

Influenza Research



ARAB REPUBLIC OF EGYPT

Damanhour Integrated Population Based Surveillance

Infectious diseases such as respiratory infections and diarrhea are among the most important causes of morbidity and mortality worldwide, especially in developing countries and among children under 5 years of age. In 2009, in collaboration with the Egypt Ministry of Health (MOH), the International Emerging Infections Program (IEIP) and the U.S. Naval Medical Research Unit No. 3 (NAMRU-3), an integrated population-based infectious disease surveillance system for multiple conditions was established in the Damanhour District of the Beheira governorate in Lower Egypt, to provide information on burden and cause of disease. The conditions include the following: acute respiratory infection (ARI), acute diarrheal illness (ADI), acute febrile illness (AFI), acute infectious neurological disease (AIND), tuberculosis, and influenza-like illnesses (ILI) was established.

Study Objectives:

Primary Objectives

- To estimate age-specific incidence rates of disease due to ARI, TB, ADI, AIND and AFI in the Damanhour District.
- To identify the main etiologies of these disease syndromes in the Damanhour District.

Secondary Objectives

The study also aims to determine the following for each syndrome:

- Detect early increases in incident cases.
- Describe the epidemiology (seasonality, geographic distribution, etc.).
- Measure resistance of bacterial pathogens to available antibiotics.
- Establish a repository of biological specimens from patients with syndromes of interest for future etiological studies in collaboration with the MOH.

Approach: Patients admitted to any of the participating hospitals or examined at the participating primary health care units and private clinics in Damanhour District are screened to determine whether they meet a case definition. Patients who meet a case definition and agree to participate in the project are asked for symptoms, demographic and risk factor information and then have appropriate samples taken to determine the etiology of their infections.

Timeline: ARI surveillance began in June 2009 in three government hospitals with AIND and AFI added in April and September of 2010 respectively. Three outpatient clinics began ILI surveillance in January of 2011, and in May 2011, ARI, AIND and AFI surveillance were expanded to two more private hospitals.

Progress and Findings: Since beginning surveillance, over 20,000 patients have been screened for ARI or ILI. Over 1,000 patients have been enrolled in ILI surveillance and over 4,800 were enrolled in ARI surveillance to date. Among 4,209 ARI naso- and oropharyngeal swabs collected, 12.7% (536) were positive for influenza virus. Among 829 ILI naso- and oropharyngeal swabs collected, 65 (7.8%) were positive for influenza virus. Additionally, 38 (4.6%) of naso- and oropharyngeal swabs from non-ARI or ILI cases tested positive for influenza virus, demonstrating that a small but significant proportion of influenza cases present with the absence of respiratory symptoms.

Conclusion: None yet.

Integrated Hospital-Based Infectious Disease Surveillance in the Greater Accra and Northern Regions

In 2002, infectious diseases accounted for 41% of all disability-adjusted life-years (DALY) lost and 41% of all deaths in Ghana. A 2004 WHO report on health status in Ghana indicated that approximately 20% of all infectious disease mortality was associated with respiratory infections, making it the third leading cause of infectious disease death in Ghana. Additionally, unexplained febrile illness and septicemia were among the five most common causes for clinic visits in children under 5 years of age. In collaboration with the Ghana Health Service (GHS), GDD/U.S. Naval Medical Research Unit No. 3 (NAMRU-3) and Noguchi Memorial Institute for Medical Research (NMIMR), an integrated hospital-based infectious disease surveillance system (IHBIDS) was established. Currently, 37 Military, Tema General, and Tamale Teaching hospitals participate as sentinel sites where surveillance for acute respiratory infections (ARI), acute diarrheal infections (ADI), and acute febrile illnesses (AFI) is ongoing.

Study Objectives:

- Determine the number of hospitalized cases due to each syndrome at participating hospital.
- Identify associated etiologies.
- Describe syndromic epidemiology.
- Strengthen local infectious disease surveillance capacity.

Approach: Demographic, and risk factor information as well as appropriate biological specimens are collected from eligible, hospitalized patients. Samples are processed at the NMIMR and NAMRU-3 (Cairo, Egypt) laboratories.

Timeline: In order to develop an effective multi-pathogen surveillance infrastructure, the study used a phased approach to initiate syndromic surveillance. ARI surveillance began in 2009 followed by ADI and AFI surveillance in 2010.

Progress and Findings: Preliminary results from IHBIDS data through September 2011 demonstrate that hospital-based influenza surveillance remains an important addition to ILI surveillance in Ghana, as it may differentiate between subtypes causing severe vs. mild illness. Among AFI patients, 20 influenza positive cases were identified (19% of all influenza positive cases). This finding demonstrates that severe influenza positive cases often present with undifferentiated febrile symptoms. Data also showed a seasonal distribution of hospitalized influenza cases peaking in June, coinciding with the annual rainy season. Influenza is a significant contributor to the etiology of both ARI and AFI.

Conclusion: IHBIDS data analysis demonstrates the effectiveness surveillance in detecting diseases of public health importance and providing the data to describe disease epidemiology, especially for AFI and ARI. In Ghana, IHBIDS syndromic surveillance can be leveraged to strengthen existing infectious disease outbreak early-warning systems and bolster efforts to gather data for sound public health decision-making.

This project is occurring in Egypt and Ghana.

BANGLADESH

Hospital Based Human Influenza Surveillance in Bangladesh

International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) collaborates with the Institute of Epidemiology, Disease Control and Research (IEDCR) of the Government of Bangladesh for the implementation of this influenza surveillance in 12 tertiary care hospitals from all seven administrative divisions of Bangladesh.

Study Objectives:

- To identify individuals and clusters of people who develop severe respiratory disease due to influenza virus.
- To characterize the diversity of influenza strains circulating in Bangladesh and causing both mild and severe respiratory disease.

Approach: Sentinel influenza surveillance in 12 hospitals (disease and virological surveillance).

Timeline: Initiated in 2007.

Progress and Findings: The surveillance is continuing in each of the participating hospitals to identify cases of influenza like illness in outpatient departments and severe acute respiratory illnesses (SARI), severe pneumonia and clusters of hospitalized cases (SARI and severe pneumonia) at the medicine and pediatric inpatient departments. The surveillance physicians at these hospitals are informing the surveillance personnel at IEDCR and icddr,b upon identification of clusters or suspected cases. During October 2010 to March 2011 a total of 1,044 samples were collected and tested in the laboratory to detect influenza A and B viruses. Among flu positive cases, there were 16 SARI patients (94%) and one severe pneumonia patient (4%). From October to March 2011, a total of 15 clusters of hospitalized severe respiratory disease were identified through this surveillance.

Kamalapur Population Based Surveillance

Community based surveillance for influenza and pneumonia in an urban slum of Dhaka, Bangladesh.

Study Objectives:

- To determine the incidence and seasonal distribution of influenza and other respiratory virus infections among all age groups, with continued emphasis on children less than 60 months old with signs and symptoms of acute respiratory illness, in a defined urban population.
- To identify the proportion of pneumonia associated with acute viral infection.
- To characterize the clinical signs associated with acute viral infection.

Approach: Active surveillance for respiratory illness among a defined urban population through weekly health screening and referral to a community clinic. Nasopharyngeal washes are collected from symptomatic participants and tested for influenza virus. A subset of influenza-positive samples are subsequently used for tissue culture and isolated viruses are typed and sent to CDC for further antigenic and antiviral resistance characterization.

Timeline: Initiated in 2004.

Progress and Findings: During March 2010 to March 2011, 3,898 children <5 years old and 1,412 persons ≥5 years old met the case definition for acute respiratory illness. Nasopharyngeal wash samples were collected from 772 children (20.0%) and 260 ≥5 years old subjects (20.0%) (every fifth child/adult who met case definition was recruited). All (100%) nasopharyngeal specimens were processed by RT-PCR to detect influenza virus. The influenza positivity rate among children was 13.7% and among ≥5 year olds was 2.7%.



Influenza B virus (Brisbane, Florida and Malaysia) was the predominant virus types circulating during this time frame. Influenza A H1N1 pdm 09 (32%) and influenza A H3N2 (7%) were less common. There was no seasonal influenza A H1N1 infection and no distinct seasonal peaks of influenza A were observed. The incidence of influenza was 10.1 children < 5 years per 100 child-years and 1.8 ≥ 5 years old per 100 person-years. All virus isolates have been cultured at icddr,b's virology lab. Monthly shipments of positive specimens have been sent to the influenza laboratory at CDC as well as monthly reports to CDC. Besides these as a part of enhanced surveillance 3,026 nasopharyngeal wash samples were collected from children <60 months and 1,061 from persons ≥5 years old from all eligible study participants who met the case definition. All samples were tested by RT-PCR. Positivity rate was 12% in children and 30% for ≥5 years old. There was co-circulation of influenza B and novel H1N1. Importantly, three human cases of avian influenza virus infection (two H5N1 and one H9N2) were identified in March 2011 among children less than 60 months old.

The surveillance site also continues to characterize the clinical signs associated with acute viral infection and serves as a critical platform for the Oseltamivir drug trial. The surveillance site also hosts an influenza vaccine trial to measure the influence of influenza vaccine on childhood pneumonia and incidence of influenza among household contacts of vaccinees. The site will continue to provide monthly influenza activity reports, as well as clinical and viral samples for vaccine surveillance.

Related Published Paper:

Brooks WA, Goswami D, Rahman M, Nahar K, Fry A, Balish A, et al. Influenza is a major contributor to childhood pneumonia in a tropical developing country. *Pediatr Infect Dis J* 2010;29:216-21.

Longitudinal Assessment of the Effect of Influenza on the Cognitive Development of Urban Poor Children, Bangladesh

Frequent, protracted, and severe influenza disease may cause cognitive developmental delays in very young children, as has been previously shown with other infectious pathogens. Substantiating whether frequent respiratory infections delay cognitive development is important because of the long term implications for affected children and their communities. This protocol proposes to study, during two years, the association between rates of influenza disease and delayed cognitive development in a birth cohort of urban poor children aged less than two years based in Mirpur.

Study Objectives:

- To assess the potential association between the frequency, duration, and severity of influenza disease and delayed cognitive development in a birth cohort.
- To assess the attributable risk of secondary tobacco exposure, indoor air pollution, and hand washing frequency, duration or severity of acute respiratory infections (ARI), and laboratory confirmed influenza infection in participating birth cohort children.
- To explore genome wide associations with frequency, duration, and severity of laboratory confirmed influenza disease in participating children.

Approach: Participants aged 3–12 months were enrolled in the study and followed for two years. During follow-up participants were evaluated for risk of delayed cognitive development, through quantifying individual incidence of ARI, laboratory confirmed influenza, and diarrhea. Annual cognitive tests were administered, and patients completed a survey in order to record a number of covariates. Field teams collected socio-demographic information from each of the enrolled participants and recorded indoor air particulate matter levels on monthly basis during 2009 for a sub-set of enrolled children. To assess the children's mental and psychomotor development, cognitive assessment teams administered the Bayley's Scale of Infant Development II. Children's behavior, language development and the quality of home stimulation were also assessed.

Study teams visited households every three days to administer a brief standard questionnaire using PDAs and to determine if participants developed ARI or acute diarrhea in the past three days. If field teams identified ARI case-patients, they recorded the onset and severity of symptoms and requested that the children be taken to the neighborhood clinic for sampling by nasopharyngeal washes. Respiratory samples were tested for influenza A and B, parainfluenza 1 and 3, RSV, adenovirus, rhinovirus, bocavirus, and human metapneumovirus by real-time RT-PCR. Blood samples were collected for genome-wide association analysis.

Timeline: This 2-year study started in April 2009 and finished at the end of March 2011.

Progress and Findings: We finished the second year of data collection and completed all field-related activities for the study on March 31, 2011. A total of 465 children aged 3-23 months were enrolled in the study. From October 2010 to March 2011, field research teams identified 400 episodes of acute respiratory infection and collected nasal and throat swab samples from them. Laboratory results demonstrated that 10% of these infections were caused by influenza. The Bayley's Scale of Infant Development has been administered on 397 children.

Between April 2009–March 2011, we followed 515 children for 730 child-years. We identified a total of 378 pneumonia episodes; 77% of the episodes were associated with a respiratory viral pathogen. The overall incidence of pneumonia associated with a respiratory virus infection was 40/100 child-years. The annual incidence of pneumonia/100 child-years associated with specific respiratory pathogens include the following: 12.5 for RSV, 6 for rhinoviruses, 6 for HMPV, 4 for influenza viruses, 3 for HPIV and 2 for adenoviruses.

Conclusion: Young children in Dhaka are at high risk of childhood pneumonia and the majority of these episodes are associated with viral pathogens. Developing effective low-cost strategies for prevention are a high priority.

Seroprevalence of Antibodies to Avian Influenza A Viruses among Poultry Market Workers

A study of poultry workers in markets found positive for presence of avian influenza virus through routine animal surveillance was performed, beginning in 2009. Multiple samples from live bird market surveillance have tested positive for H5 and unsubtypeable influenza A since the beginning of the project. These signals help study teams time the collection of sera from poultry workers 21 days after presumed exposure. Both pre-exposure and post-exposure paired sera specimens will be available to test for the antibodies to avian influenza A viruses. Serum specimens will be tested for neutralizing antibody against avian influenza viruses (H5, H2, H7 and H9) by microneutralization assay and confirmed by Western blot assay and/or modified haemagglutination-inhibition assay at CDC.

Study Objectives:

- To quantify the baseline seroprevalence of antibodies to avian influenza virus (e.g. H5, H9, H7, and H2) among Bangladeshi poultry market workers.
- To calculate the incidence of seroconversion during a one year study period.

Approach: Paired serosurvey among poultry workers.

Timeline: August 2009–April 2012.

Progress and Findings: Baseline sera was collected from over 400 poultry market workers from 11 live bird markets; baseline sera was also collected from 101 controls. Follow-up sera were collected from the enrolled workers in markets where environmental or poultry samples tested positive for avian influenza as part of ongoing animal surveillance. Serum samples have been shipped to the laboratories at CDC, where

serologic assays will be performed. Forthcoming laboratory test results will be merged with epidemiologic data for analysis. Investigators will calculate the incidence of seroconversion among poultry workers to H5N1 and other avian influenza viruses an important issue in a country in which 69% of the population raise poultry as an important protein source. The findings of the proposed study are expected to guide the response to H5N1 outbreaks and prevent the generation of novel influenza strains with pandemic potential.

Estimate Burden of Influenza Associated Mortality through Severe Acute Respiratory Infection in Matlab Population-based Surveillance

A recent study performed within a hospital-based surveillance platform documents a burden of 9 influenza cases per 100 person-years among persons of all age groups in Bangladesh. This influenza incidence rate is similar to rates calculated from a population-based surveillance site in urban Dhaka (10 per 100 person-years) and rates throughout Southeast Asia. These population-based efforts, however, have been unable to estimate influenza mortality. This longitudinal study aims to estimate the incidence of severe acute respiratory infection (SARI) and the mortality rate attributable to respiratory viral infections in Matlab, a rural sub-district of eastern Bangladesh during five months of the 2010 influenza season.

Study Objectives:

- To estimate the mortality rate due to influenza and other viral respiratory infections in this community-based surveillance site.
- To determine the sensitivity of commonly used influenza case definitions.
- To identify subpopulation at risk of dying with influenza.
- To quantify co-infections with preventable or treatable pathogens.

Approach: From June through October 2010, field staffs made weekly home visits and daily hospital visits to report potential severe acute respiratory infection (SARI) case-patients in the community or at the hospital. Criteria for reporting SARI case-patients included sudden onset of subjective fever, cough or sore throat and shortness of breath or difficulty breathing for persons over 5 years of age; and severe pneumonia or very severe disease or pneumonia for children under 5 years. A study physician visited potential case-patients to confirm diagnosis for enrollment, administered a standardized questionnaire to collect socio-demographic information, medical and treatment history, performed physical examination and obtained a nasopharyngeal swab from all enrolled case-patients. The physician also collected blood for culture from hospitalized case-patients. All swabs were tested for influenza A and B viruses, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), adenoviruses and human parainfluenza viruses (HPIV) type 1, 2 and 3 by real time RT-PCR. Blood was tested for *Streptococcus* species, *Staphylococcus* species, *Hemophilus* species, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Acinetobacter* species, *Pseudomonas* species and *Candida* species. Deaths of any SARI case-patient were identified and a nested case-control study was planned to identify potential risk factors for death among SARI case-patients.

Timeline: Data and sample collection was completed from June–October 2010. Data analysis was performed in October 2010–September 2011 and write-up is currently ongoing.

Progress and Findings: During the study period, 172 SARI case-patients were identified in a community of 113,660 persons including 13,115 children of less than 5 years of age. Among the case-patients, 141 were children less than 5 years of age. Respiratory viruses were detected in 82 case-patients, with most of them being under 5 years of age. The incidence of SARI attributable to respiratory viruses among children aged <5 years was 19.3/100,000 child-week for RSV, 3.5/100,000 child-week for HPIV3, 3.2/100,000 child-week for influenza and 1.8/100,000 child-week for adenovirus. The incidence of SARI attributable to respiratory viruses among those aged ≥5 years was 0.2/100,000 person-week for influenza and 0.04/100,000 person-week for RSV. No death among SARI case-patients was reported.

Conclusion: Respiratory viruses, and in particular RSV are commonly associated with severe acute respiratory infection among children under five years in this rural setting. Further studies to provide influenza mortality data are critical to understand the burden of influenza disease in Bangladesh and to appropriately prioritize influenza prevention and control efforts in a country with many competing health priorities.

Characterization of Children Hospitalized with Respiratory Illness

Study Objectives:

- To describe the clinical characteristics, viral etiology, and demographics of children admitted with respiratory illness.
- To identify what factors associated with prolonged hospitalization among children admitted with respiratory illness.

Approach: Building upon ongoing surveillance in four hospitals, surveillance staff enrolls children admitted to hospitals residing in the primary catchment areas of the hospital two times per month. Children <5 years of age with any of the following two and/or more symptoms: fever, cough, difficulty breathing consistent with pneumonia or respiratory distress syndrome are eligible for the study. Investigators collect information on existing chronic medical condition, symptoms, treatment prescribed, and outcome of each hospitalized child. All study methodology will remain unchanged. We will continue to collect throat and nasal swab samples from these children. The respiratory samples will be tested for influenza A and B including rhinovirus, RSV, human metapneumovirus and adenovirus using RT-PCR. Findings will be compared 1:1 to children of the same age (in years) with influenza-like illness who visit the outpatient department but are not admitted.

Timeline: October 1, 2010–September 31, 2012.

Progress and Findings: We have completed six months of data collection from children presenting with acute respiratory symptoms at the outpatient and inpatient departments of the four surveillance hospitals where the study is being conducted (i.e. Jahurul Islam Medical College Hospital, Bajitpur, Comilla Medical College Hospital, Comilla, Shahid Ziaur Rahman Medical College Hospital, Bogura and Sher-e-Bangla Medical College Hospital, Barisal). Between October 2010 and February 2011, we have collected data and respiratory samples from 75 hospitalized children and 243 children attending the outpatient department. The data collection will continue until September 2012. All of the respiratory samples collected are being tested for influenza A and B, RSV, human metapneumovirus, adenovirus, parainfluenza viruses and rhinovirus. A monthly report is disseminated to co-investigators and collaborators within icddr,b, the Government of Bangladesh and CDC.

Conclusion: The study will help characterize the complications and determine the associated factors that result in hospitalization of children with acute respiratory tract infection. It will also help us estimate the burden of respiratory viruses on hospitalized children. The findings of the study will be shared with the government and the participating hospitals. Investigators will summarize the findings for publication and presentation.

Advancing Hospital Infection Control Systems to Detect and Contain Emerging Infectious Diseases

Timeline: Initiated in 2007.

Progress and Findings: We have been conducting surveillance for hospital-acquired respiratory illness in three tertiary care facilities in Bangladesh since 2007. Beginning in 2008, we started collecting biological specimens from patients with new onset of respiratory disease during hospital stay as well as from health care workers with respiratory illness and tested these specimens for the presence of influenza viruses. We have estimated the incidence of hospital acquired respiratory disease in these facilities. Data from the first year of surveillance activities was published in 2010. Currently we are conducting our surveillance activities in three tertiary hospitals and one district hospital.

Between October 2010 and February 2011, we identified 106 cases of hospital-acquired respiratory illness and collected nasal and throat swabs for influenza testing. None of them were positive for influenza viruses. However, this reporting period spans a period outside of the regular influenza season in Bangladesh. During our hospital surveillance activities of 2008–2009, we had identified other respiratory viruses such as respiratory syncytial virus, human metapneumovirus, and parainfluenza virus which had been circulating in these facilities. Based on these findings, we plan to test this year's samples for other respiratory viruses as well. In addition to hospital acquired respiratory illnesses, we have leveraged the nosocomial surveillance platform and have been collecting information on the onset of hospital-acquired diarrhea in the study hospitals since 2008. We have estimated the incidence of hospital acquired diarrhea and anticipate publishing a manuscript on this topic later this year.

Related Published Papers: Gurley ES, Zaman RU, Sultana R, et al. Rates of Hospital-Acquired Respiratory Illness in Bangladeshi Tertiary Care Hospitals: Results from a Low-Cost Pilot Surveillance Strategy. *Clinical Infectious Diseases* (2010), 50 (8):1084-1090

Effectiveness of Pandemic (H1N1) 2009 Influenza Vaccine in Bangladesh, 2010: Program Evaluation

During 2010, WHO and other international partners donated 15.5 million doses of 2009 pandemic influenza A H1N1 (pH1N1) monovalent vaccine to Bangladesh in an effort to protect those at highest risk of complications from influenza illness. While the Government of Bangladesh vaccinated high-risk groups, we took the opportunity to estimate the effectiveness of the vaccine (VE) among health care workers (HCW), pregnant women and children. Measuring vaccine effectiveness is critical for a new vaccine as this estimation will help to recommend or decide for the use of the vaccine.

Study Objectives:

- To estimate the vaccine effectiveness among health care workers, pregnant women and children who received pH1N1 vaccine during the vaccination campaign.

Approach: Local health officials implemented a vaccination campaign during May–July 2010 with the technical assistance of WHO Extended Program on Immunization experts targeting prioritized high-risk groups (HCW, pregnant women and children more than 6 months to 5 years of age). Starting in May 2010, 12 geographically diverse hospitals and the vaccination centers in three unions (i.e. administrative units of Bangladesh with about 10,000 persons each) nearest to those hospitals were visited during the vaccination campaign, to randomly enroll participants recorded as vaccinated. Unvaccinated individuals were enrolled at a 1:1 ratio from the same hospitals and the same vaccination centers as vaccinated participants. Following enrollment, investigators telephoned and/or visited participants weekly to obtain nasal and oro-pharyngeal swabs during episodes of influenza-like illness (ILI, defined as sudden onset of fever with cough or sore throat) during the influenza season (May–October). Samples were tested for influenza by real-time RT-PCR.

Timeline: The field study was performed from May to October 2010, spanning the annual influenza season in Bangladesh. Data analysis was performed November 2010–June 2011 and manuscript writing is currently ongoing.

Progress and Findings: The data collection of this project was completed on October 31, 2010. Over 500 vaccinated HCWs, 1,000 pregnant women and 300 children and their controls were enrolled in the study. None of the participating children tested positive for pH1N1. Study participants who tested positive for influenza were infected with influenza A H3N2 and pH1N1 viruses as well as influenza B virus. Of seven HCWs who were infected with pH1N1, two were vaccinated compared to five unvaccinated. Of 11 pregnant women who were infected with pH1N1, five were vaccinated compared to six unvaccinated.

We are currently working on the final data analysis which will incorporate other components such as the total number of pandemic vaccine recipients and the cost for the vaccination campaign, and the knowledge, attitude and practices' regarding influenza and influenza vaccines among health care workers. We expect to finalize a manuscript summarizing our key findings over the next few months.

Conclusion: Although the vaccine seemed effective, low circulation of pH1N1 hampered our ability to differentiate the proportion of pH1N1 infections among vaccine recipients and those unvaccinated. Further studies remain to be performed to fully evaluate the vaccine effectiveness of influenza vaccines considered for distribution in Bangladesh.

CENTRAL AMERICA AND PANAMA (CDC-CAP)

Surveillance of Incidence and Disease Burden for Influenza-Like Illness (ILI) and Severe Acute Respiratory Infections (SARI) for Influenza and Other Respiratory Viruses in Population Cohorts in Central America

This study will increase the knowledge of influenza epidemiological data in the Central American Region, particularly on the incidence of influenza-like illness (ILI) and severe acute respiratory infection (SARI) associated to influenza and other respiratory diseases, their association to socio-demographic characteristics, and their risk factors. The study will provide valuable information that will complement sentinel surveillance in the area.

Study Objectives:

- Estimate the incidence of ILI associated with influenza and other respiratory viruses in two Central American cities.
- Estimate the burden of ILI associated with influenza and other respiratory viruses in two Central American cities.
- Identify protective and risk factors associated with the development of ILI and SARI associated with influenza and other respiratory viruses in two Central American cities.

Approach: This is an observational study prospective cohort study. Approximately 600 households in each site will be enrolled through multi-stage random sampling. Each participating household will be visited twice a week to detect new cases of ILI or SARI. Nasal and throat swabs will be taken from each enrolled case. The respiratory sample will be tested for influenza and other respiratory viruses through IFA and RT-PCR. Other respiratory viruses will be detected through IFA. Each confirmed case will be followed until symptoms subside.

Timeline: Household enrollment in Cartago, Costa Rica and San Marcos, Guatemala was during June, 2011. There have been a total 41 weeks of follow up to date and it will continue until August 31st.

Progress and Findings: Five hundred and ninety households are enrolled in Guatemala, 51% in San Pedro and 49% San Marcos. Since June, 2011, 64 respiratory samples have been collected, 25 (39%) were found positive for a respiratory virus, 6 (9%) for influenza A H3, 6 (9%) for influenza A H1N1, 8 (13%) for influenza B, 1 (2%) for parainfluenza 1 and 1 (2%) for parainfluenza 3.

Six hundred and seventy households are enrolled in Cartago, Costa Rica, 40% in Paraiso, 36% in Santa Lucia and 25% in Orosi. From June 2011, 301 respiratory samples have been collected. From these samples, 26 (9%) were found positive for a respiratory virus, 8 (3%) for influenza A, 17 (6%) for adenovirus, and 1 (0.3%) for parainfluenza 1. Respiratory samples are still pending laboratory analysis.

Conclusion: Although the last influenza season was weaker than expected, the cohort was able to detect several cases of ILI in both sites. Most of them were confirmed as influenza cases. The cohort continues to follow the enrolled families weekly and we suspect a stronger flu season approaching during 2012.

This project is occurring in Costa Rica and Guatemala.

Household Survey to Estimate the Prevalence and Burden of Disease, Symptoms, Associated Risk Factors, and Health Care Utilization Practices for Severe Acute Respiratory Infection and Influenza-like Illness in Chiriquí, Panama and San Pedro Sula, Honduras

The study aimed to characterize the prevalence, burden of disease, risk factors, and health care utilization practices of cases with severe acute respiratory infection (SARI) during the previous 12 months and cases of influenza-like illness (ILI) during the previous 30 days before the date of onset of the survey among the resident of the catchment area of the José Domingo de Obaldía Hospital (JDOH).

Study Objectives:

- To quantify the proportion of persons living in the catchment area of the JDOH who seek care at these sentinel sites when ill with respiratory illness through household surveys.
- To use health utilization data to estimate influenza-associated rates of SARI and ILI.
- To assess the economic burden of influenza-associated SARI and ILI among the study population through interviews about the direct medical costs and indirect medical costs (e.g. work absenteeism).
- To describe household characteristics, health care utilization practices, illness signs and symptoms, and risk factors in individuals identified with SARI and ILI among the study population.

Approach: We conducted a household survey using cluster sampling, stratified by urban and rural area. Clusters were selected proportionate to size. A questionnaire comprised of seven forms was administered to all household members (if present) in order to record the occurrence of SARI cases (in the last 12 months) and ILI cases (in the last 30 days), defined according to *PAHO-CDC Influenza Surveillance Generic Protocol*. For each disease syndrome we asked for symptoms, burden of disease, type of health care services that were sought, reasons for not seeking health care services, and related risk factors. Specimens were not collected.

Timeline: Data collection and analysis was conducted between August 2011 and February 2012.

Progress and Findings: Preliminary results showed that influenza vaccination coverage was 42% in Panama and 32% in Honduras (informed by the interviewees); just 17% in Panama and 9% in Honduras had a vaccination card. In general population ILI prevalence was 0.75% (95%CI: 0.16-1.34) in Panama 2.5% (95%CI: 2.02-2.96), slightly higher in women 0.88% in Panama (3.04% Honduras) and those aged 50-60 1.36% in Panama and those aged 20-30 (2.77%) in Honduras). In general population SARI prevalence was 0.28% (0.1-0.45) in Panama and 0.7% (0.41-0.99) in Honduras. Two persons died due to SARI (adult and child < 5 years old) both of them in the urban area in San Pedro Sula, Honduras. ILI prevalence in households was 2.73% (0.64-4.82) in Panama and 8.4% (6.97, 9.83) in Honduras. Urban prevalence was higher (2.99%) especially in some districts in Panama and rural prevalence was higher (19.35%) in Honduras. SARI household prevalence was 0.94% (0.33-1.56) in Panama and 2.53% (1.54, 3.53) in Honduras. Rural households had higher prevalence. 74% percent of ILI cases had difficulties to perform daily activities in Panama (51% in Honduras). The median for total days lost due to disability was two, partially lost one day in Panama and 4(p25-p75: 2, 8) in Honduras. 11% of the cases needed a person to take care of them 68% in Honduras. Care taker incomes loss was \$132 Balboas in Panama and \$11 in Honduras. SARI cases could not performed daily activities in 70% in Panama (77% Honduras) of them, 40% could not attend school in Panama (29% Honduras); 30% couldn't do housekeeping in Panama (24% Honduras) and another 30% did not work in Panama (18% Honduras). The median of days lost due to a SARI episode was seven days (p25-p75: 5, 8) in Panama and five days in Honduras; the median for days partially lost was five in Panama (three days Honduras). SARI case 90% of the patients had additional expenses in Panama and 73% of them such as transportation, medical attention, food, medication and diagnostic tests in Honduras.

Conclusion: When compared SARI and ILI prevalence preliminary results with those of the health utilization surveys previously conducted in Guatemala, El Salvador and Costa Rica, we found that these are lower probably due a low circulation of the virus. A data review of hospital discharges for the same weeks in previous years in comparison to 2011, showed a decreasing trend. Nevertheless, SARI and ILI prevalence's found in Honduras are higher than those found in Panama.

This project is occurring in Panama and Honduras.

Nicaraguan Influenza Birth Cohort Study

The proposed pediatric influenza cohort study will build upon resources and infrastructure established through the Nicaraguan Influenza Cohort Study (NICS) in Managua. NICS is performed in a low- to middle-income district of Managua served by the Health Center Sócrates Flores Vivas (HCSFV), to characterize the incidence and severity of influenza virus infection in children under the age of 2 years.

Study Objectives:

- Characterize the burden of influenza in children aged 0 to 2 years in Nicaragua.
- Examine risk factors for influenza virus infection and severity of disease.
- Investigate sequential infections.

Approach: Subjects will be recruited in the catchment area of the Health Center Sócrates Flores Vivas (HCSFV) in District II of Managua, and socio-demographic data will be collected. GPS data from subject households will be recorded, and respiratory (nasal and throat swabs) and serological samples will be collected. These specimens will be tested for influenza virus and antibodies and sera samples to determine Vitamin D levels. A weekly visit will be conducted to participant households to identify suspect cases and collect samples and to provide care or refer to the hospital. Anthropometric measures will be taken to establish nutritional status.

Timeline: The cohort was established in August 2010, and data collection will continue through August 2013.

Progress and Findings: As of March 31, 2012, 154 participants have been enrolled; study acceptance has been consistently high. 89.0% of the parent/tutors approached gave permission for their newborn's participation after a thorough consent process. All participants were aged 4 weeks or less at enrollment. The median age at enrollment was 17 days. At present, the cohort is composed of 147 participants, 78 females and 69 males. Respiratory samples were collected from all participants meeting the influenza-like illness criteria. All samples collected were nasal swabs. A total of 81 samples were collected until March 31, 2012. To date, 100% of the samples have been processed. Two samples were positive for Influenza A H1N1 2009. Respiratory infections are rare in extremely young children, but become more common as children age. All participants transferred to the hospital for pneumonia (7) have been screened by PCR for other respiratory viruses, namely Respiratory Syncytial Virus (RSV), Parainfluenza 1-3, Rhinovirus, Metapneumovirus and Bocavirus. One participant tested positive for Rhinovirus.

Conclusion: None yet.

Viral Circulation and Influenza Seasonality in Central America

This study aims to describe influenza and other respiratory virus circulation in the Central American Region from 2002 to 2010. The systematization and interpretation of this information will allow improving influenza epidemiological knowledge in tropical developing countries for a better decision making process on prevention and control of influenza and other respiratory viruses.

Study Objectives:

- Describe the viral circulation of influenza in the Central American Region during 2002–2010.
- Describe the viral circulation of other respiratory viruses in the Central American Region during 2002–2010.
- Determine influenza seasonality in the Central American Region during 2006–2010.

Approach: Through a descriptive observational ecological study, the National Influenza Centers' databases from 6 countries from the region were analyzed. The aggregated or individual monthly databases from Guatemala, Costa Rica, Nicaragua, Honduras, Panama and El Salvador were analyzed. An influenza positive case was defined as any case positive for influenza by any laboratory diagnostic test (IFA, RT-PCR, viral culture). A case positive for any other respiratory virus was defined as any sample positive for this virus through IFA, the currently established diagnostic method. Monthly positivity proportions were calculated for each virus. GLM and ARIMA models were constructed to define influenza seasonality.

Timeline: January 2011 to July 2012.

Progress and Findings: From 2002–2010, the National Influenza Centers from these six Central American countries processed a total of 67,757 respiratory samples. Until 2008, the annual average increase of respiratory samples was of 648 ($R^2=0.70$). On 2009 due to the pandemic effects, there was a significant increase in the number of samples analyzed in the region. The regional increase in 2009 was of 546.39% compared to 2008. The proportion positive for any respiratory virus was 27.4%. The percent positive was over 15% all year long, but it peaks during the months of July to October. Regionally during 2002–2010, monthly average influenza positivity percent was of 12.6%, 7.4% for respiratory syncytial virus, 4.4% for parainfluenza and for 3.6% for adenovirus. With the available data, influenza seasonality statistically proven for El Salvador, Nicaragua, Panama and Guatemala. Influenza circulation increases in the middle of the year, similar to the southern hemisphere seasonality. But in each country, influenza seasonality pattern is slightly different. The data available is limited and affected by the pandemic; more annual data should be collected to better describe seasonality in the region.

Conclusion: For the first time, circulation of respiratory viruses has been described for Central America. This analysis shows the improvement the region has made on respiratory viruses' diagnosis, particularly influenza. The analysis demonstrates that respiratory viruses circulate all year long in tropical countries, keeping monthly average positivity rates over 15%. But influenza circulates with different patterns throughout the region.

This project is occurring in Guatemala, Costa Rica, Nicaragua, Honduras, Panamá and El Salvador.

Demographics and Clinical Characteristics of Influenza A H1N1pdm Deaths in Central America and Dominican Republic during the 2009–2010 Pandemic

The epidemiology of influenza A H1N1pdm09 among low-income tropical countries during the last pandemic is not well documented. Based on data from national surveillance systems of 7 countries in Central America, we identified persons who died with influenza A H1N1pdm09 infection during 2009–2010. Using unified methodology and a web based database we explored the demographics and clinical characteristics. We include areas such as the use of antivirals, antibiotics, radiology, timing from onset to a visit to a health care provider, and pre-existing medical conditions.

Study Objectives:

- Explore the demographics and clinical characteristics of persons who died with influenza A H1N1pdm09 infection during 2009–2010.
- To identify potential risk factors for severe outcomes among influenza A H1N1pdm09 deaths in Central America and the Dominican Republic.

Approach: We identified influenza-associated deaths by hospital-based surveillance of severe acute respiratory infection (SARI). A case of influenza-associated death was defined as a person with SARI (defined as sudden onset of temperature $>38^{\circ}\text{C}$, cough or sore-throat, and shortness of breath or difficulty breathing requiring hospitalization) who tested positive for influenza A H1N1pdm09 rtRT-PCR in the two weeks prior to death. We then abstracted decedents' demographic and clinical information from medical records and described these characteristics through proportions using Chi square, T-test and ANOVA as appropriate.

Timeline: May 2009 to June 2010.

Progress and Findings: We identified 185 cases of influenza A H1N1pdm09 decedents. The median age was 33 years and 48% were aged 15–44 years. One-hundred and two (55%) were female of which, 21 (18%) were pregnant, and 12 (14%) were in their puerperium. Nine (43%) of 21 pregnant women had an underlying medical condition (5 [24%] had asthma, 2 [10%] were obese, 2 [10%] had cardiac disease, and 1 [5%] had diabetes). Of the 113 cases (61%) that had a pre-existing medical condition, 26 (23%) were obese, 25 (22%) had diabetes, 22 (19%) had asthma, 16 (14%) had other chronic metabolic diseases, 11 (10%) had chronic obstructive pulmonary disease, 11 (10%) had seizure disorder, and 7 (6%) had cerebral palsy. Sixty-nine percent of cases received treatment with oseltamivir, but only 9% received it within the first 48 hours of symptoms onset. There was no statistically significant difference in the average amount of time elapsed between symptom onset and health seeking by, age groups ($p=0.4$), or country ($p=0.2$). Among the 75 case-patients with CXR, 24 (32%) had consolidation or complete opaqueness at the time of admission and 19 (25%) developed these later during their hospitalization.

Conclusion: The pandemic affected the young and those with pre-existing medical conditions. Most patients sought health care too late for oseltamivir to provide much benefit. Based on the results it is recommended to review the indications and availability of oseltamivir in the country.

This project is occurring in Guatemala, El Salvador, Honduras, Costa Rica, Nicaragua, Panamá and the Dominican Republic.

Influenza Vaccine Effectiveness in El Salvador in 2010

Despite the substantial increase in the uptake of influenza vaccines since 2004 in Central America, no assessment of its effectiveness has been published to date. In order to support gains in vaccine coverage and explore the value of expanding vaccination, we conducted a retrospective case-control study among vaccination target groups in El Salvador to estimate effectiveness for 2010.

Study Objectives:

- To measure the effectiveness of the trivalent seasonal influenza vaccine against laboratory-confirmed severe acute respiratory infections (SARI) cases due to influenza, among children aged 6–23 months and adults aged over 60 years hospitalized at influenza sentinel hospitals in El Salvador in 2010.

Approach: We conducted a retrospective case-control study in 25 sentinel surveillance hospitals in El Salvador. The study population consisted of several target groups for vaccination: children aged 6–23 months and adults aged over 60 years. We reviewed SARI surveillance and national reference laboratory data. A SARI patient was defined as having suffered sudden onset of fever, cough or sore-throat, and difficulty breathing that required hospitalization. We identified influenza-positive hospitalized SARI case-patients and influenza-negative controls enrolled in surveillance. We used RT-PCR results for influenza illness confirmation. We compared the proportion of vaccinated among case-patients and among controls. We calculated vaccine effectiveness as 1-odds ratio. For each influenza case-patient, we matched a control of the same age. We collected information on demographics, SARI symptoms, underlying conditions, previous and current influenza vaccinations, respiratory sample taken and laboratory results.

Timeline: September 2010–2011.

Progress and Findings: We identified 227 SARI case-patients with complete information (SARI status, vaccination status, laboratory results); 157 children aged 6–23 months and 70 older adults aged over 60 years. Of 227, 43 case-patients had a positive test for influenza; 29 children and 14 older adults. Baseline characteristics did not differ between influenza-positive case-patients and influenza-negative controls. Twenty-two case-patients (51%) and 29 controls (67%) were vaccinated. Among children, 16 case-patients (55%) and 22 controls (76%) were vaccinated and among older adults, 6 case-patients (43%) and 7 controls (50%) respectively. Overall, vaccine effectiveness was 54% (95% confidence interval [CI] -21– 83%); 60% (95% CI -27–87%) among children and 34% (95% CI -21–89%) among older adults.

Conclusion: Results suggest a higher rate of vaccination among influenza-negative controls compared to influenza case-patients. However, results lack precision as the availability of vaccination status significantly reduced sample size. We are currently collecting further data (2011–12) in order to provide more precise estimates.

DOMINICAN REPUBLIC

Clinical Trial of Safety and Immunogenicity of Needle-free Jet Injection of Reduced-dose, Intradermal Influenza Vaccine Administered to Children aged 6 to 24 Months-old in the Dominican Republic

This was a sequential phase I and II, controlled, double-blinded study to determine whether immune responses suggesting protection against influenza can safely be induced in young children by two reduced doses one month apart of a trivalent inactivated influenza vaccine (INF) administered by the intradermal (ID) route with an investigational ID spacer on a needle-free jet injector (JI) (0.1 mL), compared to two standard intramuscular (IM) doses by needle-syringe (N-S) (0.25 mL).

Study Objectives:

- The primary endpoint of this study is to measure the percentage of participants with seroconversion on hemagglutination inhibition (HI) assay of serum collected at least one month after two doses of influenza vaccine (INF) administered in a reduced-dose volume of 0.1 mL intradermally (ID) by needle-free jet injector and intramuscularly (IM) by conventional needle-syringe (N-S), compared to standard IM injection of full 0.25 mL doses.
- Secondary objectives of this study are to determine for the above comparison seroprotection rates, geometric mean titers, and the extent and frequencies of local and systemic reactions.

Approach: Healthy participants were recruited from a large, public tertiary care children's hospital. Participants were randomly assigned to receive two doses of Sanofi-Pasteur Vaxigrip® influenza vaccine by one of the three study arms: a) Group "IM-NS-0.25" (controls)-two full 0.25 mL doses administered IM by standard N-S; b) Group "ID-JI-0.1" (investigational)-two reduced 0.1 mL doses administered ID by needle-free jet injector (JI), and c) Group "IM-NS-0.1" (investigational)-two reduced 0.1 mL doses administered IM by N-S. At the conclusion of the study, participants in the two investigational groups (reduced doses) received a third "insurance" dose via the conventional route, method, and dose. Six months later, all participants received a fourth "bonus" booster dose for protection during the following influenza season.

Timeline: This study started on October 2006 and the completion of data collection was November 2009. Final serologic results became available in final quarter of 2010. Currently working on final analysis and publication, estimated study end date is July 2012.

Progress and Findings: Our preliminary still-blinded results, show that local pain was mild in 111 (25%), moderate in 9 (2%), and severe in 5 (1%) participants (n=450). Moderate adverse event (AE) sizes of ≥ 10 - < 25 mm occurred for erythema (1%), and swelling ($< 1\%$). Systemic AEs were diarrhea (39%), fever $\geq 38.0^\circ\text{C}$ (33%), loss of appetite (32%), vomiting (32%), sleepiness (18%), unusual crying (19%), irritability (13%), and convulsions (1%). Serious AEs (n=25) included: 10 asthma-related, 4 varicella, 4 febrile convulsions, 1 death (trauma), and 6 miscellaneous others. All but one possibly-related convulsion were deemed unrelated by DSMB. HAI inverse GMTs after dose 2 for multiple formulations and strains were 84 for H1N1, 62 for H3N2, and 100 for B. Seroconversion was 65%, 62%, and 75%; seroprotection 68%, 70%, and 85%, respectively.

Conclusion: Based on these preliminary results, we conclude that local AEs were tolerable, and immunity generally good. Definitive assessment awaits unblinded analysis by group of all participants.

GHANA

Dodowa Influenza Population-Based Surveillance (DIPS)

A population-based surveillance study is currently being implemented at the demographic surveillance site of Dodowa in the Dangme West District; this is a collaborative project between CDC, NAMRU-3, the Ghana Health Service and the Noguchi Memorial Institute for Medical Research. This population-based surveillance will take place in a well-defined population in a demographic surveillance site (DSS), and will serve as a platform for several influenza studies, including evaluating the disease burden and economic burden of influenza and identifying risk factors for severe influenza morbidity and mortality in Ghana. Initial activities planned in Dodowa include a health utilization survey to improve knowledge of health seeking behaviors and ensure that surveillance health facilities will identify the majority of ILI and SARI cases occurring in the population.

Study Objectives:

- Estimate the incidence of acute lower respiratory tract infections and the proportion attributable to influenza virus and to other pathogens in Ghana.
- Establish rates of severe respiratory disease and identify risk factors for severe influenza and other respiratory infections such as age, underlying medical conditions, malnutrition, co-infection with other pathogens, and environmental exposures.
- Evaluate case definitions for influenza and other emerging pathogens and identify clinical predictors of severe influenza and other respiratory infections in target populations.
- Characterize influenza and other respiratory infections in persons with coinfections (such as persons infected with HIV, tuberculosis, or malaria) that may place them at increased risk for severe influenza.

Approach: ILI and SARI screening at all health facilities and community health posts in the DSS.

Timeline: Surveillance initiated at the district hospital in April 2011.

Progress and Findings: N/A

Conclusion: N/A

INDIA

Addressing Emerging Infectious Diseases in the Republic of India: Influenza Disease

This HHS/CDC cooperative agreement was established five years ago (9/2008–9/2013) to estimate the burden of disease related to influenza virus infection in India, through a population-based longitudinal study at three sites with demographic surveillance systems.

Study Objectives:

- Estimate the incidence of laboratory-confirmed influenza among persons hospitalized with acute respiratory illnesses and acute exacerbations of chronic medical conditions.
- Determine risk factors for severe disease due to influenza, including underlying chronic conditions, demographics, smoking, and socio-economic status.
- Estimate the annual mortality rate due to severe respiratory disease and influenza in the population.

Approach: Persons living in the DSS areas in one of the three study areas (Ballabgarh, Vadu, and Vellore) that seek inpatient medical attention and meet the study enrollment criteria are enrolled, and clinical and epidemiologic information and respiratory specimens are collected from all consenting persons. Each site is also conducting a community-based survey, to gather health care utilization data as well as household risk factors and socioeconomic status. Additionally, two sites (Ballabgarh and Vadu) are conducting outpatient screening and enrollment to estimate the burden of non-hospitalized medically attended influenza disease.

Timeline: April 2009–September 2013.

Progress and Findings:

- The facility based surveillance for medically attended inpatients among 300k population has varied greatly both in enrollment and percent positivity for influenza from site to site. While Vadu/Pune revealed influenza positivity of 21% (699/3,325), Delhi had positivity of only 7% (62/923). Almost half of all influenza virus infections at each site are due to the A(H1N1)pdm09 virus.
- A preliminary estimate of influenza related hospitalization based on eligible cause hospitalization rate of 86/10,000 population/year and 8–10% influenza positivity has allowed extrapolation of total hospitalizations to approximately 1 million hospitalizations related to influenza.

Conclusion:

- >5,000 hospitalizations recorded with influenza positivity ranges from 7–21%.
- Rates of hospitalization vary significantly and almost 1/5 hospitalizations due to influenza occur during peak monsoon season.
- Incidence of influenza varied between sites (6–44/10,000).
- Annualized incidence rate in Vadu comparable to rates in U.S.
- Age specific differences from site to site.

Spectrum of Respiratory Virus Infections in Acute Respiratory Tract Infection (ARI) Among Children in India

This study has been carried out by utilizing the hospital based surveillance to estimate the incidence of influenza in Ballabgarh, Haryana.

Study Objectives: The main objectives of the study are to estimate burden of pneumonia, due to important viral (e.g., influenza, Respiratory Syncytial Virus (RSV)) and bacterial pathogens (*H. influenzae* and *streptococcus pneumoniae*), as well as drug sensitivity patterns of the causative agents in a rural community.

Approach: A subset of febrile acute respiratory illness (FARI) specimens collected from October–December 2010 were tested for various pathogens using a real-time RT-PCR.

Timeline: October 2010–to date.

Progress and Findings: Analysis of 137 specimens revealed 60 (44%) specimens were positive for one of the viruses tested: RSV (17%), rhinovirus (13%) and PIV3 (4%). Studies are underway to determine the incidence of each of these viruses in this population-based study.

Conclusion: This study provides evidence that respiratory viruses singly or in mixed infections are detected in >50% of medically attended hospitalized children from a rural community in India using sensitive detection methods like RT-PCR. These findings will help guide efforts to reduce the disease burden due to viral ARIs in developing countries.

Surveillance for Influenza and Infection with Other Respiratory Viruses Among Persons with Chronic Obstructive Pulmonary Disease, Kashmir, India

Surveillance data from other countries suggest that persons hospitalized with exacerbations of underlying chronic lung disease account for a substantial proportion of persons hospitalized with influenza. However, data are needed to establish the magnitude of acute exacerbations of COPD (AECOPD) due to severe influenza in India to inform local and national government response efforts to influenza epidemics, including targeting of influenza prevention efforts in areas with a high prevalence of COPD, and to guide local clinicians' management and treatment of persons hospitalized with AECOPD.

Study Objectives:

- Determine prevalence of influenza in hospitalized patients with acute exacerbation of COPD.
- Assess risk factors, spectrum of clinical illness, and case definitions.
- Determine prevalence of other respiratory viruses.

Approach: Sher-I-Kashmir Institute of Medical Sciences (SKIMS) is the main tertiary care hospital providing care for persons with COPD in Srinagar, the capital of Kashmir, and admits the majority of COPD patients with exacerbations requiring more than just supportive care. SKIMS will join the existing Indian Influenza Surveillance Network and begin conducting surveillance for influenza-like illness (ILI) among its outpatient population in September 2010, using Influenza Surveillance Network standard operating procedures. Additional surveillance for persons hospitalized with AECOPD will be conducted at this facility, due to the high burden of such patients, to better understand the contribution of influenza virus infections among persons hospitalized with AECOPD at SKIMS in Srinagar beginning in September 2010.

Timeline: September 2010–2013.

Progress and Findings: From the beginning of the project until September 2011, 318 patients of AECOPD were enrolled; two-thirds were aged 65 years or more. The influenza positivity rate is 8.2% (23) out of the 282 samples tested. Among the positive samples, 52.2% (12) were tested for influenza A/H1N1pdm09, 8.7% (2) for influenza A/H3N2 and 30.4% (7) for influenza B.

Conclusion: The preliminary data shows Influenza to be an important cause of hospitalization due to acute exacerbation of COPD among elderly population.

Direct and Indirect Protection by Influenza Vaccine Given to Children in India

Through collaboration between the All India Institute of Medical Sciences (AIIMS), CDC, and the University of Alabama, a study to determine whether immunization of young children with trivalent influenza virus vaccine (TIV) protects immunized children and older non-immunized household members is being conducted in three villages near New Delhi, India. Children aged 6 months–10 years are randomized at the household level to receive TIV or a control vaccine—Inactivated Polio Vaccine (IPV). Weekly household surveillance for febrile acute respiratory illness (FARI) is conducted among all study household members.

Study Objectives:

- Measure direct protection of children by influenza vaccine.
- Measure indirect protection against influenza among family members of influenza vaccine recipients.
- Define contribution of influenza virus to illness within three villages in rural Ballabgarh.
- Assess risk factors for more severe disease due to influenza virus.
- Establish surveillance system for influenza virus infections that will be used to assess outcomes in subsequent influenza virus vaccine study.

Approach: A prospective, household randomized, controlled, observer-blinded study is being conducted in 3 peri-urban villages outside of Delhi, India. The vaccination of children aged 6 months to 10 years with either Trivalent Influenza Vaccine (TIV) or the control vaccine (IPV) will be carried out for 3 years, followed by weekly household surveillance for febrile acute respiratory illness (FARI) to assess the efficacy of influenza vaccination. Additionally, a small subset of vaccinated children (n=200) will be enrolled to measure their immune response to vaccination and risk factors affecting immunogenicity.

Timeline: September 2008 to September 2011; extended till September 2014.

Progress and Findings:

- Enrollment, vaccination, and surveillance activities have been successfully implemented in October 2009 and 2010 with high rates of vaccine acceptance (91% of 3,700 eligible children received at least one dose of vaccine), and exceptionally high rates of community acceptance for surveillance enrollment (90% of 17,000 persons consented to participate in weekly surveillance).
- Influenza positivity was identified among 350 (17%) of the 2,030 FARI episodes during 2010 across all age groups. Pandemic H1N1 accounted for half of the influenza positivity.
- While the predominant circulating strain in 2009 was pandemic H1N1, co-circulation of both influenza B and pH1N1 was observed in 2010.
- Influenza seasonality in Delhi coincides with rainy season in July, plans underway to change the vaccination using SH vaccines resulting in policy changes.

Conclusion:

- Enrollment and immunization rates for influenza vaccine are high, reflecting high community acceptance.
- Likely no benefits of vaccination in first year (due to vaccine mismatch), need to continue three year vaccination.

- Emergence of pandemic H1N1 emphasized the importance of multi-year studies of influenza vaccine.
- Weekly FARI Surveillance in 18,000 villagers providing incidence data.
- Measurement of cell mediated immune response and nutritional factors in subset of the participants.
- Vaccine efficacy yet to be determined.

A similar vaccine effectiveness study is underway in Senegal.

Understanding Host Innate Immune Responses Against Influenza A Virus: An ICGEB-CDC Collaboration

Influenza virus has evolved complex translational control strategies as part of an innate defense mechanism exhibited by the infected cell. The influenza virus has evolved complex cap-dependent translation initiation mechanisms and involve the recruitment of both viral and host-cell proteins to preferentially synthesize viral proteins and prevent activation of antiviral responses.

Study Objectives:

- To explore cellular factors that are activated or involved with influenza virus (including H5N1) replication, assembly or release.
- Study viral-host factors associated with Influenza pathogenesis.

Approach: We undertook a comprehensive analysis of these virus-host interactions to better understand the viral drift resulting in a molecular evolution that results in its adaptability to infect different hosts. Discovering these new host-viral protein-protein interactions holds great promise for further research leading to new anti-viral targets.

Timeline: 2009–2014.

Progress and Findings:

- The NP protein of influenza A viruses (H1, H3, H1pdm09) down regulates the PKR pathway via interaction with HSP-40 (PLoS ONE published).
- The influenza A virus Neuraminidase protein via up regulation of Src signaling thereby enhancing cell survival through interaction with CEACAM6. (JBC, submitted).
- Influenza A virus Nucleoprotein interacts with Clusterin and inhibits its anti-apoptotic function, likely by preventing Bax movement into the mitochondria. This may lead to Cytochrome c release from mitochondria and subsequent induction of apoptosis.
- Role of Actinin-4, a cytoskeleton scaffolding protein postulated to be involved in viral trafficking within an infected cell. Actinin-4, being a cytoskeleton protein, can be hypothesized to facilitate transport of viral components during entry and exit of viral particles and can possibly have a role in intracellular trafficking of viral components thus controlling viral assembly and budding.

Conclusion: Complex interplay of viral proteins with innate host factors likely will uncover unique pathways that can be exploited for anti-viral approaches.

KENYA

Population-based Surveillance for Influenza and Other Respiratory Diseases in Nairobi and Kisumu, Kenya

The Influenza Program in collaboration with the International Emerging Infections Program (IEIP) under KEMRI/CDC currently conducts population-based disease surveillance (PBDS) for severe acute respiratory illness (SARI) and influenza-like illness (ILI) in two sites in Kenya; Kibera, an informal urban settlement in Nairobi, and Lwak, a rural community in western Kenya. Approximately 25,000 residents are enrolled in each of the two sites.

Study Objectives:

- Characterize etiologies of acute respiratory illness in a rural community and an urban community in Kenya.
- Evaluate the burden of medically attended and home-reported influenza and other respiratory diseases.
- Provide a platform to evaluate interventions such as vaccine.

Approach: Community interviewers visit each household bi-weekly to ask residents questions about illness symptoms in the past week. In addition, residents have access to a free clinic, where surveillance is conducted for respiratory illness, including influenza, and a number of other disease syndromes. Patients meeting the case definition for SARI and ILI have a nasopharyngeal and oropharyngeal specimen collected. Specimens for SARI and ILI are tested at the CDC/KEMRI laboratory in Nairobi for influenza and other viral pathogens using real-time RT-PCR. Data from weekly household visits, visits at the free clinics, and laboratory results are stored in a central database in CDC-Nairobi and at KEMRI/CDC, Kisumu.

Timeline: Surveillance for respiratory illness in the two sites began in 2006. A five year review of data is underway so that the seasonality, epidemiology and burden of influenza can be well-understood, and interventions such as vaccines can be evaluated.

Progress and Findings: Influenza virus has been shown to circulate year-round with a peak in activity between July and October of every year. From March 2007–February 2010 the adjusted rates of medically attended influenza-associated acute lower respiratory infections per 1,000 person-years in Kibera (urban informal settlement, Nairobi) and Lwak (rural western Kenya) were 13.7 and 23.0, respectively.

Conclusion: Influenza circulates throughout the year in Kenya with peaks during the cool and rainy seasons. Influenza constitutes a preventable fraction of the overall burden of respiratory illness in Kenya.

Hospital-based Surveillance for Multiple Respiratory Pathogens in Nyanza Province, Kenya

The Influenza Program in collaboration with the CDC Global Disease Detection program under KEMRI/CDC currently conducts comprehensive hospital-based surveillance for multiple respiratory pathogens at Siaya District Hospital, located within the Health and Demographic Surveillance System site in Nyanza Province, Kenya. This area has been under systematic health and demographic surveillance since May 2007.

Study Objectives:

- Characterize etiologies of hospitalized respiratory illness at Siaya District Hospital.
- Monitor the impact of influenza in the context of other respiratory viruses and underlying comorbidities.

Approach: Patients hospitalized with respiratory illness have a nasopharyngeal and oropharyngeal specimen collected. Specimens are tested at the CDC/KEMRI laboratories for influenza A and B, respiratory syncytial virus (RSV), parainfluenza (PIV)-1, -2 and -3, adenovirus (AdV) and human metapneumovirus (HMPV) by real time reverse transcriptase polymerase chain reaction. More recently Taqman Array Card (TAC) multiplex PCR technology has been used to test specimens for seven bacterial and 13 viral pathogens. Comprehensive clinical data are also collected.

Timeline: Surveillance is ongoing.

Progress and Findings: During 2009–2011 the respiratory viruses most commonly detected in hospitalizations using conventional PCR methods were AdV, PIV-3, RSV, HMPV and Flu A. Flu-associated hospitalization rates were highest in children 0<2 years old; similarly, other pathogen-associated rates remained high in this age group. Taqman array analyses have found *Streptococcus pneumoniae*, rhinoviruses, respiratory syncytial viruses, adenoviruses, enteroviruses, and influenza viruses to be most commonly detected in hospitalized patients. Median CT values in patients with detectable *S. pneumoniae* were significantly lower in fatal compared to surviving patients, potentially suggesting a higher density of the pathogen. This is consistent with previously reported associations between high NP/OP density and pneumococcal pneumonia.

Conclusion: TAC Multiplex PCR technology identified one or more pathogens in 85% of respiratory hospitalizations in Western Kenya. Fatal outcomes are associated with multiple pathogens. In patients with *S. pneumoniae* a greater pathogen density of *S. pneumoniae* was detected in the NP swabs of fatal as compared to non-fatal cases.

Seasonal Influenza Vaccine Effectiveness Study

Influenza vaccine has been shown to reduce influenza-associated acute respiratory illnesses (ARIs) in developed countries. However, little is known about the effectiveness of influenza vaccine in the developing world. KEMRI/CDC-Kenya, with support from the Kenya Ministry of Public Health and Sanitation, is conducting a three-year observational influenza vaccine effectiveness study using the commercially available Southern Hemisphere seasonal vaccine in two sites in Kenya; Lwak—a rural site in western Kenya, and Kibera—an informal urban settlement in Nairobi. The International Emerging Infections Program (IEIP) under KEMRI/CDC currently conducts population-based disease surveillance (PBDS) for severe acute respiratory illness (SARI) and influenza-like illness (ILI) in these two sites.

Study Objectives:

The objectives of the study are to evaluate the following:

- Effectiveness of the vaccine in preventing laboratory-confirmed disease.
- Effectiveness of the vaccine in preventing medically attended ILI and SARI, and symptomatic ILI and SARI reported in the community.
- Indirect effect of the vaccine in preventing laboratory-confirmed influenza, and ILI and SARI in non-immunized household members.
- Acceptability of influenza vaccination among community residents.

Approach: The vaccine is offered on a voluntary basis to infants from 6 months of age through children up to 10 years old enrolled in the IEIP study site. Sanofi Pasteur-France has donated Southern Hemisphere trivalent influenza vaccine for the study. Prior to the vaccination campaign a vaccination awareness campaign was conducted to sensitize the community to the benefits and availability of the influenza vaccine.



After vaccination, the study participants are followed up through the routine IEIP surveillance which includes weekly home visits where field workers ask household members questions about recent illnesses and deaths. The study participants also have free access to a medical clinic where free care is provided. At the clinic, specimens are collected from patients who have SARI or ILI. Samples are tested at the CDC laboratory using real-time RT-PCR for influenza virus. At the end of every year patients who received vaccine will be compared to patients who did not receive vaccine.

Timeline: Data from the past three years of influenza surveillance has shown that the influenza season in Kenya peaks July–October, and therefore most closely mirrors the Southern Hemisphere influenza season. The vaccine is available every year in Kenya beginning in March to coincide with the Southern Hemisphere influenza season. The study is entering its third and final year.

Progress and Findings: There has been over 40% uptake in both sites during the 1st and 2nd year of vaccine campaign. A preliminary estimate of 43% vaccine effectiveness has been observed, which is consistent with the use of TIV at other sites. To date there has been a 100% match between locally grown viruses from existing surveillance and the Southern Hemisphere vaccine formulations. Children living more than 5km radius from the nearest vaccination facility have been significantly less likely to get vaccinated. Families with mothers aged 25–34, 35–44, >45 years have all been more likely to participate in the vaccination campaign than families with mothers aged <25 years. Mothers whose occupation requires them to be away from home have been less likely to participate than mothers who did not work or whose nature of work did not require that they be away from home. While formal evaluation of vaccine effectiveness will occur after year three, these findings also point to the essential role that mothers play in the vaccination of their children with seasonal influenza vaccine. Future campaigns will need to consider ways to adapt vaccination schedules to working mothers, and community mobilization efforts may need to target alternative family members who may bring children for vaccination if working mothers are unavailable. These findings also support the notion that future campaigns may need to consider opening additional vaccination centers if large portions of the targeted population will have to travel greater than 5km for vaccination.

Conclusions: To date the uptake of influenza vaccine has approached 50%, with 46% vaccine effectiveness against lab-confirmed influenza observed. There has been a 100% match between circulating and vaccine strains.

Surveillance for Avian Influenza Viruses in Live Bird Markets in Kenya

Influenza viruses circulating in poultry in Kenya have not been described. Influenza surveillance in live bird markets (LBM) has been recognized as an effective tool in detecting influenza subtypes circulating in the poultry population. Qualitative risk assessment studies carried out in Kenya in 2008 identified live bird markets as high-risk points, not only for virus introduction and circulation among birds, due to the practices employed, but also for introduction of virus to humans working with poultry. Conducting surveillance at LBMs is vital to early detection of the introduction of avian influenza to the poultry population in Kenya.

Study Objectives:

- To identify and characterize avian influenza viruses circulating in poultry traded in live bird markets in Kenya.
- Investigation of market practices that would contribute to mixing and transmission of virus within the market.

Approach: Between March 2009 and February 2011, we collected samples from birds presented for sale in the five live bird markets in Kenya. We visited each market once a month, and collected tracheal and cloacal samples from 25 birds per market visit. We also collected five environmental samples in each market at every visit. All the specimens were tested for influenza A matrix gene by real-time reverse transcription-polymerase chain reaction at the BSL-3 KEMRI/CDC laboratory in Kisumu. All influenza A positive samples were sent to CDC's Atlanta laboratory for virus isolation and subtyping.

Timeline: This field work was carried out from March 2009 through February 2011 but the project is ongoing.

Progress and Findings: From March 24, 2009 through February 28th, 2011, we collected a total of 5,221 cloacal and tracheal samples during 22 monthly visits to the five markets. Of these 4,176 (80%) were from chickens, 321 (6.1%) from ducks, 382 (7.3%) from turkeys, and 342 (6.6%) from geese. Of the 5,199 (99.6%) samples tested, influenza A virus was detected in 42 (0.8%) of the samples. Influenza A was detected in 35 of 4,166 (0.8%) swabs from chicken, three of 381 (0.8%) turkeys, four of 335 (1.2%) geese, and 0 of 317 (0%) ducks as shown in. Overall, influenza A was detected in 33 (1.3%) and nine (0.4 %) of oropharyngeal and cloacal swabs respectively. Virus isolation and subtyping of influenza A positive swabs is ongoing to determine the influenza subtypes circulating in poultry species in Kenya.

Conclusion: Current work suggests a relatively low prevalence of influenza circulating in poultry at live bird markets. However additional work has suggested higher prevalence in pigs, suggesting slaughterhouses as an important area for future monitoring of occupational exposures.

Evaluation of Length of Specimen Storage on Influenza PCR Test Results: An Analysis of Influenza Surveillance Specimens in Kenya, 2008–2010

Little is known about the optimal time specimens can be refrigerated before being tested for influenza by real time reverse transcription-polymerase chain reaction (rtRT-PCR). Existing guidelines recommend that samples be stored at 4°C for up to 96 hours.

Study Objectives:

- To determine the relationship between the numbers of days a specimen was in refrigeration and influenza positivity as well as the rRT-PCR Cycle Threshold (Ct) values for influenza specimens.

Approach: We collected nasopharyngeal and oropharyngeal specimens from patients with respiratory illness at influenza sentinel surveillance sites in Kenya. Specimens were stored in viral transport medium (VTM) at 2-8°C, transported to Nairobi, and tested for influenza A and B using rRT-PCR. We used multivariable logistic regression to determine the relationship between the number of days a specimen was refrigerated and influenza positivity influenza surveillance data from Kenya (2008–2010). We conducted ordinal logistic regression to evaluate the relationship between refrigeration days and the rRT-PCR Cycle Threshold (Ct) values of influenza-positive specimens.

Timeline: Analysis of sentinel surveillance data, 2008–2010.

Findings: Of the 7,833 samples included in the analysis, 940 (12.0%) were positive for influenza. In the multivariable analysis, there was a decline in positivity when samples were stored for six days or longer. We found that samples could remain in storage for at least five days without affecting the proportion-positive of samples. Ct values of influenza-positive specimens did not vary significantly by storage days.

Conclusion: These findings suggest that respiratory specimens can be refrigerated for up to five days in Kenya without substantially influencing the detection of influenza viruses.

A Comparison of Smart Phones and Paper-Based Questionnaires for Surveillance Data Collection using Influenza Sentinel Surveillance Sites in Kenya, 2011.

Manual data collection and data entry using paper-based questionnaires can be time consuming and prone to errors. Smartphones and other hand held electronic devices have potential to improve speed and accuracy of data collection, transmission, and entry. We introduced smartphones in four hospital-based influenza sentinel surveillance sites in Kenya. We compared smartphone-collected data to paper-based-collected data previously collected by the same surveillance officer.

Study Objectives:

- To compare the quality and timeliness of surveillance data collected using smart phones and paper-based questionnaires.

Approach: Since 2006, the Kenya Ministry of Health and the Kenya Medical Research Institute/CDC-Kenya have conducted sentinel influenza surveillance at 9 to 11 health facilities in Kenya. At each site, surveillance officers identify patients with respiratory illness and administer a brief (18 question) questionnaire that includes demographic and clinical information. From May–June 2011, we pilot-tested an electronic data collection system using Field Adapted Survey Toolkit (FAST) on HTC Touch Pro2 smartphones at four sentinel sites. For each site, we compared questionnaires collected using smartphones to an equal number of paper-based questionnaires collected by the same surveillance officer. We evaluated completeness of data collection, errors in data entry and time taken to enter collected data into the central database. We projected costs of running the two systems for a period of two years and compared them. In addition we sought the surveillance officers' experiences on using these data collection tools.

Timeline: Analysis of sentinel surveillance data, 2010–2011.

Findings: A total of 1,019 paper-based questionnaires were collected at the four sites from Dec 14, 2010–June 6, 2011 and 1,019 smartphone questionnaires were collected at the same four sites from May 3, 2011–Aug 26, 2011. In all, 5% (95% CI: 4.5%-5.0%) of paper-based questionnaires were determined to be incomplete compared to 3% (95% CI: 2.8%-3.2%) of smartphone questionnaires. Of the questions requiring mandatory responses in the smart phone questionnaire, 4% of them were unanswered in paper-based questionnaires. Seven paper-based questionnaires had duplicated patient identification numbers, while no duplication was seen in smartphone data. Smartphone data was uploaded into the database within 8 hours of collection, and paper-based data took an average of 24 days to be uploaded. For two years, costs of establishing and running paper-based data collection system is approximately \$61,830 USD compared to approximately \$45,546 USD for smart phone data collection system. Fixed costs incurred in establishing the two systems were estimated at \$12,990 USD and \$16,480 USD for paper and smartphone systems respectively. All surveillance officers reported that smartphones were much easier, faster and more convenient to use as data collection tools. Electronic data collection using smart phones has potential to improve data integrity and reduce resource costs.

Conclusion: Electronic data collection using smart phones has been demonstrated to improve the timeliness of data analysis, to improve data integrity and to reduce resource costs in Kenya.

MADAGASCAR

Viral Etiology of SARI in Madagascar

In order to describe epidemiology and etiology of various viruses known to be responsible for SARI cases, we selected two hospitals in the SARI surveillance system in Madagascar. Samples are analyzed at the NIC for influenza detection and characterization, but also for detection of other respiratory viruses, using a multiplex real-time PCR implemented at the NIC.

Study Objectives:

- Describe epidemiology of SARI cases.
- Study viral etiology of SARI cases.
- Identify risk factors for hospitalization.
- Estimate economic burden of SARI for Malagasy population.

Approach: Based on the SARI surveillance system implemented in 18 hospitals in Madagascar, we selected two hospitals for an active SARI surveillance (Antananarivo and Moramanga). Every hospitalized patient with clinical features of SARI syndromes is included in the study. Respiratory specimens are tested for a panel of 14 viruses developed at the NIC. Genetic studies are conducted to characterize Malagasy strains and see if there is a correlation between genotype and severity of the disease.

Timeline: The active survey begins in November 2010 and is ongoing.

Progress and Findings: From October 1, 2010 to September 30, 2011, the NIC tested 222 samples of SARI cases for 14 respiratory viruses (influenza A and B; respiratory syncytial virus; human Coronaviruses HKU1, OC43, NL63 and 229E; human Metapneumovirus; human Rhinovirus; Parainfluenza Virus type 1, 2 and 3; Adenovirus, Bocavirus). Among them, 20.7% (46/222) were negative for all respiratory viruses tested. Influenza A was the most common virus detected with 34.2% of positivity rate, followed by RSV (27.5%), HRV (18.5%) and influenza B (10.8%). Most patients included were less than five years old (>50%).

Conclusion: The part of viral infection in SARI hospitalized patients is important. Some viruses may have a role in the severity of disease, particularly in the younger (<5 years old).

Excess Mortality Associated with the 2009 A(H1N1)v Influenza Pandemic in Antananarivo, Madagascar

Assessing the burden of influenza epidemics on mortality is difficult in tropical countries. In Africa, until recently, the burden of influenza was believed to be negligible. The purpose of this study was to assess the impact of 2009 influenza epidemic on mortality in Madagascar.

Study Objectives:

- Evaluate the impact of the recent pandemic A(H1N1) 2009 virus on mortality among inhabitants in Antananarivo.



Approach: The study was carried out in Antananarivo, the capital city of Madagascar. We obtained death certificate data from 2007 to 2009 from the three urban centers in Antananarivo, where all deaths are reported in paper-based registers. Death certificates in Antananarivo and its suburbs must be obtained before casketing. In hospital, few cases of acute respiratory disease were laboratory-confirmed as influenza infection, but no deaths. Furthermore, there is no routine analysis of mortality data in Madagascar. Each death certificate, including the decedent's date of birth, sex, date of death, address and the cause of death declared, was classified according to the International Classification of Diseases (10th revision). Data were entered using Microsoft Access and were analyzed using the statistical package R. Mortality was measured by the number of deaths per month or per year, for the total population or each subpopulation, distinguished according to age.

Timeline: We analyzed all deaths reported in 2009 and compared them with the expected number of deaths calculated from the average rate of deaths of the two preceding years (2007, 2008).

Progress and Findings: We observed 20% more deaths than expected among people in Antananarivo, Madagascar, during the influenza pandemic in November 2009 with an excess of mortality for age group ≥ 50 years (RR=1.41). Statistical analyses showed that pulmonary diseases were more frequent than other causes of deaths.

Conclusion: These results suggest that the pandemic A(H1N1) 2009 virus may have been accompanied by increased mortality.

NEW ZEALAND

Southern Hemisphere Influenza and Vaccine Effectiveness Research and Surveillance Study (SHIVERS)

The Influenza Division is cooperating with New Zealand public health laboratories and universities to conduct a five year study in the southern hemisphere on influenza and other respiratory diseases; their burden, epidemiology, transmission, risk factors, and the effectiveness of vaccination.

Study Objectives:

The SHIVERS study has two Primary Objectives:

- Severe disease: Estimate the incidence rate, prevalence, clinical spectrum, pathogenesis and outcomes of severe pneumonia and severe acute respiratory infection (SARI) caused by influenza and other respiratory pathogens in the Auckland population, including the Maori and other indigenous groups.
- Vaccine effectiveness: Assess the annual effectiveness and/or efficacy of influenza vaccines in preventing laboratory confirmed influenza in the population of Auckland and targeted subpopulations.

Approach: At the end of September 2011, the U.S. Department of Health and Human Services awarded the Institute of Environmental Science and Research (ESR) with a five year research grant, *Southern Hemisphere Influenza Vaccine Effectiveness Research and Surveillance (SHIVERS)*. It is a multi-centre and multi-disciplinary collaboration amongst ESR, Auckland District Health Boards, University of Otago, University of Auckland, the U.S. Centers for Disease Control and Prevention and the WHO Collaborating Centre at St. Jude Children's Hospital in Memphis, USA.

Timeline: The research cooperative agreement was awarded in September 2011 and the first few months of work focused on establishing the platform to conduct the study.

Progress and Findings: The cooperative agreement was awarded at the end of FY 2011.

Conclusion: The SHIVERS study is expected to answer many questions related to the epidemiology of influenza in a southern hemisphere setting through enhanced real-time surveillance in sentinel practices and hospitals.



The SHIVERS Project Team meet in Upper Hutt, New Zealand.

PERU

Influenza Seasonality and Incidence in Four Ecologically Diverse Regions of Peru

Most influenza burden estimates are based on passive surveillance data in temperate countries. These estimates lack population denominators necessary for the determination of disease incidence. Population-based epidemiologic and laboratory data on influenza would be useful for describing the impact of this disease and formulating effective public health strategies for prevention and control. We therefore implemented a multisite, prospective cohort study with active community-based household surveillance in four ecologically distinct regions of Peru.

Study Objectives:

- To estimate the incidence of human influenza in four distinct ecological regions of Peru.
- To estimate the economic burden of influenza in the study population in the four locations.

Approach: A total of 8,000 persons (2,000 per site) representing approximately 2,000 households from four distinct geographic locations of Peru (Lima: desert valley, Puerto Maldonado: rainforest, Cuzco: highlands, and Tumbes: tropical coast) are participating. Field workers enroll household members and perform active prospective surveillance to identify ILI cases through household screening visits three times per week. Once an ILI case is identified, field workers administer a questionnaire about influenza risk factors and economic burden. Nasal and throat swabs are obtained for viral diagnostic testing by PCR. All ILI cases are followed for 15 days to allow for adequate data collection to optimize disease burden estimation.

Timeline: This study is ongoing.

Progress and Findings: A total of 5,457 ILI cases were identified in all four sites between June 2009 and March 2012. The percentage of ILI episodes positive for influenza A(H1N1)pdm09 was 15.3%, 12.6%, 9.3%, and 7.3% in Lima, Tumbes, Puerto Maldonado, and Cusco, respectively. From 5,432 samples processed to date, 626 (11.5%) were positive for influenza A (H1N1)pdm09, 4 (0.1%) for A (H1N1), 661 (12.2%) for A (H3N2), 329 (6.1%) for influenza B, and 3,812 (70.2%) were negative for influenza virus (testing of these samples for other pathogens is underway). Preliminary results from June 2009 and December 2010 show an overall influenza incidence rate of 140/1000 person years (py). Of these, 66/1000py sought care and 1.1/1000py required hospitalization. The incidence of influenza was higher during 2009 (160/1000py, 95% CI 150–180) than during 2010 (130/1000py, 95% CI 120–140). During 2010, when influenza A (H3N2) and influenza B were predominant, incidence of influenza was highest among children aged 12–23months (31/100py, 95% CI 23–43). Tumbes consistently had the highest incidence of influenza (19/100py, 95% CI 17–21), followed by Lima (17/100 py, 95% CI 15–18), Madre de Dios (12/100 py, 95% CI 11–13) and Cuzco (8/100 py, 95% CI 8–10).

Conclusion: Our findings show that children had higher influenza incidence rates than adults. This may be explained by a lower cumulative immunity to influenza than other age groups, stressing the importance of a targeted vaccination strategy among children. Incidences rates were different in all sites, suggesting an influence of environmental or cultural variables on virus transmission.

Incidence and Prevalence of Exposure to Influenza Virus in Peru

Despite the many studies on influenza A(H1N1) pdm09 virus since the 2009 pandemic, there are few data on the incidence or prevalence of infection with this virus in the general population of Peru. Sero-epidemiological data are important to estimate true infection rates and to determine which strains are prevalent. Moreover, such data will allow calculation of rates of asymptomatic infection and vaccine effectiveness when combined with existing clinical and vaccination data.

Study Objectives:

- To estimate the incidence and prevalence of exposure to various influenza virus strains in humans at four study sites in Peru.

Approach: This study is nested in the influenza cohort study described in Entry One. Blood samples were taken from the cohort study participants in June 2011 and again in April 2012 and are being tested for evidence of exposure to influenza virus by hemagglutination inhibition assay.

Timeline: Samples collection is completed. Laboratory testing has been performed on 30% of the samples to date.

Progress and Findings: A total of 5,936 serum samples have been collected in the four sites in the two cross-sectional samplings. Results from the first sampling in 2011 show that 40.6%, 35.4%, 24.1%, and 15.4% tested positive for antibody to influenza A(H1N1)pdm09 virus in Lima, Tumbes, Cusco, and Puerto Maldonado, respectively. With regard to antibody to influenza A H3N2 virus, preliminary results show 46% of the study population is positive in Lima and 32.1% in Cusco. Results for H3N2 from Tumbes and Puerto Maldonado, as well as all results from the second sampling, are pending.

Conclusion: The prevalence of exposure to influenza virus varies significantly between the four study sites in Peru. Calculations on the incidence of exposure will be conducted when the laboratory analyses are complete.

Influenza Virus Transmission between Humans and Animals in Backyard Farms in Peru

Domestic animals, especially swine and fowl, play an important role in the evolution and ecology of influenza A virus. Pandemic influenza A(H1N1)pdm09 virus is thought to have been first introduced into humans from pigs. Transmission from humans to swine has also been reported by the World Organisation for Animal Health (OIE) in more than 15 countries. Recently our group, in collaboration with the San Marcos University Veterinary School, has found serologic evidence of influenza A(H1N1)pdm09 virus infection in 9.1% of pigs in backyard farms in Tumbes, Peru, with virus isolated from 1% of the animals. These findings highlight the potential risks of influenza virus transmission between humans and backyard animals.

Study Objectives:

- Describe epidemiology of influenza virus transmission between humans and backyard animals (poultry, swine and guinea pigs) in Tumbes, Peru.
- Determine potential risk factors for influenza virus infection associated with animal contact.

Approach: This study is nested in two of the study sites, Tumbes and Cusco, included in the influenza cohort study described in Entry One. A large percentage of the population of these areas lives in close proximity/contact with domestic pigs, poultry and guinea pigs, often in unsanitary conditions. A questionnaire regarding animal living conditions, diseases, and interaction with humans was administered and nasopharyngeal swabs taken from persons meeting a standard case definition for ILI as well as an age and sex-matched household control. Nasal swabs for pigs/guinea pigs and cloacal/pharyngeal swabs for poultry were collected when a human case of ILI was identified. Swab specimens were tested for evidence of influenza A virus by RT-PCR. Serum samples were also taken from animals to test for IgG antibody by hemagglutination inhibition to assess from previous exposure to influenza virus.

Timeline: Sample collection is completed. Data entry and laboratory testing are ongoing.

Progress and Findings: We have enrolled 141 cases of ILI and their matched controls. Swabs from 282 humans and 565 animals have been obtained, as well as 1,164 serum samples. Laboratory testing on the human cases shows 22% to be positive for influenza A H3N2 virus and 1.8% positive for influenza

A(H1N1)pdm09 virus. Only one animal sample, collected from a guinea pig, is positive for influenza A (sub-typing pending). Results from the swabs from human controls and serologic results from animals are pending.

Conclusion: During the period of study, exposure to domestic animals in backyard farms did not appear to increase risk of influenza A virus infection to humans, primarily because very few animals were infected. It is likely that the one infection detected in a guinea pig was acquired from a human. Serologic testing of the animals will give an indication of the risk of influenza A infection in animals over a broader period of time.

Serological Correlates of 2009 Pandemic H1N1 Influenza A Virus Immunity and Cross-Protection in a Neotropical Zone

Population-based data on the incidence of influenza A virus infection and the infection-to-disease ratio in the Amazon region of South America are limited. To address this knowledge gap, we conducted a prospective community-based cohort study to estimate the incidence of influenza A infection, antibody prevalence, and seroconversion rate (i.e. seroincidence) in Iquitos, a city in the Peruvian Amazon.

Study Objectives:

- Determine the age-specific prevalence of antibody to influenza A virus following the 2009 pandemic in Iquitos, Peru.
- Determine the extent of mild or asymptomatic infection and the effect of previous influenza virus infection on diseases severity.

Approach: We capitalized on an existing cohort study of dengue fever in Iquitos, Peru in which participants are monitored three times per week for acute febrile illness, including influenza-like illness. Serum was taken from a subset of these participants every six months, timed to represent the periods prior to, during, and following the first wave of pH1N1 transmission. Samples were tested by hemagglutination inhibition (HI) for evidence of antibodies to pH1N1, seasonal H1N1 (sH1N1), and H3N2 influenza A viruses.

Timeline: Samples were collected between early 2009 and mid-2010, and laboratory testing was conducted between January and June 2011.

Progress and Findings: A total of 3,375 samples from 1,606 participants were collected and screened for antibodies. Between the baseline and the end of the first pandemic wave, the highest proportion of seroconversion against pH1N1 was observed among younger participants: 39.8% of participants younger than 15 years seroconverted compared with 20.5% overall. Another 9.9% of the population seroconverted against pH1N1 during a second smaller wave. The overall ratio of subclinical: symptomatic infections was approximately 5:1, with subclinical infection more frequent in older age groups. We did not observe a clear effect of pre-pandemic pH1N1, sH1N1, or H3N2 antibodies on the odds of experiencing symptomatic infection.

Conclusion: Seroincidence rates of pH1N1 in Iquitos 2009 were similar to those reported for other countries. The attack rate was elevated in younger populations, suggesting that observed increased incidence of disease in this group is the result of increased exposure as opposed to a reflection of pre-existing immunity in older populations. The ratio of subclinical: symptomatic infection was 5:1. The prevalence of exposure to sH1N1 and H3N2 was low.

Occupational Exposure to Zoonotic Influenza Virus in Peru

As pandemic influenza A H1N1 virus continues to circulate, more questions arise about how this and other novel influenza viruses appear and are introduced into human populations. We have implemented an active surveillance cohort study to explore the frequency and epidemiology of zoonotic influenza virus transmission in humans with occupational risk of infection with swine and avian influenza viruses. We are also conducting surveillance in birds and swine.

Study Objectives:

- Determine the prevalence of antibodies against avian and swine influenza among humans regularly exposed to swine and birds.
- Estimate the incidence of zoonotic influenza in the exposed humans.
- Determine risk factors for zoonotic influenza infection in the exposed humans.
- Determine the prevalence of influenza virus infection in birds and swine.

Approach: Although this project leverages CDC-funded activities, it is primarily funded by NIH. Active and passive surveillance was established in three cities in Peru (Pucallpa, Tumbes, and Lima) to monitor humans with occupational exposure to poultry and swine through exposures in backyard farms, slaughterhouses, live bird markets, game bird breeding, and through clinical veterinary medicine. Subjects are followed weekly by phone to report ILI cases in the humans and animals. A monthly follow-up visit is also made. When human or animal ILI is reported trained personnel visit the site to collect nasal and oropharyngeal swabs from the humans and animals. Swabs are tested for influenza virus by RT-PCR. Blood samples are also collected every six months from each participant and tested for anti-influenza antibody by ELISA, with positive samples tested by hemagglutination inhibition assay against specific swine and avian influenza virus strains. Lastly, regular blood collections and serologic testing is conducted on pigs arriving at a slaughterhouse in Lima from various areas of Peru.

Timeline: Enrollment, sample collection, and testing are ongoing.

Progress and Findings: To date 415 human participants have been enrolled from whom 38 swabs and approximately 650 sera samples have been collected. Thirty-four (89.5%) of the swabs have been tested, of which 2.9 % are positive for influenza A(H1N1) pdm09, 8.8 % for influenza A H3N2, and 5.9 % for influenza B. Of the 650 serum samples, 345 (53%) have been tested, with 25%, 18% and 12% positive for antibody to influenza A(H1N1) pdm09 virus in Tumbes, Pucallpa and Lima respectively. None of 44 swabs collected from animals are positive for influenza virus, but 22% are positive for New Castle disease virus. The Ministry of Agriculture was notified. Serological testing on the animals is pending.

Conclusion: All evidence of influenza virus infection in the human population to date appears to be related to human-to-human or human-to-animal transmission. To date we have not identified episodes of novel zoonotic influenza virus infection in humans or animals, but surveillance is ongoing.

The Economic Cost of SARI in Hospitals in Peru

According to the World Health Report, the disease burden of respiratory tract infections is estimated at 94 million DALYs and 3.9 million deaths. However, there is limited information on the economic cost of severe acute respiratory illness (SARI) in Peru and the factors that influence this cost. We propose a prospective hospital-based study to assess the direct and indirect costs of influenza-associated SARI in hospitals in Peru.

Study Objectives:

- Calculate the health care costs associated with SARI using an incidence-based approach.
- Describe the characteristics affecting the health care costs of influenza-associated SARI in Peru.

Approach: We will collect information from randomly selected SARI cases admitted to three hospitals in Lima, Peru (one private, one from the Ministry of Health, and one from the Social Security system). Study staff will record the information for all direct and indirect costs associated with the SARI episode through daily monitoring. Sources of information will include interviews with patients or their relatives, medical records, accounting records, and daily interviews with hospital staff. Data collection will include patient demographic information and information related to the disease, previous and current therapy, time of onset, duration of illness, and symptoms. In addition to clinical information, the use of health care resources to manage the event will be collected. When known, the biological etiology of the SARI case will be noted.

Timeline: The study protocol was designed in 2011 and will be implemented in 2012.

Progress and Findings: Pending.

Conclusion: Pending.

The Effect of Micronutrient Deficiencies on Frequency, Severity, and Outcome of Influenza-like Illnesses

Micronutrient deficiencies have been associated with increased infectious disease morbidity rates, particularly diarrheal and respiratory infections. Children under the age of 5, who are anemic and who have previously developed diarrhea, are three times more likely to develop pneumonia. In general, there is a clear dose-response pattern between malnutrition and increased morbidity and mortality rates due to acute lower respiratory infections (ALRI)/pneumonia. However, data demonstrating decreased nutritional status as risk factor for acquiring an ILI or as a predictor of severity of illness is limited.

Study Objectives:

- Estimate the effect of micronutrient deficiencies on severity, frequency and outcome of an influenza-like illness (ILI) infection among individuals living in semirural communities of Peru.

Approach: All enrolled participants will be asked to join the nested study and provide a blood sample and a stool sample after giving their consent. Hemoglobin tests will be performed to address health status. Levels of Vitamins A, C, D, and the minerals Iron and Zinc will be detected from sera. Parasitological status will be assessed from analyzing the stool samples. This study aims to determine whether or not abnormal levels of micronutrients are associated with increased frequency, severity and outcome due to an ILI infection.

Timeline: On hold, awaiting funding.

Progress and Findings: Protocol approved by IRB.

Conclusion: None yet.

SENEGAL

Assessment of the Effectiveness of Seasonal Trivalent Influenza Vaccine (TIV) Among Children in Senegal

Effective influenza vaccines have been available for decades, but they have neither been studied nor used in tropical developing countries. A number of reports from developed countries indicate that influenza vaccine, when given to a limited number of persons most responsible for transmission, usually children, has the potential to interrupt transmission and reduce the overall influenza burden of a community. This study will determine whether immunization of young children (6 months to 9 years) with influenza vaccine will protect not only the immunized children but also the infants, older children and adults who are around them.

Study Objectives:

- Evaluate the total effectiveness of TIV in reducing rates of laboratory-confirmed symptomatic influenza among vaccinated children from villages where TIV is introduced, compared to rates among vaccinated children from villages where inactivated polio vaccine (IPV) is introduced.
- Evaluate the age-specific indirect effectiveness of TIV in reducing rates of laboratory-confirmed symptomatic influenza among unvaccinated persons, as well as the total (population) effectiveness in reducing community-wide rates of influenza in villages where TIV is introduced, compared to compared to rates in villages where inactivated polio vaccine (IPV) is introduced.
- Evaluate age-specific post-vaccination immune responses and describe the safety profile of TIV among a subset of vaccinated children.

Approach: This research is a partnership with PATH, the Institut de Recherché pour le Développement (IRD), and Institute Pasteur Dakar. The study is an observer-blinded Phase IV cluster-randomized trial with villages randomized into two groups. Children aged 6 months to 9 years in these villages receive either TIV or a control, inactivated polio vaccine (IPV). In addition, some of the vaccinated children will be enrolled into an immunogenicity and safety subset which will measure the immune response to vaccination and assess reactions to the study vaccines among Senegalese children. A combined approach of active and passive influenza surveillance will be used to assess laboratory-confirmed influenza among vaccinated children and consenting unvaccinated persons in order to evaluate the effects of the vaccines in this population.

Timeline: July 2008 to June 2014.

Progress and Findings: From 2009 to 2011, three vaccination rounds have taken place, with full vaccination of between 7,600 and 9,500 eligible children each spring. Surveillance activities, including administration of health questionnaires and nasopharyngeal specimen collection, are ongoing. As of 2011, over 20,000 febrile respiratory episodes have been identified and tested for influenza through community surveillance. Influenza A(H1N1)pdm was first detected in the study villages in February 2010, and preliminary findings were shared at the International Conference on Emerging Infectious Diseases (July 2010) and Options for the Prevention and Control of Influenza (September 2010). Data on the vaccination status of participants remain blinded; analyses of direct, indirect, and total effectiveness are pending unblinded analysis.

Conclusion: Vaccination rates are high among eligible children in this community, and influenza viruses are a major cause of febrile respiratory illness in the population.

SOUTH AFRICA

Respiratory Viral Co-infections Identified by a 10-plex Real-time Polymerase Chain Reaction Assay in Patients Hospitalised with Severe Acute Respiratory Illness— South Africa, 2009–2010

Use a newly developed multiplex assay to investigate 10 respiratory viruses including influenza (INF) A and B, parainfluenza (PIV1-3), respiratory syncytial virus (RSV), enterovirus (EV), human metapneumovirus (hMPV), adenovirus (AdV) and rhinovirus (RV) as causes of SARI during 2009–2010.

Study Objectives:

- Develop a real-time multiplex reverse transcriptase PCR assay to detect the most common respiratory viruses (influenza A and B, RSV, EV, hMPV, AdV, RV, PIV 1, 2 and 3) in nasopharyngeal aspirates and nose and throat swabs.
- Use the developed test assay to investigate the role of the most common viral agents as aetiological agents in patients hospitalised with SARI in South Africa.

Approach: The multiplex assay was developed to detect 10 respiratory viruses including influenza (INF) A and B, parainfluenza (PIV1-3), respiratory syncytial virus (RSV), enterovirus (EV), human metapneumovirus (hMPV), adenovirus (AdV) and rhinovirus (RV), followed by influenza subtyping. Nasopharyngeal and oropharyngeal specimens were collected from patients hospitalized with pneumonia at six hospitals during 2009–2010.

Timeline: From February 2009 up to December 2010.

Progress and Findings: Out of the 8,173 patients tested in this period 3,240 (40%) had single-infections, 1,426 (17%) co-infections and 3,507 (43%) were negative. The most common viruses were: RV (2,034, 25%), RSV (1,169, 14%), (1,083, 13%), influenza A (704, 9%). RSV, hPMV and influenza had seasonal patterns while AdV and RV were detected throughout the year. RV and RSV were associated with most single infections in children 0–1 years.

Conclusion: The data provide a better understanding of the viral aetiology of hospitalized cases of pneumonia and demonstrate the usefulness of this multiplex assay in respiratory disease surveillance in South Africa.

Evolutionary Dynamics of 2009 Pandemic Influenza A(H1N1) in South Africa from 2009-2010

Genotypic characterization of A(H1N1)pdm09 strains from influenza-like illness (ILI) and severe acute respiratory illness (SARI) cases from South Africa during 2009–2010.

Study Objectives:

- To investigate the evolution pandemic H1N1 in South Africa from July 2009 to December 2010.
- To describe the effect of hosting the FIFA Soccer World Cup in 2010 on the circulation of specific lineages.
- To describe the geographic and temporal distribution of strains, genetic and antigenic drift in HA genes and investigate genotypic markers of mild and severe disease and the presence drug resistant strains.

Approach: Amplify and sequence the HA1 region of the HA gene. Perform hemagglutination inhibition assays on viral isolates and sequence of the PB2 and NA genes to investigate pathogenicity and resistance mutations.

Timeline: September 2009–July 2011.

Progress and Findings: We investigated 9,792 and 6,915 specimens from patients with influenza-like illness (ILI) or severe acute respiratory infection (SARI) symptoms across South Africa for 2009 Pandemic Influenza A(H1N1) in 2009 and 2010 and conduct a molecular epidemiological investigations of 96 strains. The pandemic strain occurred as a second epidemic peak following seasonal H3N2 cases in 2009 and in 2010. Progressive drift away from the A/California/7/2009 vaccine strain was observed at both the nucleotide and amino acid level with 2010 strains clustering separate to 2009 strains although antigenically these strains were still similar to the vaccine strain. No resistance or known pathogenicity mutations were detected.

Conclusion: Pandemic H1N1 cases occurred in both 2009 and 2010 as a second wave following seasonal H3N2 cases in South Africa. Molecular epidemiological analyses suggest multiple introductions of the pandemic H1N1 strain in 2009 and at least two clusters in 2010 that are distinctly separate from 2009 strains. Progressive genetic drift away from the original vaccine strain is apparent; however antigenic investigations showed that these strains are still similar enough to A/California/07/2009 vaccine strain.

Risk of Death Amongst TB patients Hospitalised with Influenza in South Africa 2009–2010

This is an analysis of influenza surveillance data to assess the relationship between influenza and tuberculosis.

Study Objectives:

There are limited published data on influenza in patients with pulmonary tuberculosis (TB). We aimed to compare the characteristics of patients admitted with TB to those without TB and to determine whether influenza virus co-infection was a risk factor for in-hospital death among patients with suspected or laboratory-confirmed TB.

Approach: Hospitalised patients presenting with severe acute respiratory infection (SARI) were enrolled prospectively at public hospitals in four provinces of South Africa. TB cases were defined as patients with either a laboratory-confirmed diagnosis of TB, or currently receiving or started on TB treatment at the current admission.

Timeline: 2009–2011.

Progress and Findings: The influenza detection rate was similar in patients with (8% (94/1,162) and without (9% (618/6,935) TB ($p=0.36$). 76% of the 862 TB patients tested HIV positive compared to 50% (2257/4,512) of those without TB, ($p<0.001$). The case-fatality ratio was 10% (114/1,175) in TB cases as compared to 5% (319/7,004) non TB cases ($p<0.001$). On multivariable analysis amongst TB cases, patients who were co-infected with influenza were more likely to die than patients who tested influenza negative (odds ratio 2.59, 95% confidence interval 1.26-5.33, $p=0.009$).

Conclusion: Preliminary data suggest influenza co-infection may be associated with increased mortality amongst patients with TB. Patients with TB are a potential risk group that may be targeted for influenza vaccination.

Increased Risk of Death amongst HIV-infected Persons Hospitalised with Influenza-Confirmed Illness

This is an analysis of influenza surveillance data to assess the relationship between influenza and HIV.

Study Objectives:

- We aimed to compare the clinical and epidemiologic characteristics of HIV-infected and -uninfected patients with influenza infection.

Approach: Hospitalised patients presenting with severe acute respiratory infection (SARI) were enrolled prospectively at public hospitals in four provinces of South Africa. Clinical and epidemiologic data were collected. Upper respiratory samples were tested for the presence of influenza virus by real time RT-PCR and blood samples tested for pneumococcal DNA using real time PCR. The study was a cohort study including all patients testing influenza-positive. Characteristics of patients testing HIV-positive were compared to those testing HIV-negative.

Timeline: 2009–2011.

Progress and Findings: Amongst 1,022 patients hospitalised with influenza-associated SARI, HIV infection status was available for 731 (72%). On multivariable analysis controlling for age-group and sex, HIV-infected patients were more likely to have confirmed pneumococcal (36/323, 11% vs. 16/379, 4%, odds ratio (OR) 2.6, 95% confidence interval [1.2-5.6]) or tuberculosis (52/325, 16% vs. 14/393, 4% OR 7.3 [3.0-17.4]) co-infection, be infected with influenza type B (vs. A) (131/331, 40% vs. 108/400, 27%, OR 1.7 [1.1-2.6]) and have prolonged duration of hospitalisation. On multivariable analysis controlling for receipt of mechanical ventilation and other underlying conditions; HIV-infection (OR 4.0 [1.4-11.2]) and tuberculosis co-infection (OR 3.7 [1.3-10.3]) were independent risk factors for death. HIV-infected individuals experienced a 4–5 times greater age-adjusted incidence of hospitalisation than HIV-uninfected individuals.

Conclusion: HIV-infected patients have increased incidence of influenza hospitalization and experience increased mortality as compared to HIV-negative individuals. HIV-infected patients should receive early antiviral therapy and should receive preventive measures such as annual influenza vaccination.

HIV Infection and Influenza Co-infection Increase the Risk of Elevated Blood Pneumococcal Loads and Associated Mortality in Hospitalised Pneumonia Patients

This is an analysis of influenza surveillance data to assess the relationship between influenza and HIV.

Study Objectives:

- There is a lack of sensitive assays for making an etiologic-specific diagnosis of pneumococcal pneumonia. We determined the prevalence of pneumococcal DNA in blood and factors associated with high bacterial load and death in patients with hospitalised pneumonia.

Approach: Hospitalised patients presenting with severe acute respiratory infection (SARI) were enrolled prospectively at public hospitals in four provinces of South Africa.

Timeline: 2009–2011.

Progress and Findings: We determined the prevalence of pneumococcal DNA in blood and factors associated with high bacterial load and death in patients with hospitalised pneumonia. Overall 7% (372/5130) tested lytA positive. On multivariable analysis the lytA-positive patients with higher blood pneumococcal loads had a higher prevalence of HIV [adjusted odds ratio (AOR): 2.5, 95% confidence interval (CI): 1.6-3.8], influenza co-infection [AOR: 1.4, CI: 1.2-1.7] and were more likely to be treated with supplemental oxygen [AOR: 1.6, CI: 1.1-2.4]. Amongst lytA-positive patients increased risk of death was associated with pneumococcal loads of $\geq 10,000$ DNA copies/ml [AOR: 3.9, CI: 1.9-8.1], controlling for oxygen treatment and late presentation to the hospital.

Conclusion: HIV and influenza infections are significant risk factors for elevated pneumococcal loads in blood, which was also associated with an increased risk of death. High pneumococcal loads at time of diagnosis may have a role in future as a prognostic marker for pneumococcal pneumonia.

THAILAND

Randomized Controlled Trial to Compare the Immunogenicity of Intramuscular versus Intradermal Trivalent Inactivated Split Virion Influenza Vaccine in HIV-infected Men who have Sex with Men in Bangkok, Thailand

This study will assess the efficacy of a new intradermal formulation of the trivalent inactivated influenza vaccine (TIV) compared to standard intramuscular TIV in HIV-infected men who have sex with men (MSM) in Bangkok, Thailand.

Study Objectives:

- Primary Objective: To assess humoral antibody responses to intramuscular versus intradermal TIV in HIV-infected MSM prior to vaccination and at 1 month, 6 months, and 12 months post vaccination. We will further characterize humoral antibody responses by low versus high CD4 cell count.
- Secondary Objective: In a subset of participants, we will characterize cell-mediated immune responses to intramuscular versus intradermal TIV in HIV-infected MSM prior to vaccination and at 1 week, 1 month and 6 months post-vaccination. We will further characterize cell-mediated immune responses by low versus high CD4 cell count.

Approach: Randomized controlled trial.

Timeline: Enrollment started November 2011, participants followed for one year.

Progress and Findings: Enrollment ongoing, investigators remain blinded.

Conclusion: None yet.

Pediatric Respiratory Infections Cohort Evaluation (PRICE)

This is a longitudinal study to follow children aged 0–36 months for two years; we will follow children with underlying disease and age and time-matched healthy children.

Study Objectives:

Primary Objectives:

- To measure the rate of influenza acquisition and duration influenza illness in a cohort of healthy children and children with underlying disease.
- To evaluate the difference in the rate of influenza acquisition and duration of influenza illness between healthy children and children with underlying disease.

Secondary Objectives:

To repeat the first two Primary Objectives for RSV.

- To assess and compare disease severity between healthy children and children with underlying disease.
- To assess the medical costs (direct and indirect) associated with influenza and RSV infections and the difference in costs between healthy children and children with underlying disease.
- To evaluate the predictive value of markers of nutrition and inflammation, particularly vitamin D, for the incidence, duration and severity of influenza virus and RSV infections, as well as how these differ between healthy children and children with underlying disease.

- To investigate the relationship between environmental tobacco smoke exposure and occurrence of lower respiratory track disease and influenza virus and RSV infections.

Approach: Prospective, observational cohort study.

Timeline: Enrollment started August 2011, participants followed for two years.

Progress and Findings: Enrollment ongoing.

Conclusion: None yet.

Etiology of Pneumonia and Influenza-like Illness in Sa Kaeo and Nakhon Phanom Provinces, Thailand

This is a prospective study to describe the etiology and epidemiology of respiratory pathogens in Sa Kaeo and Nakhon Phanom provinces of Thailand.

Study Objectives:

- To perform population-based surveillance to determine the etiology and burden of acute respiratory diseases among patients hospitalized with community acquired pneumonia

Approach: Prospective, population-based.

Timeline: Enrollment started in 2003 in Sa Kaeo and 2004 in Nakhon Phanom.

Progress and Findings: Enrollment ongoing.

Conclusion: Established incidence, cost, seasonality and risk factors for influenza; data informed vaccine policy.

VIETNAM

Burden of Influenza-related SARI in 3 District Hospitals in Vietnam

Seasonal influenza viruses circulate in Vietnam throughout the year, however the understanding of influenza burden in Vietnam remains limited, especially the burden associated with hospitalized severe acute respiratory infection. Surveillance is carried out to detect severe acute respiratory infections (SARI) in three district hospitals from north, central and south Vietnam over a 12 month period. These data will be used to estimate disease burden of seasonal influenza virus in hospitalized severe acute respiratory infections (SARI).

Study Objectives:

- To describe morbidity and mortality of influenza-related SARI in hospitalized patients in district hospital in Vietnam over a 12 month period.
- To describe clinical, virological and epidemiologic characteristics of influenza-related hospitalized SARI cases in Vietnam.
- To compare influenza-related SARI to other hospitalized SARI at district hospital level in Vietnam.
- To evaluate the socioeconomic impacts of SARI for patients.

Approach: Data were collected to describe epidemiologic and clinical features at the district hospital level in Vietnam. Adults and children admitted to general medical wards of 3 selected district hospitals with SARI will be asked to participate in this surveillance. It is estimated that about 900 patients will be systematically sampled for evidence of influenza infection by RT-PCR testing of throat swabs taken at time of hospital admission. Epidemiologic, virological and clinical information from these patients will also be collected to estimate disease burden of hospitalized SARI in Vietnam.

Timeline: April 2011–March 2012.

Progress and Findings: Training sessions on burden study procedures, data collection, specimen collection and shipment were conducted for 63 field staffs at 3 selected district hospitals at the start of the study. After four months of field implementation, a total of 564 SARI patients were enrolled into the study and swabbed for RT-PCR testing. Thirty-two of the 564 tested specimens (6%) were positive for influenza infection (11 positive for influenza B virus and 21 positive for pA/H1N1/2009). A data entry system has been developed based on EpiData Version 3.1. The data on burden of influenza will be cleaned and analyzed at the end of the 12 month of data collection.

Conclusion: Interim results show that influenza viruses contribute to the burden of severe acute respiratory disease in district hospitals in Vietnam. Full analysis of the entire 12 month time period is forthcoming. Further studies looking at more severe respiratory disease in provincial hospitals is planned.

Animal Human Interface Studies: Pilot Extension Project for Influenza Viruses Infecting Humans and Animals in Vietnam

Vietnam has one of the highest densities of avian reservoirs of influenza A in the world, along with susceptible swine and human populations, all living in close contact. In domestic animals in Vietnam, influenza A is endemic. Avian strains circulating in fowl in Vietnam include H4N6, H5N2, H5N1, and H9N3. There is limited serological evidence of H5N1, H3N2, and H1N1 in swine in Vietnam. In humans, Vietnam has sporadic cases of avian influenza A/H5N1, and year-round transmission of seasonal strains, including H1N1 2009, H3N2, and B. Influenza virus isolates of H5N1 in humans and animals in Vietnam have varied genetically, producing a number of different clades, and suggesting a complex virus co-evolution. Given population densities, biosecurity practices, and close geographic proximity of animals and humans, opportunities for influenza virus genetic mutation and for cross-species transmission may be prevalent in Vietnam.

Study Objectives:

- To identify and characterize the circulating influenza viruses in humans, swine and poultry living in close proximity in a rural community in Vietnam.
- To determine the dynamics of animal-human interface transmission of influenza viruses in a rural community.
- To determine the phylogenetic relationships and genomic characteristics of virus isolates.

Approach: Participating households in a commune of the Red River delta area of north Vietnam with swine and poultry farming were surveyed to determine the relation by time and place of infections with influenza viruses in humans and animals. The households were visited every two days for three months to observe for human influenza-like-illness (ILI), and for sick pigs or poultry with respiratory symptoms. Blood samples and swabs from humans with self-reported ILI, and from poultry and pigs with reported respiratory illness, were tested for influenza infection. If influenza A was found in any species, humans and animals in the same households were followed up with blood and swab collection at the time of the first influenza confirmation and two weeks (for household members) and four weeks (for household animals) later. Paired human serum samples were also collected at the baseline of the study and three months after follow-up. RT-PCR was performed to identify influenza viruses in human and animal swab samples. HI was employed for testing human paired sera for H1N1 2009 or H5N1 antibodies; and ELISA and HI were used to test for immunity against influenza viruses from blood samples from pigs and poultry. Isolated influenza viruses from humans and animals will be further serologically and genetically analyzed in a subsequent study.

Timeline: April–July 2011.

Progress and Findings: A total of 446 people from 186 households had baseline serum samples collected and were observed for ILI and for pig and poultry respiratory illness. There were 220 serum samples collected three months later. The incidence rate of ILI was 28 episodes per 1,000 person-weeks (95% CI 17-45). From the follow-up, 27 episodes of ILI were observed in humans, seven respiratory episodes in poultry, and no illness in pigs. Of two sick pigs and 13 sick poultry, none were positive with influenza. Of the 27 human swabs, influenza pA/H1N1/2009 accounted for two cases (7%) and influenza B was found in three cases (11%). Of household and animal contacts of two H1N1 2009 positive persons, none of seven humans, one pig, and four poultry were positive for influenza after two weeks. Further virological and serological testing and genetic analyses are being done.

Conclusion: There was limited ILI in humans, and little respiratory illness in poultry and pigs, during this pilot study. Preliminary findings suggest that multiple strains of influenza viruses were identified in people and animals in a rural community. Sub-clinical human infection with H5N1 from poultry, and reverse zoonotic transmission of H1N1 2009 from humans to swine, are two of many animal-human interface variables that potentially may lead to continued genetic changes in influenza viruses, and uncertainties of the next pandemic strain in Vietnam or globally.