovirus/Rhinovirus
2	08	RSV
3	13	Parainfluenza-3
4	7	Parainfluenza -4
5	1	Parainfluenza -2
6	1	Parainfluenza -1
7	5	Adenovirus
8	4	Influenza B
9	6	Coronavirus OC43
10	3	Coronavirus HKU1
11	3	Coronavirus NL63
12	4	Metapneumovirus
13	48	Negative
14	7	Inconclusive
15	10	In Process

Fig 3: Pie chart distribution of data for nasopharyngeal swab test results with a single etiologic pathogen from Table 7 identified in cases of ARI in newborns

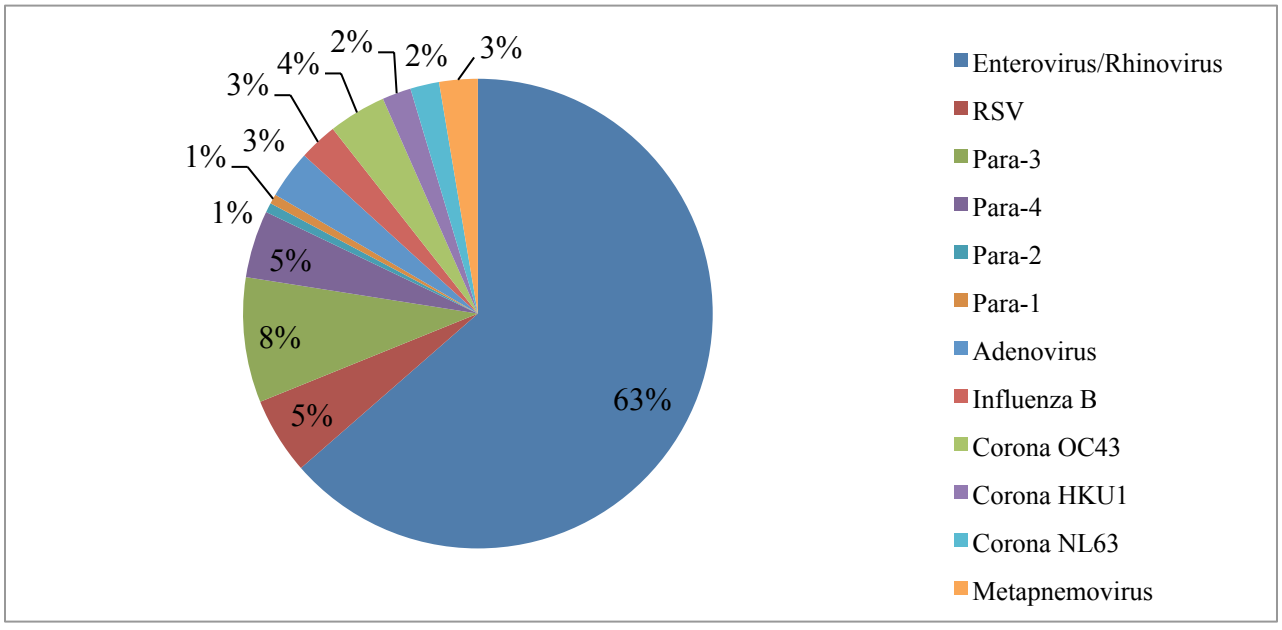
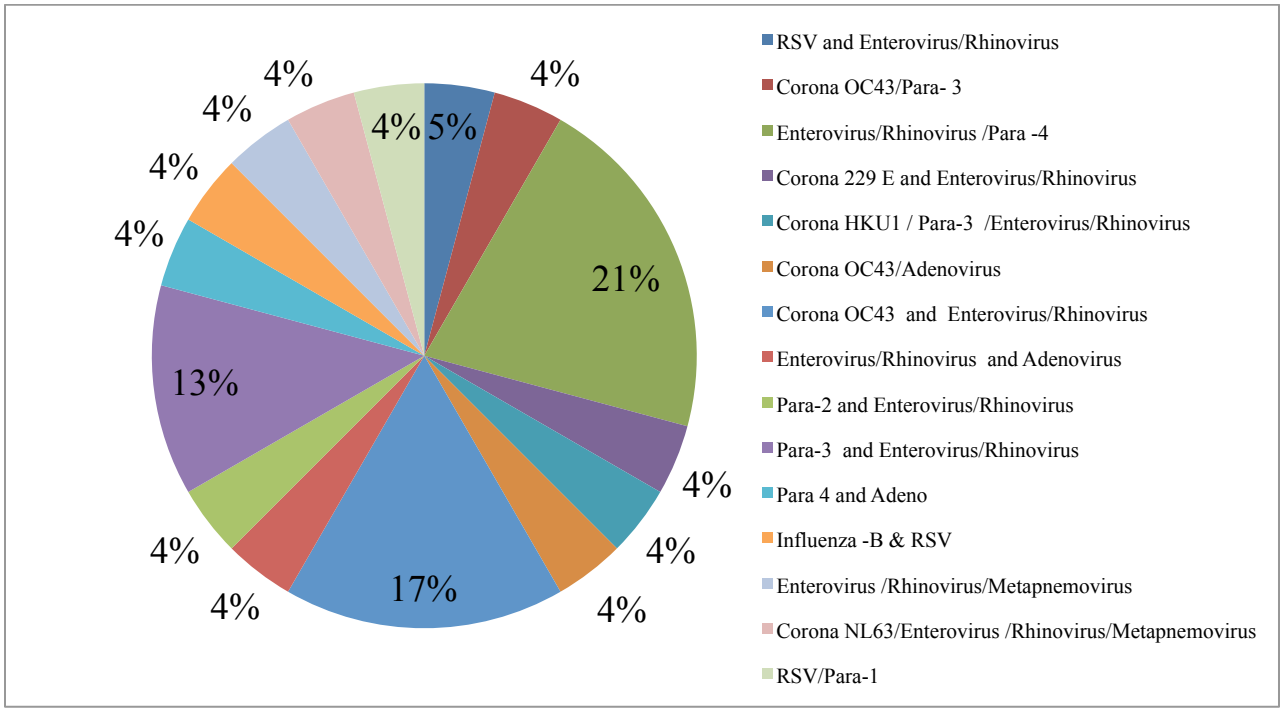


Table 8: Nasopharyngeal swab test results with >1 potential etiologic pathogen (co-infections) identified in cases of ARI in newborns

S #	NUMBER OF INFECTIONS	TYPE OF INFECTIONS (MIXED)
1	1	RSV and Enterovirus/Rhinovirus
2	1	Corona OC43/Parainfluenza 3
3	5	Enterovirus/Rhinovirus /Parainfluenza 4
4	1	Corona 229 E and Enterovirus/Rhinovirus
5	1	Corona HKU1 / Para influenza 3 /Enterovirus/Rhinovirus
6	1	Corona OC43/Adenovirus
7	4	Corona OC43 and Enterovirus/Rhinovirus
8	1	Enterovirus/Rhinovirus and Adenovirus
9	1	Parainfluenza 2 and Enterovirus/Rhinovirus
10	3	Parainfluenza 3 and Enterovirus/Rhinovirus
11	1	Parainfluenza 4 and Adenovirus
12	1	Influenza B & RSV
13	1	Enterovirus /Rhinovirus/Metapneumovirus
14	1	Corona NL63/Enterovirus /Rhinovirus/Metapneumovirus
15	1	RSV/Parainfluenza 1

Fig 4: Pie chart distribution of nasopharyngeal swab test results from Table 8 with >1 potential etiologic pathogen (co-infections) identified in cases of ARI in newborns



In adults, sixty nasopharyngeal swab samples have been analyzed to date (see Table 9). Of these sixty, two samples were positive for Enterovirus/rhinovirus, seven samples were positive for Influenza A, two samples were positive for influenza B, four samples were positive for SwH1/H3, and three samples were negative.

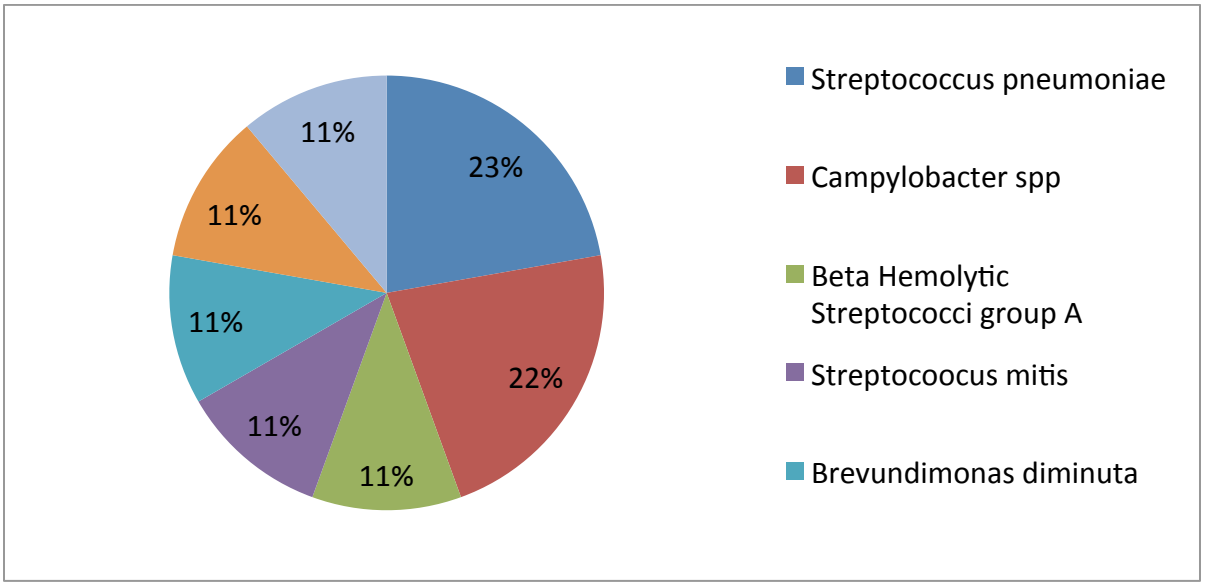
Table 9: Nasopharyngeal swab test results in age group 15 to 45 years old

S #	NUMBER OF INFECTIONS	TYPE OF INFECTIONS (MIXED)
1	02	Enterovirus/Rhinovirus
2	07	Influenza A
3	02	Influenza B
4	04	SwH1/H3
5	03	Negative

Blood cultures were positive in a total of nine cases, with two cases each of *Streptococcus pneumoniae* and *Campylobacter spp*., one case each of Group A beta-hemolytic streptococcus, *E. coli*, Streptocoocus mitis, and *Brevundimonas diminuta*. One case of *Salmonella spp.* bacteremia was detected in the older children age group.

In this past year (2013-2014) reporting period, we detected only one positive blood culture. We found fewer cases of high grade fewer and severe pneumonia as compared to last year, mainly due to increasing age of study children. In Pakistan, the neonatal sepsis rate is approximately 5 to 10%; therefore, it will be important to compare the neonatal sepsis rate in this study, which is being conducted in a rural area of country. At this point, the data indicate that the neonatal sepsis rate is lower in this rural area of the country.

Fig 5: Blood culture results



Malaria is common in this area of Pakistan, and this year, we detected 5 additional cases since our last reporting period, thus bringing the total number of malaria cases to 14. These cases were diagnosed on the same day as the blood sample for diagnosis was collected, so the study subjects were all promptly prescribed appropriate antimalarial drugs. All cases involved young children, and they all recovered well. **Malaria ICT showed *Plasmodium vivax* infection in 13 cases with one solitary case of *Plasmodium falciparum*. Malaria ICT results were then confirmed by examination of thick & thin blood smears at the main Aga Khan University laboratory in Karachi.**

These malaria data are surprising and of particular interest since the cases are being detected in young infants who otherwise would not even be tested for malaria by standard clinical approaches since this age group has traditionally not been thought to have malaria at such a young age. This malaria data may serve as a driving force for a change in clinical diagnostic algorithms in Pakistan and perhaps elsewhere.

Anthropometric data have been entered for all children enrolled in this study, and the data are currently being examined and undergoing error check process at this time (thus, data not shown yet). The anthropometric measurements are collected on a monthly basis, and the anthropometry/growth data will be correlated with ARI and febrile illnesses. Graphical presentation of height and weight data for one child is shown below to depict how anthropometric data are being collected for other children as well. This child had an episode of ARI/severe pneumonia and diarrhea in December 2013 and febrile illness in April 2014.

Figure: 6

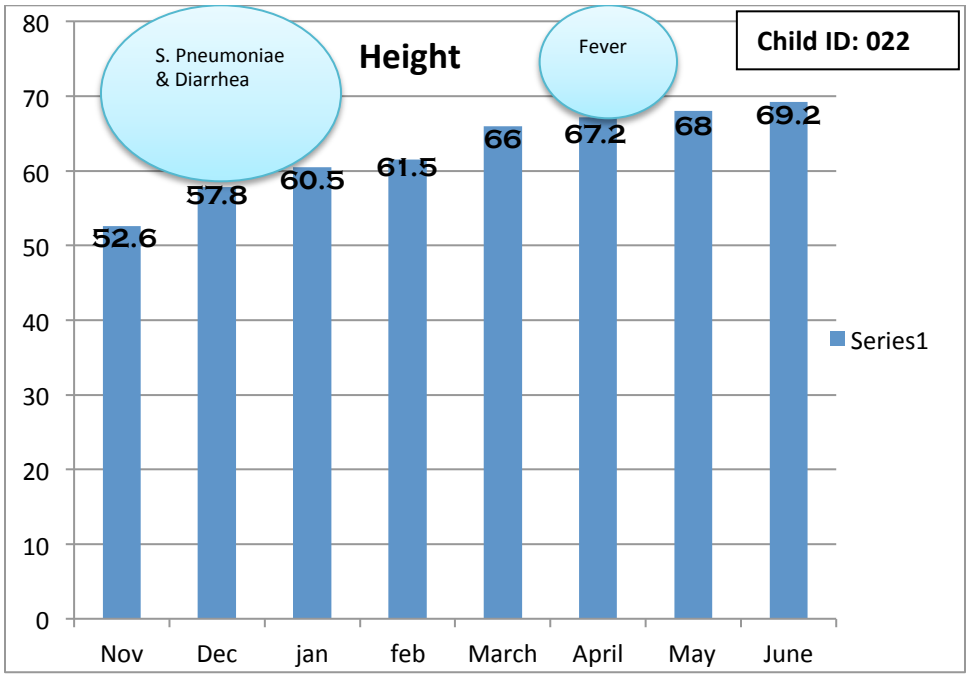
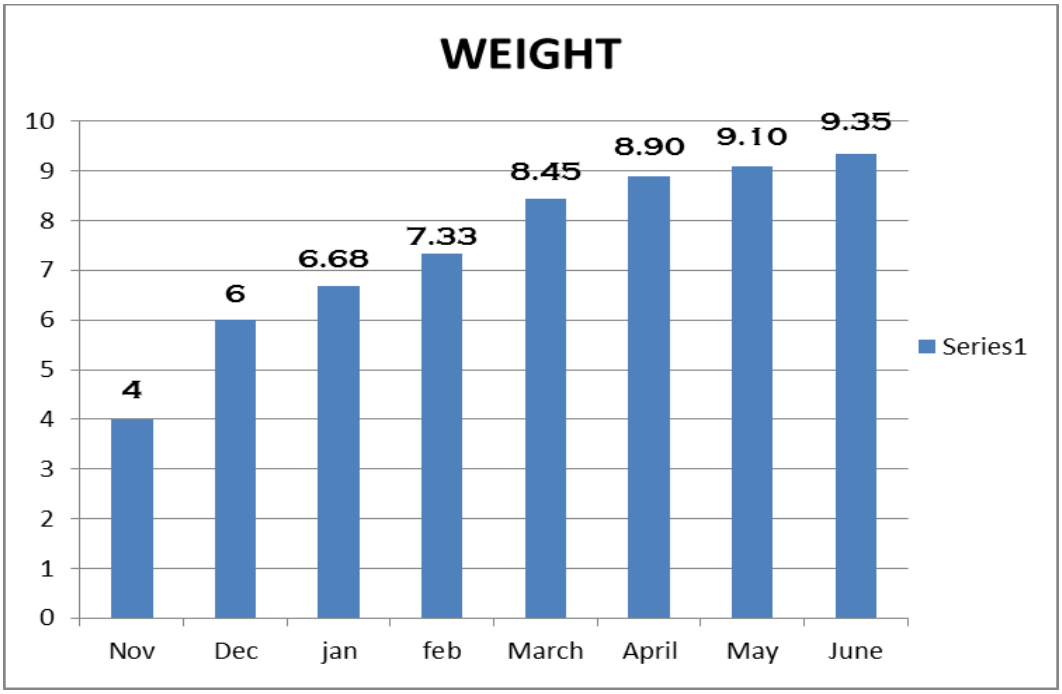


Figure: 7



14. Challenges faced in this study

- Adults & older children were often not present at home or at least at the time of the visit, resulting in failure to draw samples when they were sick
- Missing of episodes when study participant got sick during middle of the follow up
- Very high volume of phone calls to the RMOs asking for medicines, most of these for minor ailments
- Habitual callers to the clinic
- Recruited 2833 study participants; hence, the number of sick study participants also increased
- Sampling (a few cases refused blood sampling)
- Avoiding contamination (during the sampling process in the field)
- Community expectations were high
- There were some cases of dog bites to the study personnel during visit to households

15. Next steps

The study was completed at the end of the 2013-2014 Optional Year 4.

Appendix A: Blood sample collection and dispensing technique check list

Blood Culture Technique Observation Check List

To be completed and signed off by the Research Medical Officer at each collection

Phlebotomist's name: _____Observer's name: _____Date: _ _- _ _ - _ _ _ _

Child Name _____ResPak ID: _____

S.No	Activity	Remarks			
1	Was the rubber top of the BACTEC bottle cleaned with 70% alcohol Prep?	**Yes**	**1**	**No**	**2**
2	Was the protective cap placed back on the top of the bottle and the alcohol allowed to dry?	**Yes**	**1**	**No**	**2**
3	Were hands washed prior to wearing gloves?	**Yes**	**1**	**No**	**2**
4.	Was clothing removed from area and vein visualized/palpated before site cleansing?	**Yes**	**1**	**No**	**2**
5.	Gloves worn before cleaning the venupuncture site?	**Yes**	**1**	**No**	**2**
6	Was area of the venupuncture site cleaned in anticlockwise manner covering an area of 2-3 inches in diameter going from center to periphery?	**Yes**	**1**	**No**	**2**
7.	Was the following sequenced followed for cleaning the venupuncture site? 1. Cleaned with alcohol swab, let dry? 2. Cleaned with Pyodine, let dry? 3. Cleaned with alcohol swab, let dry?	**Yes**	**1**	**No**	**2**
8.	Was there any break in the sterility between cleaning the site and puncturing the skin? If yes, go to 8 a. If no, go to Q9.	**Yes**	**1**	**No**	**2**
8a	Vein puncture touched again after cleaning?	**Yes**	**1**	**No**	**2**
9	Was the blood dispensed in BACTEC bottle first?	**Ye**	**1**	**No**	**2**

		s			
10	Was the butterfly removed and a fresh needle used before putting blood in the BACTEC bottle?	**Yes**	**1**	**No**	**2**
11	In your opinion what was the level of difficulty in obtaining blood in this child?	**1.Easy** **2. Moderately difficult** **3. Very difficult**			

Additional instructions for observers:

1. Should not leave the alcohol swab on the bottle
2. Should clean the gloves with Purell and let dry before beginning site cleaning
3. Should not return to the center of the site once swab has moved outwards to the periphery and let the area dry completely
4. Once the site has been cleaned, should maintain aseptic precautions. If touches the site again for palpation should re-start the cleaning sequence of alcohol-pyodine-alcohol
5. Should dispense blood in the Bactec Bottle first

Not blood culture related but important for specimen collection

- Should gently release 1 ml of blood in EDTA tube through the rubber cap, slightly invert the EDTA tube up and down 2-3 times to mix the anti-coagulant
- Should discard sharp in the puncture resistant Danger Bin
- Should ensure proper specimen labeling and CRF completion

Observer Signature: _____

Appendix B: Validation Sheet used by team leaders

Instructions: To be filled by Team Leader for 20% of HH visited by CHW, on same day

Name of TL: _____ UC: _____

S.No	Name of child/study participant	Date	Address with Village	Name of house hold head	CHW visit date	CHW name	Did the CHW visit the HH? Yes/no *In case of No, confirm from at least 2 household member s*	Remarks
01								
02								
03								
04								
05								
06								
07								
08								
09								
10								

Appendix C: Weighting Machines (Biomedical) Calibration Log Sheet (To be filled by teal leader)

S #	Date	Standard Wt: (KG)	Variations (Kg)	Remarks/ Adjustment made to the machine?
1				
2				
3				
4				
5				
6				
7				
10				

Name of Staff: _____ Signature: _____

Appendix D: Medicine list

RESPAK Study (Medicines)			
Department of Paediatrics & Child Health Aga Khan University			
S.N	**Description**	**Field Site**	**Required**
1	Amoxil Forte suspension 250 mg		
2	Amoxil drops 125mg/ml		
3	Cefim susp 100mg/5ml (30ml pack)		
4	Ceclor Syrup Suspension +drops		
5	Panadol drops 80mg/ml		
6	Calpol syrup 120mg/5ml		
7	Triaminic flu		
8	ORS		
9	Zincat syrup (60ml pack)		
11	Flagyl susp 200mg/5ml		
12	Combantrin susp (20ml pack)		
13	Nilstat oral drops		
14	Ventolin Syrup 60ml		
18	Fucidin Cream		
19	Lotrix Cream		
20	Syp Gravinate		
21	Polyfax skin ointment		

22	Syp.Augmentin 156 mg		
23	Syp Nivaquin P		
24	Syp Sytron		
25	Normal Saline Nasal Drops		
27	Syp Acefyl Respiratory		
28	Syp Ridgix		
29	Tab Panadol		
30	Tab Tirlor		
31	Cefspan DS		
32	Tab Novidate 500 mg		
33	Cap Amoxil 500 mg		
34	Tab Stemetil		
35	Vi-dalyn drops		

Appendix E: Refresher Training Syllabus

Days	Topics	Time	Facilitators /Co Facilitators
1.	Introduction to project aims and objectives	9.30 – 9.45	Dr. Tauseef
	Responsibilities of CHWs, field workers phlebotomists, team leaders, RAs and RMOs	9.45 – 10.15	Dr. Tauseef
	Anthropometric Measurements (Including tea break) / practice	10.15 – 11.00	Dr. Tauseef/ Dr. Fayaz & Khadim Hussain
	Introduction to CRFs & consent form	11.00 – 11.30	Dr. Tauseef
	Tea Break	11.30 – 11.50	ALL
	Study CRFS	11.50 – 4.00	Dr. Tauseef

Characterize Respiratory Pathogens Endemic to Pakistan

Study Site: Bilal Colony, Karachi, Pakistan

Final Report

DTRA Sub-Award Principal Investigator

Dr. Syed Asad Ali

Assistant Professor

Department of Pediatrics & Child Health

Aga Khan University, Pakistan

Annual/Final Progress Report for Year 4 (2013 - 2014)

Study Title: Characterize Respiratory Pathogens Endemic to Pakistan

1. Study Aims:

1.1 Primary Objective:

- To determine the etiology of acute respiratory infection (ARI) and febrile illness in different age groups in Pakistan.

1.2. Secondary Objectives:

- To determine the risk factors of respiratory infections in children of different age groups in Pakistan.
- To identify respiratory pathogens (single, mixed infections) with the greatest impact on child growth and nutrition
- To identify the vulnerable times in early childhood that causes most disruption to growth and development
- To determine the effect of respiratory infections on nutritional status and growth from birth through 24 months of age.
- To determine the effect of nutritional factors on respiratory infections from birth through the first 24 months of life
- To determine the combined effect of respiratory infections and nutritional status on physical growth through the first 24 months of life

2. Study Tasks (by year):

2.1 Year 1 (2010-2011) - ***completed***

Task 1: Complete the local IRB and DTRA Human Research Oversight Board (HROB) protocol approval process before any human subject research begins in this study - *completed*

Task 2: Organize the cohort study sites (one rural site and one urban site), organize Infrastructure and personnel, train personnel for initiation of study - *completed*

Task 3: Recruitment of subjects to be initiated during the last 6 months of Year 1 in order to begin study cohort surveillance in Year 2 and recruitment to be continued through- *completed*

2.2 Year 2 (2011-2012) - ***completed***

Task 4: Surveillance for acute respiratory illness (ARI) and febrile illness episodes

Task 5: Sample collection/data measurements

Task 6: Microbiology characterization

Task 7: Data analyses

2.3 Year 3 (Year 2012-2013) - *completed*

Task 4: Surveillance for acute respiratory illness (ARI) and febrile illness episodes

Task 5: Sample collection/data measurements

Task 6: Microbiology characterization

Task 7: Data analyses

2.4 Year 4 (Year 2013-2014) - *completed*

Task 4: Surveillance for acute respiratory illness (ARI) and febrile illness episodes

Task 5: Sample collection/data measurements

Task 6: Microbiology characterization

Task 7: Data analyses

3. STUDY BACKGROUND

Approximately 90,000 of all under-5 deaths in a year are associated with acute respiratory infections, especially pneumonia in Pakistan. There have been no comprehensive studies of the etiology of severe pneumonia in Pakistan since the US-sponsored BOSTID studies conducted in urban Rawalpindi in the 1980s. Understanding this will provide valuable information on pathogens responsible for ARI in a region of the world where there are huge gaps in surveillance for respiratory infections. In this study we hypothesize that acute respiratory infection ARI and febrile illnesses in children and adults in Pakistan will differ in etiology, frequency, and antimicrobial resistance patterns from those of children and adults in the western countries. Therefore the goals of this proposal are to determine the etiology of ARI and febrile illness in different age groups in Pakistan, using cutting edge diagnostic modalities including real-time PCR and automated culture methods. We are conducting a longitudinal observational study at Bilal, semi urban area of Karachi, where we are following a cohort of approximately 350 pregnant women from the first trimester and then their newborns from birth through 24 months of age. The participants are visited at home once every week to record any fever or respiratory symptoms and are then referred for treatment of observed illnesses. Each episode of fever (100.4^0F/38^0C), influenza-like illness (in pregnant women), or ARI/severe pneumonia (in children) is eligible for laboratory testing of blood; if any respiratory symptoms are present, then a nasopharyngeal swab is collected.

4. RESPAK PROJECT TEAM

4.1 Sub-Award Principal Investigator:

Dr. Ali has been at the Aga Khan University (AKU), Pakistan since September 2008. Initially he was appointed as a Senior Instructor and was then promoted to Assistant Professor on January 1, 2010. He has devoted his efforts to this project as well as other respiratory and enteric pathogens research, other scholarly activities, and patient care.

4.2 Research Supervisor

Dr. Umber Zaman was hired for this project. She has a Masters in Epidemiology and Biostatistics from Aga Khan University and M.B.B.S from Dow University of Health Sciences. She has been affiliated with the Pediatric Research in Aga Khan University Hospital since 2006. She was responsible for monitoring overall field activities (area mapping, baseline surveys, subject recruitments, episode enrollment, data management, quality control measures, project liaison and networking, etc.), and to ensure the completeness and proper filing and archiving of the CRFs. Dr. Zaman reported directly to the Principal Investigator on a regular basis.

4.3 Research Medical Officer

Two Research Medical Officers, one each in Pediatrics and Gynecology/Obstetrics, contributed their time in this project. Dr. Farrukh Abbasi has been affiliated with the Department of Pediatrics at Aga Khan University for the past ten years. He is working as a Senior Research Medical Officer in a neonatal sepsis trial and contributed his efforts with this RESPAK study. Dr Naheed has been conducting the Gynecology/Obstetrics outpatient clinics for the past twelve months, and she contributed her efforts in this RESPAK study. The role of the Research Medical Officers was to take the subject's history, perform clinical assessment of the sick pregnant women/child coming at the referral healthcare facility, and provide appropriate management. Research Medical Officers work with the Research Officer to complete the clinical details of the illness in the study forms, including case information, lab specimen information, refusals, and or any other tasks pertinent to case enrollment.

4.4 Research Officer

One Research Officer, Ms. Samana Zaidi, has a Masters by qualification and has been affiliated with the Pediatric Research Department, Aga Khan University Hospital for at least two years. She was responsible for conducting quality control measures (re-interviews, observing interviews, spot checks, and forms editing). She also supervised the community health workers (CHWs) to ensure that data collection was done according to the stated instructions. She reported to the Research Supervisor on a daily basis about field activities, operational or administrative problems, and any issues faced at field sites; she also reported to the Principal Investigator whenever required. She was responsible for preparing the weekly reports of the field activities.

4.5 Community Health Workers (CHWs)

Six community health workers (CHWs) were initially hired for this project, and one more was hired in June 2012 due to the increased workload of newborn recruitments in the project. The role of the CHWs was to collect data by visiting households on a regular, scheduled basis. The roles and responsibilities of the CHWs in this project also included visiting recruited households at the study site on twice weekly basis, collecting information for all pregnant women and their newborns, and entering the data on a daily basis for the records collected from the households. The CHWs reported to the Research Officer about community-based activities and any problems and or issues faced at the community level. The CHWs reported to the Research Supervisor whenever required. They were responsible for preparing the daily reports of the field activities.

4.6 Phlebotomist

One phlebotomist, Ms. Misbah Mehmood, was recruited as a member of our RESPAK team in March 2012. She was responsible for taking a brief history of the sick pregnant women and /or sick child, who was directly seeking medical care at the sentinel healthcare facility or was being referred by a CHW from the community. She was responsible for explaining the procedure of the required tests to the pregnant women or parents of the child, collecting the specimens and sending them to the AKU laboratory safely, and maintaining log registers for all of the specimens collected. She reported directly to the Research Officer on a daily basis for her completed tasks and/or for any problems faced at the sentinel health care facility; she reported to the Research Supervisor whenever required.

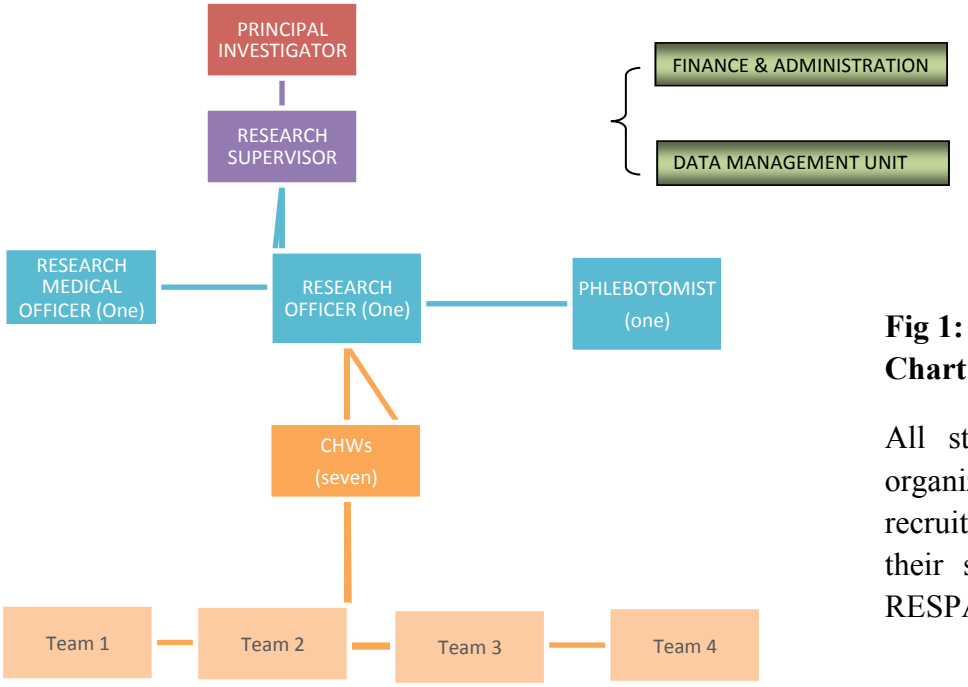


Fig 1: Team Organization Chart

All staff members in the organization chart were recruited and trained for their specific roles in this RESPAK study.

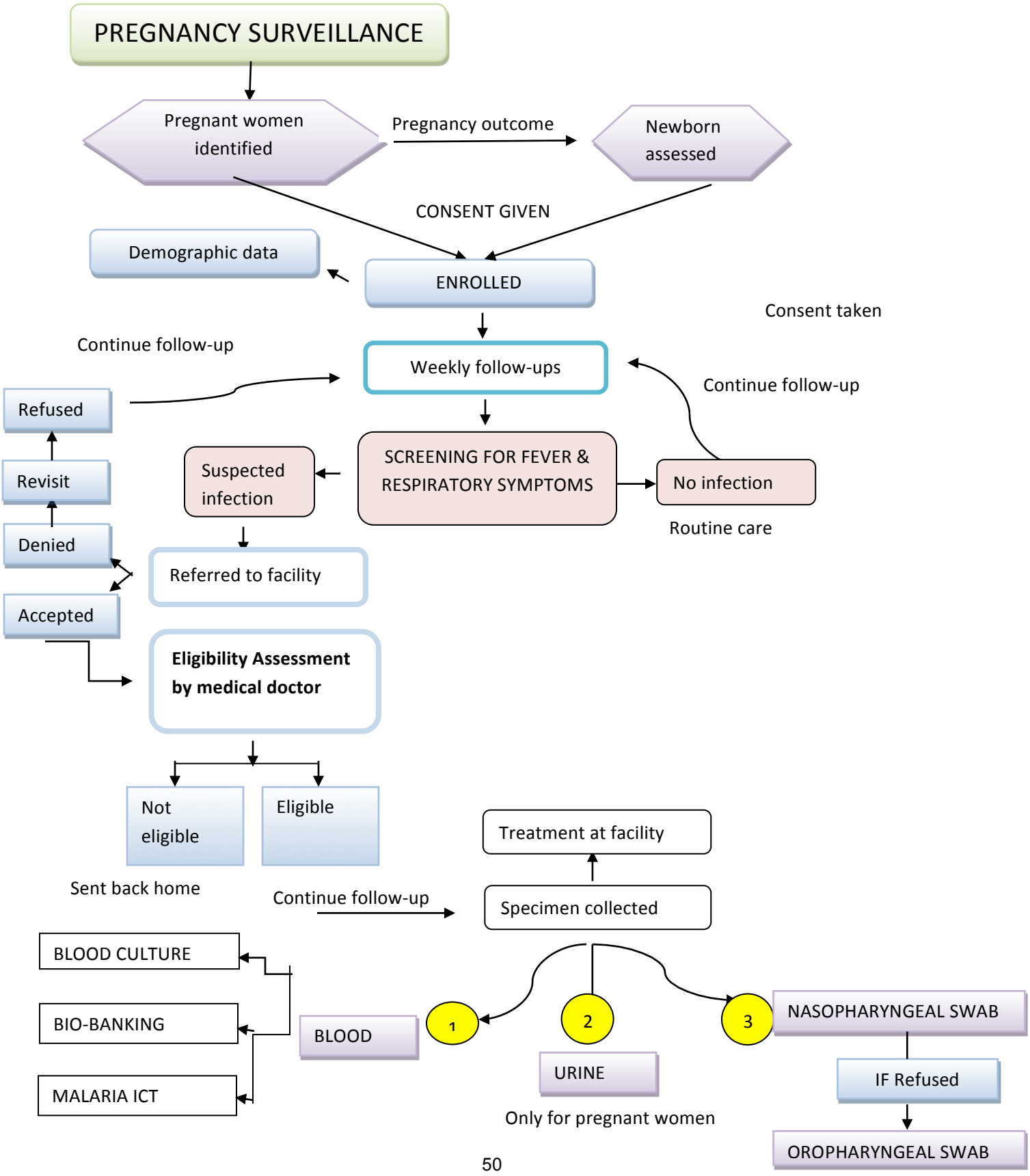
5. RESPAK STUDY METHODS

5.1 Study Procedure

A random sample of approximately 350 pregnant women was selected from the community for active surveillance after informed consent and a questionnaire regarding subject's socio-demographic and antenatal status have been obtained. The pregnant women were actively followed for any episode of ***influenza-like illness*** or ***fever (38°C)*** through once-weekly home visits by the CHWs. The study physicians evaluate any child who was brought to the AKU PHC by their parents/caregivers themselves or upon the advice of the CHWs. If a pregnant woman was eligible for any episode of ***influenza-like illness*** or ***fever*** based on the inclusion and exclusion criteria stated below, informed consent was obtained, and a questionnaire regarding the subject's clinical condition was administered. Blood was collected for culture and malaria immunochromatography test (ICT), a nasopharyngeal swab was collected for ARI workup, and the samples were immediately sent to the AKU research laboratory for processing and storage.

The pregnant women were followed throughout the study until the outcome of their pregnancies; the newborns of the women were recruited in the study and then followed from birth until 24 months of age. The CHWs visited the mother and child once every week to observe and take a history for respiratory illnesses.

Fig 2: Methodology flow diagram



5.2 Eligibility for the Study Enrollment

5.2.1 Inclusion Criteria

- ☐ Healthy pregnant women
- ☐ Captured at first trimester
- ☐ No plans to move out of our surveillance area
- ☐ Newborns of the recruited pregnant women in our study

5.2.2 Exclusion Criteria

- ☐ Parents of children who do not agree to participate in the study
- ☐ Pregnant women / Children having the same old episode for which they have been enrolled in episode once

  (A new episode of ARI was diagnosed only if the child was free of symptoms of respiratory infection for at least 1 week between the old and new episodes)

5.3 Eligibility for the Episode Enrollment

5.3.1 Inclusion Criteria for Pregnant women

- ☐ Influenza-like illness: Fever ≥ 100^0F, and cough and/or sore throat
- ☐ Fever ≥ $100.4^0F/38^0C$ only, without respiratory symptoms

5.3.2 Inclusion Criteria for Children

- ☐ Severe pneumonia (fast breathing with chest in-drawing present) with or without fever
- ☐ Fever ≥ $100.4^0F/38^0C$ only
- ☐ Fever associated with danger signs *(for 0-2 months only)*

(1) Chest in drawing (2) Central cyanosis (3) Unable to feed and drink (4) Lethargy (5) Unconsciousness (6) Vomiting (7) hypothermia

6. RESPAK STUDY DATA UPDATE

6.1 Study enrollment of pregnant women

The pregnant women surveillance has been completed, and the outcomes of 401 pregnancies are known, out of which 299 babies were delivered live. Thirteen deliveries were stillbirths, and 31 abortions were recorded, which is comparable to our national rates of termination of 1 in 7 pregnancies in abortions. No deaths were related to this study. A total of 689 episodes of sick pregnant women were recorded during the study, out of which there 9 eligible episode enrollments. There are 0.05 eligible episodes per pregnancy years of follow up (Table 1).

6.2 Study enrollment of newborns

From the live deliveries captured, 294 newborns were enrolled in this study. To date, 228 infants were followed via CHW household visits once every week for respiratory symptoms. A total of 623 episodes of all kinds of illnesses, and 67 eligible episodes in newborns were recorded. Eligible episodes per child years of follow up calculated to be 0.24 in the total of 277 years of follow up completed (Table 2).

Table 1: Study enrollment status of pregnant women *(as of 102nd week of RESPAK Study)*

	Total
Total number of recruitments of pregnant women	401
No. of end lines of pregnant women	401
Total # of live deliveries	299
Total # of abortions	31
Total # of still births	13
Total # of shifted/moved out of area	46
Total # of refusals	10
No. of currently followed pregnant women	00
Total number of sick pregnant women	689
No. of sick pregnant women referred BY CHWs	130
No. of sick pregnant women came by self	244
No. of NON-ELIGIBLE episode enrollments	681
No. of ELIGIBLE episode enrollments	9
Pregnancy weeks of follow up	9165
Pregnancy months of follow up	2291.25
Pregnancy years of follow up	190.93
Episodes per pregnancy years of follow up	3.61

Non-eligible episodes per pregnancy years of follow up	3.57
Eligible episodes per pregnancy years of follow up	0.05
No. of follow ups done	8903
No. of QC done	333
% of QC DONE for the number of follow ups DONE	3.74

Table 2: Study enrollment status of newborns *(as of 102nd week of RESPAK Study)*

	Total
Total number of newborns recruited	294
No. of end lines of children enrolled	66
Total # of deaths	15
Total # of shifted/moved out of area	40
Total # of refusals	09
No. of currently followed children	228
Total number of sick children	623
No. of sick children referred BY CHWs	352
No. of sick children came by self	271
No. of NON-ELIGIBLE episode enrollments	558
No. of ELIGIBLE episode enrollments	67
Child weeks of follow up	13305
Child months of follow up	3326.25
Child years of follow up	277.19
Episodes per child years of follow up	2.25
Non-eligible episodes per child years of follow up	2.01
Eligible episodes per child years of follow up	0.24
No. of follow ups done	10996
No. of QC done	635
% of QC DONE for the number of follow ups DONE	5.77

7. STUDY RESULTS UPDATE

7.1 RESPAK Eligibility episodes

In total, 9 eligible episodes of pregnant women were recorded, which included 4 episodes of influenza-like illness and 5 episodes of fever ≥ 100.4^0F/38^0C. In newborns. 67 eligible episodes were recorded as of the 102nd week of the study. These episodes included 49 episodes of fever ≥ 100.4^0F/38^0C, 17 episodes of any fever with danger sign, and only 1 episode of ARI/severe pneumonia.

Table 3: Eligibility Episodes *(as of 102nd week of RESPAK Study)*

S #	STUDY SUBJECTS	INFLUENZA-LIKE ILLNESS	FEVER ONLY	TOTAL ELIGIBLE EPISODES
01	Pregnant women	04	05	09

S #	STUDY SUBJECTS	SEVERE PNEUMONIA	FEVER ONLY	FEVER WITH ANY DANGER SIGNS	TOTAL ELIGIBLE EPISODES
01	Newborns	01	49	17	67

7.2 RESPAK Laboratory samples

The pregnant women samples collected during this study included 8 blood samples for culture, 8 urine samples for microscopy, and 4 nasopharyngeal swabs. In newborns, there have been 56 blood cultures and 55 nasopharyngeal swabs sent for testing. Some study subjects refused having the laboratory samples collected and evaluated.

Table 4: Laboratory samples *(as of 102nd week of RESPAK Study)*

S #	LABORATORY TESTS	PREGNANT WOMEN	NEWBORNS
01	Blood Culture	08	56
02	Urine microscopy	08	---
03	Nasopharyngeal Swab	04	55
04	Refused for sample	01	08

7.3 RESPAK Results of laboratory samples

Results were negative for all blood cultures and urine microscopy examinations of samples collected from the pregnant women in this cohort. Of the 4 nasopharyngeal swabs that were collected from pregnant women who met eligibility criteria for work up of ARI, there was 1 Influenza B positive sample out of the 4 tested by real-time PCR.

Table 5: Nasopharyngeal swab real time PCR (pregnant women) results

S #	NUMBER OF INFECTIONS (OUT OF 4 TOTAL) WITH POSITIVE TEST RESULT	TYPE OF INFECTION
1	00	Influenza A
2	01	Influenza B

The results of newborns samples of blood culture also showed no growth of any organism. The nasopharyngeal swabs, however, were positive in a number of cases - the results are detailed below in the following Tables 6 and 7.

Table 6: Nasopharyngeal swab test result: Single pathogen identified as potential etiology of infection in newborns evaluated for ARI

S #	NUMBER OF INFECTIONS	TYPE OF INFECTIONS (SINGLE)
1	11	Enterovirus/Rhinovirus
2	02	*Bordetella pertussis*
3	01	Parainfluenza-3
4	03	RSV
5	01	Corona NL63
6	01	Influenza A
7	01	Parainfluenza-4
8	01	Metapneumovirus
9	14	Negative
10	14	To be processed

Table 7: Nasopharyngeal swab test – Mixed infection or multiple pathogens identified as potential etiologies of infection in newborns evaluated for ARI

S #	NUMBER OF INFECTIONS	TYPE OF INFECTIONS (MIXED)
1	02	Rhinovirus/ *Streptococcus pneumoniae*
2	01	Rhinovirus/ Streptococcus pneumonia/ *Bordetella pertussis*
3	01	Rhinovirus/ *Bordetella pertussis*
4	11	Enterovirus /Rhinovirus
5	05	RSV/Enterovirus /Rhinovirus
6	01	RSV/*Streptococcus pneumoniae*/Rhinovirus
7	01	Rhinovirus/CYMV/*Streptococcus pneumoniae*
8	02	Enterovirus /Rhinovirus & Adenovirus
9	01	Enterovirus /Rhinovirus & Adenovirus & Bocavirus
10	01	Influenza B/ Adenovirus

8. RESPAK ANALYSIS PLAN

The results of this study will help in the establishment of empiric treatment algorithms for ARI and febrile illness in Pakistan and will provide a basis for future fundamental research.

Through this study, we will be able to provide information on the frequency of self-reported symptoms and frequency of physician-identified episodes of ARI or febrile illness during pregnancy in a semi-urban cohort of women.

In addition, data on weight gain during pregnancy will help optimize mother's weight gain chart showing percentiles of weight gain in our settings. Further associations of these risk factors with the various outcomes of pregnancy will be identified.

We are in the process of analyzing the data to determine the frequency of various symptom episodes (both eligible and non-eligible), physician visits, seasonal distribution of episodes, incidence and etiology of ARI during infancy in a semi urban cohort of newborns. Additionally, we will be able to generate child growth charts and percentiles of weight and height in our settings using the anthropometric data of our study infants. Along with the risk factors of respiratory infections and malnutrition in children, the associations between symptom episodes in mothers and children and growth in mothers and children will be explored.

8.1 RESPAK PREGNANT WOMEN DATA ANALYSIS

Analyses to identify whether maternal respiratory illness during pregnancy is a risk factor for infant's low birth weight (LBW) are underway.

The results of our analyses to date of continuous variables for the pregnant women samples are provided in Tables 8 and 9 below.

Table 8: Analyses of continuous variables of the pregnant women data

	LBW infants (n=51)		Normal weight infants (n=192)	
Continuous Variables	Mean(SD)	Median(IQR)	Mean(SD)	Median(IQR)
No of follow-up weeks of pregnant women	22.7(4.4)	23(12-32)	25.6(4.7)	26(11-35)
Age of child when birth weight is captured (in days)	4.7(3.4)	4(0-14)	5(3.7)	4.5(0-14)
Age of pregnant women (years)	23.0(3.8)	22(18-35)	24.4(4.5)	25(17-40)
Trimester of pregnancy		1(1-2)		1(1-2)
Age at first pregnancy (years)	18.8(2.2)		18.9(3.6)	
Para (number of pregnancies that led to birth)	1.97(1.3)	1(1-6)	2.8(1.79)	2(1-9)
Gravida (total number of pregnancies)	3.0(1.7)	3(1-8)	3.7(2.28)	3(1-11)
Weight at first visit (kg)	49.0(9.0)		53.6(11.7)	
Height of pregnant women (cm)	153.1(4.5)		152.6(11.5)	
No of people living in house hold	8.9(5.5)	8(2-25)	8.7(5.5)	7(2-34)
No of room in the household	2.5(1.5)	2(1-7)	2.4(1.4)	2(1-9)
No of children less than 5 years in household	1.2 (0.5)	1(1-3)	1.5(0.8)	1(1-8)
No children less than 5 years in household under pregnant women care	1.2 (0.5)	1(1-3)	1.5(0.6)	1(1-5)
Total score of household assets	154.3(141.9)	142(0-511)	201.1(179.0)	142(0-990)
Previous child gestational age at birth (in weeks)	28.3(14.1)	37(0-37)	29.1(14.1)	37(0-37)
This child gestational age at birth	36.6(1.2)	37(32-37)	36.7(3.9)	37(33-44)
Self-reported symptom episodes				
Fever episodes per 100 wks of f-up	7.2(7.4)	5(0-25)	6.8(7.9)	4.5(0-65.5)
Cough episodes per 100 wks of f-up	10.2(9.4)	10(0-35.2)	8.5(7.3)	7.1(0-32)
Difficulty breathing episodes per 100 wks of f-up	10.6(10.9)	8.6(0-41.1)	8.2(7.7)	6.8(28.5)
Runny nose episodes per 100 wks of f-up	10.7(8.5)	9(0-36.3)	10.9(7.3)	10(0-33.3)
Sore throat episodes per 100 wks of f-up	10.0(9.1)	8.3(0-33.3)	9.1(8.1)	7.4(0-8)
Headache episodes per 100 wks of f-up	18.7(13.3)	17.2(0-59)	20.4(13.1)	20(0-65)
Chills episodes per 100 wks of f-up	6.6(6.1)	5.5(0-23)	5.3(6.2)	3.8(0-40)
Myalgias episodes per 100 wks of f-up	19.0(9.8)	16.6(0-38.8)	18.0(9.0)	17.9(0-42.3)
Decreased activities episodes per 100 wks of f-up	16.9(8.6)	16.1(0-38.8)	15.3(8.6)	15.1(0-55.5)
Diarrhea episodes per 100 wks of f-up	3.3(4.3)	0(0-20)	4.6(6.8)	3.1(0-36.8)
Vomiting episodes per 100 wks of f-up	6.2(9.0)	3.5(0-32)	6.2(8.9)	3.3(0-45)
Infant anthropometrics				
Weight of the infant(kg)	2.1(0.2)		3.0(0.4)	
Height of the infant (cm)	45.6(2.5)		49.5(3.2)	
MUAC of infant (cm)	8.4(0.9)		9.8(0.8)	
OFC of infant (cm)	31.7(1.3)		34.2(1.4)	

Table 9: Analyses of the categorical variables of the pregnant women data

	LBW infants (n=51)		Normal weight infants (n=192)	
Categorical Variables	n	(%)	n	(%)
Using boiled water	10	19.6	40	20.8
Using flush toilet	0	0.00	2	1.0
First pregnancy	8	15.6	33	17.1
Antenatal visits during last pregnancy				
No previous pregnancy	8	15.6	33	17.1
Yes	15	29.4	75	39.0
No	27	52.9	78	40.6
Outcome of last pregnancy				
No previous pregnancy	8	15.6	33	17.1
Alive birth	33	64.7	136	70.8
Abortion	5	9.8	16	8.3
Still birth	3	5.8	4	2.0
Last child delivery place				
No previous pregnancy	8	15.6	33	17.1
Home	27	52.9	91	47.4
Maternity home	16	31.3	65	33.8
Piped water in house	16	31.3	71	36.9
Antenatal visits during this pregnancy	15	29.4	52	27.0
Number of antenatal visits during this pregnancy				
0	36	70.59	140	72.92
1	5	9.80	9	4.69
2	4	7.84	23	11.98
3 and above	5	9.80	16	8.33
Pregnant women education				
No education	35	68.63	126	65.63
Below matric	8	15.69	38	19.79
Matric and above	6	11.76	24	12.50
Main source of drinking water				
Piped into the house	16	31.37	71	36.98
Bought from tankers	16	31.37	63	32.81
Bought from drums	19	37.25	57	29.69

Characterize Respiratory Pathogens Endemic to Pakistan

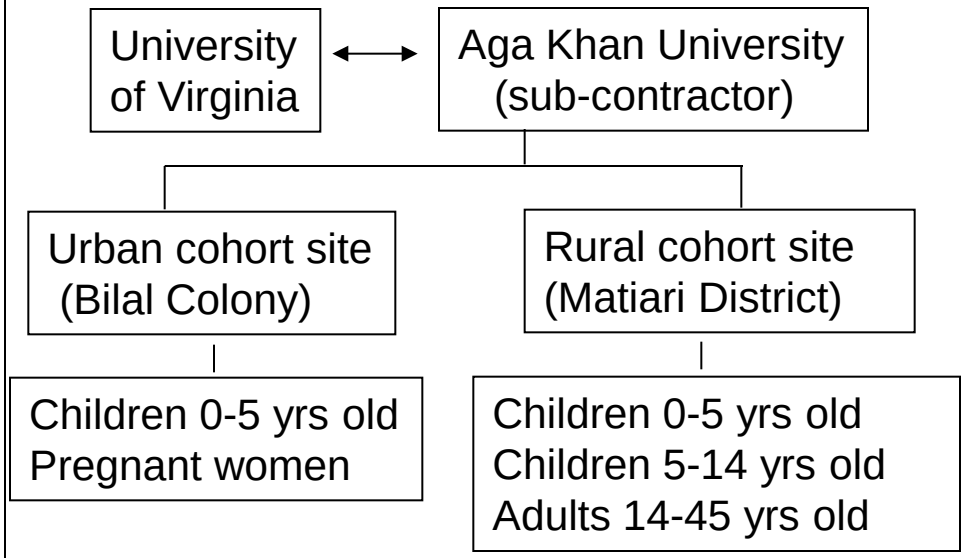
William A. Petri, Jr., University of Virginia

Grant number HDTRA1-10-1-0082

Description of Effort: The etiology of acute respiratory infection (ARI) and febrile illness in different age groups in Pakistan will be determined, using cutting edge diagnostic modalities including real-time PCR and automated culture methods. The study will be conducted in an urban and a rural field site in Pakistan.

Challenges:

- Identify infectious pathogens causing ARI and febrile illness in Pakistan.
- Distinguish endemic infectious causes of ARI from those caused by WMD and those caused by emerging infections such as H1N1 influenza.



Status of Effort: (1) Differentiation of ARI or febrile illnesses due to endemic infectious diseases vs. WMD; (2) Development of medical countermeasures to infectious diseases; (3)Training/technology transfer to Pakistani scientists to promote sustainable capability for infectious diseases fundamental research. **Personnel Support:** 2 faculty at UVA (Dr. William Petri; Dr. Molly Hughes) & 3 faculty at AKU (Dr. Zulfi Bhutta; Drs. Anita Zaidi, and Asad Ali). **Meetings of PIs:** Pakistan 7 visits, ASTMH (Atlanta10/10, Phila 11/11, Atlanta 11/2012, DC 11/2013); **Publications & workshops/symposia**:

4 posters (ASTMH), 2 manuscripts submitted, 1 manuscript in preparation, 12 workshops/symposia

Major goals/milestones by Project year:

Year 1: Complete IRB/HROB approval process, organize study cohorts, and begin recruiting subjects

Years 2-4: Surveillance and data analyses for ARI and etiologies of febrile illness

Funding profile:

Year 1: 08/01/10-07/31/11, $330,000 total project costs
Year 2: 08/01/11-07/31/12, $330,000 total project costs
Year 3: 08/01/12-07/31/13, $330,000 total project costs
Year 4: 08/01/13-07/31/14, $330,000 total project costs

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