

INFLUENZA RESEARCH



Influenza Research

BANGLADESH

Hospital Based Human Influenza Surveillance

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

Study Aims and Objectives:

- To identify individuals and clusters of people with life threatening infections or severe respiratory disease caused by influenza virus and other respiratory pathogens.
- To characterize the diversity of strains of influenza in circulation in Bangladesh.

Approach: Sentinel influenza surveillance in 12 hospitals. In each surveillance hospital, physicians systematically identified patients aged ≥ 5 years with severe acute respiratory illness (SARI), or patients aged < 5 years with severe pneumonia (SP), or presenting as outpatients with influenza-like illness (ILI). Staff collect demographics and clinical history data and respiratory specimens for real time reverse transcription polymerase chain reaction (rRT-PCR) influenza testing.

Timeline: Surveillance was initiated in May 2007 and is ongoing.

Progress and Findings: During May 2007–August 2013 we collected 19,964 specimens. Among them, 2,912 (14.6%) tested positive for influenza virus. Among the influenza positive cases, infection due to influenza A and B were 1,984 (68%) and 924 (32%) respectively. Among the influenza A positive cases 137 (7%), 928 (47%) and 922 (46%) were due to A/H1, A/H3 and A(H1N1)pdm09 respectively. The highest flu positivity was observed among those of 5–14 years of age. Before the 2009 pandemic, we observed distinct influenza peaks from May to September. But after the pandemic, there is no distinct seasonality observed.

During October 2012–August 2013 we collected 3,115 specimens. Among them 552 (17.7%) tested positive for influenza. Among the influenza positives, most were infected with influenza A (478/87%). Among the influenza A positives highest frequency was due to A/H3 (306/64%). Among the collected specimens most were from ILI cases (1,333 /43%). The highest influenza positivity was among SARI cases (257/24%) and the lowest among SP cases (42/6%).

Conclusion: Influenza affects all age groups in Bangladesh, but most frequently those between 5 to 14 years old. Both influenza A and B are circulating in Bangladesh. Among the influenza A type, most prevalent circulating sub-type is A(H3). No human cases of H5N1 infection have been detected through this surveillance. Influenza seasonality may have altered after the 2009 pandemic.

Related Published Paper: Zaman RU, Alamgir ASM, Rahman M, Azziz-Baumgartner E, Gurley ES, et al. (2009) Influenza in Outpatient ILI Case-Patients in National Hospital-Based Surveillance, Bangladesh, 2007–2008. PLoS ONE 4(12): e8452. doi:10.1371/journal.pone.0008452.

Dispensing Practices and Impact of Educational Intervention for Acute Respiratory Illness among Drug Sellers, A Pilot Study in Bangladesh

Pharmacies are the primary source of health advice and medicines in Bangladesh but very little information is available regarding availability of oseltamivir as well as medication purchase patterns and drug dispensing practices in pharmacies for respiratory illness. Thus, this pilot study was undertaken to collect this data as well as to assess the adoption of treatment guidelines for acute respiratory illness by pharmacies in Dhaka city.

Study Aims and Objectives:

- To describe medications provided for acute respiratory illness (ARI) by drug sellers in pharmacies across Dhaka, Bangladesh.
- To describe factors driving patients' for health care seeking from pharmacy.
- To assess the availability of oseltamivir in pharmacies.
- To develop and assess the adherence and sustainability of an educational intervention for the empirical treatment of acute respiratory illness through pharmacies in Dhaka, Bangladesh.

Approach: icddr,b in collaboration with the government of Bangladesh and funding from CDC is conducting this cross sectional and interventional pilot study among 100 randomly selected pharmacies in Dhaka city. Field staffs acting as surrogate relatives of patients suffering from ARI with different clinical scenarios were deployed to each of the selected pharmacies to assess dispensing practices. Availability of oseltamivir at each selected pharmacy was also surveyed. Real-life pharmacy customers were interviewed to explore health care seeking drivers. After assessment of dispensing practices, a training manual for an educational intervention to improve the knowledge of the drug sellers for the management of ARI was developed with government and medical association partners. Pharmacists were trained and follow-up assessments will be performed to assess the adherence of the drug sellers to the training material and the sustainability of the educational intervention.

Timeline: May 2012–December 2013.

Progress and Findings: Among 100 selected pharmacies; none had oseltamivir available for sale during the survey. Drug sellers dispensed drugs for the patient in 64% of the field staff visits as surrogate relatives, recommended that the patient be seen by a physician in 31% of the visits and refused to give drugs without the patient present in 6% of the cases. Among all the dispensed drugs, acetaminophen was the most frequently dispensed drug (40%), followed by antibiotics (37%), antihistamines (30%), and bronchodilators (17%). According to WHO management guidelines for ARI, 70% of the dispensed drugs were inappropriate but not harmful, 23% were appropriate, 5% could not be judged appropriate or inappropriate (herbal medicine) and 2% were inappropriate and potentially harmful.

Conclusion: Drug sellers were unfamiliar with and did not stock oseltamivir. Their dispensing practices for ARI were often not in accordance with ARI management guidelines.

Related Published Papers: In Progress.

Surveillance for Human Infections with Avian Influenza A Viruses among Live Bird Market Workers and Their Household Members in Dhaka City Area, Bangladesh

Live bird market (LBM) workers in Bangladesh are at risk of avian influenza A virus (AIV) infection due to ongoing circulation of these viruses among market poultry and their occupational exposure. LBM workers handle, slaughter and process poultry and offal, mostly without personal protective equipment. We initiated active influenza surveillance among a closed cohort of LBM workers and their household members to identify human cases of AIV infection, to detect circulating AIVs and to assess serological evidence of AIV infections.

Study Aims and Objectives:

- To identify live bird market workers and their household members infected with avian influenza A viruses.
- To characterize avian influenza A virus strains causing human infections and to compare them to strains currently circulating among poultry in live bird markets.
- To assess the serological evidence of avian influenza infection among live bird market workers and their household members.

Approach: Surveillance was implemented in 16 LBMs with ongoing avian influenza surveillance of poultry since 2009. Among LBMs, four are wholesale (operating 24 hours/day) and 12 are retail. We enrolled 750 workers and 1,830 household members proportionately from each market through random sampling, based on the total number of LBM workers. Enrolled workers were visited twice a week at the market to determine if they or their household members experienced illness compatible with AIV infection since the last visit. A possible case of AIV infection was defined as: fever (subjective, measured or reported) and/or any respiratory signs or symptoms or conjunctivitis. For all possible cases, nasopharyngeal and oropharyngeal and conjunctival (if conjunctivitis was present) swabs were collected in viral transport media. The samples were tested by real-time RT-PCR (rRT-PCR) for influenza viral RNA and further subtyped if positive for influenza A (H1N1pdm09, H3, H5, H9). Acute (<7 days since illness onset) and convalescent serum (>21 days after acute serum collection) were collected from laboratory-confirmed influenza A participants and tested for influenza H5N1 and H1N1pdm virus specific antibodies by microneutralization and hemagglutination inhibition assays.

Timeline: This project was initiated in February 2012 and is ongoing.

Progress and Findings: From February 2012 to September 2013, a total of 1,183 poultry workers or their household members experienced illness compatible with AIV infection. Of 1,183 swab samples tested, 107 (9%) tested positive for influenza A and 25 (2%) tested positive for influenza B by rRT-PCR. Of 107 samples positive for influenza A, 33 (30%) were subtyped as H1N1pdm09, 23 (21.5%) as H3, 15 (14%) as H9N2, 10 (9%) as H5N1, 19 (18%) were unsubtypeable, and 4 (4%) were inconclusive. No worker with detectable H5 viral RNA developed severe illness.

Thirty paired (acute and convalescent) blood samples have been collected from the influenza A+ cases for serological testing by hemagglutination inhibition and microneutralization assays. Among available paired serum testing results from 15 participants, including two with H5 viral RNA detected in respiratory specimens, none demonstrated seroconversion to H5N1, four LBM workers had evidence of H5N1-specific antibodies suggesting previous infection, and three had evidence of seroconversion to H1N1pdm09 virus.

We have shared the positive H5N1 results with the Government of Bangladesh and CDC. All avian flu positive samples were shipped to CDC for further characterization.

Conclusion: Among symptomatic poultry workers in Bangladesh LBMs, we detected seasonal influenza A and B viruses, and viral RNA from AIVs, including H5 and H9. Use of serological testing on paired serum can help to assess acute infection versus exposure to AIVs and inform the interpretation of detection of AIV RNA in LBM poultry workers exposed to AIVs. Surveillance in LBMs is useful for detecting and monitoring persons with occupational exposure to AIVs and increasing understanding of transmission of AIVs at the animal-human interface.

Incidence of Influenza-associated Mortality in Bangladesh, 2010–2012

Influenza-associated mortality estimates can help assess the burden of disease, identify high risk groups and assist policy makers determine if an annual influenza immunization should be a prioritized public health intervention.

Study Aims and Objectives:

- To estimate the incidence of influenza-associated mortality in Bangladesh during 2010–2012.
- To describe health seeking behavior for severe acute respiratory illness (SARI).

Approach: During July to December 2012, we randomly selected 22 unions, the smallest administrative units in Bangladesh, from the catchment areas of 11 tertiary hospitals and collected mortality data in the unions. We employed a social networking approach to identify people who died in these unions in May 2010 to April 2012. We interviewed the household members, who had taken care of the decedents during their illness and asked about decedent's demographics, medical history, symptoms, and health seeking 14 days before death. For children aged <5 years, we classified a death as being associated with acute respiratory illness (ARI) if caregivers reported that decedents developed sudden onset fever and cough or difficulty breathing within 14 days of death. For people aged ≥5 years, we classified a death as being associated with ARI if caregivers reported that decedents developed sudden onset fever and cough or sore throat within 14 days of death. As part of the ongoing national hospital based surveillance in 11 hospitals, physicians routinely collected throat and nasopharyngeal swabs from case-patients. We only considered swabs from children <5 years who presented with fever and cough or difficulty breathing with one danger sign; and from patients presented with fever and cough or sore throat if aged ≥5 years. We tested the samples for influenza and its subtypes. To calculate the incidence of influenza-associated mortality, we multiplied the monthly proportion of samples from the hospitals that were laboratory-confirmed for influenza in May 2010 to April 2012 by the number of decedents in the community with a history of sudden onset of ARI 14 days prior to death, and divided this numerator by the age-specific 2011 census population of the unions.

Timeline: May 2012–February 2013.

Progress and Findings: We identified 10,043 deaths; 1,191 (12%) were associated with ARI. Of those who died with ARI, 150 (13%) were children <5 years, 30 (2%) were aged 5–19 years, 211 (18%) were aged 20–59 years and 800 (67%) were aged ≥60 years; 738 (62%) were males. A total of 360 (13%) patients were laboratory-confirmed for influenza; the proportions of samples that were confirmed for influenza among people aged ≥5 years were 19% and 4% among children <5 years. The annual cumulative incidence of influenza-associated mortality was 11.1 (95% CI 10.4–11.7) per 100,000 population for all ages. The annual incidence of influenza-associated mortality among children <5 years was 3.7 (95% CI 3.1–4.3); for people

aged 5–19 years it was 1.3 (95% CI 0.8–1.8); for 20–59 years it was 5.3 (95% CI 4.6–6.1) and for persons aged ≥60 years the incidence was 108.5 (95% CI 101.1–116.0) per 100,000 persons. After extrapolating the incidence rate to the 2011 census population of Bangladesh, the estimated deaths associated with influenza were 15,989 (95% CI 14,981–16,853) per year among all ages; it was 12,503 (95% CI 11,650–13,367) among ≥60 years annually.

Conclusion: The highest burden of influenza mortality in Bangladesh appears to be among persons aged ≥60 years. Vaccination among elderly persons may reduce the burden of influenza in the country.

Related Published Papers: In Progress.

Identifying Zoonotic Transmission of Swine-borne Diseases in Bangladesh

Zoonotic infections from swine pose a worldwide public health concern because of their potential to infect humans and lead to pandemics. In addition, pigs are potential pathogen reservoirs and amplifiers for transmission. Through this study, we would like to understand zoonotic transmission of influenza and rotavirus viruses in swine and swine raisers, as well as to create a platform to conduct surveillance on swine-borne zoonoses in Bangladesh.

Study Aims and Objectives:

- To establish active surveillance to identify and characterize influenza A viruses in pigs and rotavirus strains circulating in piglets
- To detect pathogens of zoonotic origin in pig raisers (influenza A viruses and rotavirus)
- To create a platform to explore pathogen transmission between pigs and people.

Approach: To understand zoonotic transmission of influenza A viruses, icddr,b in collaboration with the government of Bangladesh and funding from CDC has been conducting swine surveillance in two pig raising communities and one pig slaughterhouse. We have been collected nasal swab samples from the pigs regardless of their health status in two pig raising communities in two sub-districts of Rajshahi (Bangladesh) to identify current subtypes and strains of influenza viruses in circulation. We are also conducting surveillance in a pig slaughterhouse in Gazipur district, where nomadic, and backyard raised pigs are brought from all over the country. Simultaneously, we are collecting nasal and throat swabs of those showing influenza-like illness (ILI) symptoms among the pig raiser household members in the two pig raising communities for detecting circulating influenza viruses. The samples are tested by real-time RT-PCR (rRT-PCR) for influenza viral RNA and further subtyped if positive for influenza A. The viral genomes will be sequenced to characterize the diversity of the viruses identified in humans and in pigs. We are also investigating the prevalence of antibodies against influenza A viruses in pigs and the pig raisers and compare with a non-pig raising population. To identify circulating rotavirus in the pig population, we have been sampling piglets (under two months of age) with diarrhea. Rotavirus will be identified by real time RT-PCR and the viral genome for further characterization by sequencing. Additionally, we have been conducted surveillance to identify children (less than five years) in the pig raising communities having diarrhea and look for rotavirus in their stool samples. Rotaviruses will be detected and sub-typed by rRT-PCR. Pig rotavirus strains will be compared with human strains identified in the pig raising community as well as through a hospital based surveillance in Bangladesh to detect possible interspecies transmission.

Timeline: Initiated in June 2013 and ongoing.

Progress and Findings: So far, we have collected 220 pig nasal swab samples, of which 160 have been tested and found 11 samples positive for influenza A (H3). We have also collected nasal and throat swabs samples from 27 ILI patients and among them we have tested six samples and found one sample positive for influenza A (H3). We have also collected 200 serum samples from the primary pig caregivers and abattoir workers but these have not yet been tested. We have collected three stool samples from the under five children with diarrhea and these sample are under laboratory processing. We have also collected two fecal samples from the piglet with diarrhea and the samples are under laboratory processing. Sample collection, processing and testing are ongoing.

Incidence of Influenza-associated Respiratory Illness among Pregnant Women in Bangladesh

Physiological changes during pregnancy puts pregnant women at increased risk for severe influenza infections and associated complications. Several studies have documented severe influenza infections in pregnant women including excess maternal mortality and loss of pregnancy. To our knowledge, there are no data describing burden of influenza among pregnant women and limited data on health impact of influenza on pregnant women during pregnancy and on infant birth weight and gestational age in Bangladesh.

Study Aims and Objectives:

- To assess the burden of seasonal influenza infection in pregnant women in Bangladesh.
- To assess the health impact of maternal influenza illness with pregnancy related complications and growth of young infants including neonates in Bangladesh.

Approach: We have been conducting this study in eight upazillas (sub-district) in Bangladesh. We identified pregnant women from the community with the help of female welfare assistants (FWA) who identify pregnant women during their door to door visit in the villages. Subsequently icddr,b field staff visited the pregnant women's household and enrolled the women in the study. After enrolment, we followed up each participant once a week over phone or through household visit. Whenever any participant mentioned having influenza-like illness (ILI) during follow-up contact, the trained field staff collected nasal swab from that participant. The swab specimen was sent to icddr,b virology lab for influenza testing. When any participant informed about her delivery, the field staff visited the household within three days of delivery to collect the weight of newborn, to enroll the newborn in this study and then started to follow-up of the newborn until six months of age for episodes of ILI.

Timeline: May 2013–September 2013.

Progress and Findings: We completed identification and enrollment of study participants during May–June 2013. We enrolled a total of 1,912 pregnant women in this study. Of those, we have been communicating with 1,811 participants once a week to identify any episode of ILI from July 2013 to second week of September 2013. We had to drop 101 participants, primarily due to migration to areas outside of the study site and a few refused to be contacted. Currently there are 1,210 pregnant women and 610 young infants under follow-up. We collected nasal specimen from 81 pregnant women who reported to have ILI and of 81 specimens, five (6%) had detectable influenza virus RNA. We have not yet identified any ILI episode among neonates.

Conclusion: This study data would describe the burden and suggest if maternal influenza infection has health impact on pregnant women and on infants in low-income settings such as Bangladesh. Moreover, the data may also help in targeting influenza vaccines for pregnant women in Bangladesh to reduce influenza burden and associated complications during the pregnancy period as well as indirectly the very young infants.

Characterization of Children Hospitalized with Respiratory Illness in Bangladesh

Limited data is currently available on the burden of influenza and other respiratory illness in Bangladesh. This study aims to characterize the burden of viral respiratory illness, particularly for illness requiring hospitalization, as well as to identify potential risk factors for severe illness in children under five years of age.

Study Aims and Objectives:

Aim

- Estimate the burden of childhood viral respiratory illness in Bangladesh.

Objectives

- Determine the incidence of medically attended influenza and other respiratory viral illness in children aged <5 in Bangladesh.
- Understand the seasonality of childhood respiratory viral illness in Bangladesh.
- Identify the risk factors for severe influenza and other respiratory viral illness in children.
- Explore the causal association between respiratory viral infection and symptomatic illness.
- Determine the association between micro nutritional deficiencies and severe influenza and other respiratory viral infections in children.

Approach: Hospital-based surveillance in combination with health utilization survey.

We have ongoing surveillance in four hospitals which will act as the main platform for the study. Twice every month research physicians enroll children admitted in the hospitals residing in the primary catchment areas of the hospital. Children age <5 years 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 children. We collect throat and nasal swab samples from these children. All the respiratory samples collected are being tested for influenza A and B, RSV, metapneumovirus, adenovirus, parainfluenza viruses and rhinovirus by real-time RT-PCR. Findings will be compared 1:1 to children of the same age with influenza-like illness who visit the outpatient department but are not admitted.

In addition we have been collecting respiratory specimens from asymptomatic hospitalized children from January 2012, which will help us better understand the causal association between influenza infection and respiratory illness. We have also started collecting blood samples from children with and without respiratory symptoms for micronutrient testing which includes Vitamin A, D and Zinc from January 2013 which will help us better understand the role of these micronutrient on influenza infection.

Timeline: October 2010–September 2014.

Progress and Findings: We have completed four years of data collection from children presenting with acute respiratory symptoms at the outpatient and inpatient department of four sentinel surveillance hospitals in Bangladesh where the study is being conducted. Between October 2010 and January 2013 we have collected data and respiratory samples from 400 hospitalized children and 1,400 children attending the outpatient department with acute respiratory symptoms. The data collection will continue until September 2014.

Based on the analysis of three years of data we estimated the incidence of ambulatory influenza illness per 100 children per year at 13.3 (95% CI 10–20) in 2011 and 11.3 (95% CI 9–19) in 2012. Similarly, incidence of hospitalized influenza illness per 1,000 children per year was 0.5 (95% CI 0.09–1.2) in 2011 and 0.3 (95% CI 2–4.3) in 2012. In addition incidence of hospitalized RSV illness per 1,000 children per year was 3.58 in 2011 and 3.16 in 2012. Similarly the incidence was 1.46 and 0.73 for parainfluenza viruses and 1.28 and 0.27 for human metapneumovirus viruses. We will conduct the risk factor analysis at the end of the study period in 2014.

A monthly report is disseminated to co-investigators and collaborators within icddr, the Government of Bangladesh and CDC.

Conclusion: The surveillance has been providing crucial information in terms of frequently occurring respiratory viral infection in children, a group who are at higher risk of morbidity and mortality associated with influenza and other respiratory viral infections. In addition, the data may also help in targeting influenza vaccines for children at risk in Bangladesh.

DOMINICAN REPUBLIC

Needle-Free Cutaneous Jet Injection of Reduced-Dose Influenza Vaccine in ≥ 6 to < 24 -Month Old Children in the Dominican Republic

Most developing countries cannot afford influenza vaccines (INF) to protect their populations. One potential strategy to lower cost is to reduce antigen mass and deliver into intradermal (ID) or intramuscular (IM) tissues. To that end, a controlled, randomized study in the Dominican Republic used jet injection, avoiding the risks and drawbacks of conventional needle-syringe (NS) to determine whether immune responses suggesting protection can be induced safely in young children by reduced 0.1 mL ID doses of INF administered ID with an investigational, disposable-syringe jet injector (DSJI), or IM by conventional NS, compared to standard 0.25 mL IM doses by NS.

Study Aims and Objectives:

- Assess non-inferiority for seroconversion (SC), seroprotection (SP), geometric mean titer (GMT) or GMT foldrise (GMTFR) of two 0.1 mL INF doses ID by investigational spacer on a Biojector® 2000 DSJI, or by two 0.1 mL doses IM by NS, compared to two full doses IM of 0.25 mL in controls.
- Determine the tolerability of local and systemic reactions of INF delivered ID by DSJI, compared to full-dose IM.

Approach: A total of 450 healthy participants ≥ 6 and < 24 months of age were recruited at the national children's hospital in Santo Domingo. Consented eligibles received two doses one month apart of trivalent, inactivated Vaxigrip® INF (Sanofi-Pasteur) in randomly-allocated investigational study arms ID-JI-0.1 (n=150) or IM-NS-0.1 (n=150), or in control arm IM-NS-0.25 (n=150). Investigators were blinded to allocation; unblinded nurses vaccinated in a closed room without parents present.

Timeline: Phase I patients first enrolled in October 2006. Data collection from final Phase II subjects occurred in November 2009. After serologic assays and data cleaning, the DSMB unblinded the investigators in quarter two of 2013.

Progress and Findings: Unblinded analyses of the Total Vaccinated Cohort found systemic reactions generally mild and similar among all study arms, while local reactions – although mostly mild – were more frequent in the ID-JI-0.1 arm. Moderate pain occurred on either injection in 3% (5/150) of ID-JI-0.1 subjects and 1% (1/150) of IM-NS-0.25 controls. Severe pain occurred in 2% (3/150) and 1% (2/150), respectively.

The immunologic endpoint of non-inferiority was not achieved for SC, SP, GMT, or GMTFR against H1N1, H3N2, or B vaccine antigen type by either the ID-JI-0.1 or IM-NS-0.1 reduced-dose study arms, compared to IM-NS-0.25 controls. All immune measures were lowest for ID-JI-0.1, intermediate for IM-NS-0.1, and highest for IM-NS-0.25. For example, post-dose-1-and/or-2 SC rates for H1N1 were 57.1%, 67.1%, and 73.6%, respectively. For H3N2, SC was 44.6%, 62.4%, and 79.7%, and for type B, 64.6%, 72.5%, and 87.8%, respectively.

Conclusion: Causes for inferior immune responses of jet-injected cutaneous delivery are hypothesized and being analyzed, including “wet” injections (noted in 26 [17%] of 150 ID-JI-0.1 subjects), which may result from variable operator technique, jet-cartridge bubbles, and short plunger stroke for 0.1 ml. The safety profile was tolerable and not a contraindication for this technique.

EL SALVADOR

Characterization of Influenza Co-infection, Dengue Fever and Other Respiratory Viruses in Patients Hospitalized in El Salvador

Prospective cohort.

Study Aims and Objectives:

- Assess the utility of the dengue and severe acute respiratory infection case-definitions during the rainy season in El Salvador.

Approach: We describe the influenza laboratory confirmed cases among dengue case-patients admitted in four hospitals, in El Salvador, during 2012 rainy season.

Timeline: 2012–2013.

Progress and Findings: We found that dengue case-patients were more likely to test positive for influenza (19% CI95% 12–26%) than severe acute respiratory case-patients (10%, CI95% 6–14%). The prevalence of influenza-dengue co-infection was 1%.

Conclusion: Health officials should explore the diagnosis of influenza and the potential value of empirically treating dengue and/or SARI case-patients with oseltamivir during influenza season, especially in those cases with respiratory signs or severe disease.

GHANA

Health Facility–based Surveillance for Influenza and Other Respiratory Viruses in Residents of Shai–Osudoku and Ningo–Prampram Districts, Ghana

After the 2009 influenza pandemic, data on acute respiratory infections started being gathered in Ghana from nascent hospital-based surveillance. Knowledge of the epidemiology of influenza and other respiratory virus infections will assist in producing reliable estimates of disease burden.

We will document the occurrence of influenza-like illnesses (ILI) and hospitalized severe acute respiratory infections (SARI), and the proportion attributable to influenza infection. These data will be combined with health utilization data to estimate the burden of influenza in the Shai-Osudoku and Ningo-Prampram Districts (SONPD).

Study Aims and Objectives:

Aim

- To describe the epidemiology, etiology and outcome of ILI and SARI caused by influenza and other respiratory pathogens.

Objectives:

- Establish enhanced health facility-based surveillance for outpatient ILI cases, hospitalized SARI cases, and deaths caused by influenza and other respiratory pathogens in SONPD.
- Measure the incidence, prevalence, demographic characteristics (including age, sex, ethnicity, and socioeconomic status), clinical spectrum and outcomes for outpatient ILI cases, hospitalized SARI cases, and deaths due to influenza and other respiratory pathogens.
- Identify etiologies of ILI cases, SARI cases, including deaths attributable to influenza and other respiratory pathogens using routine methods (Polymerase Chain Reaction and culture).
- Determine risk factors for severe influenza.
- Evaluate case definitions for influenza and other respiratory pathogens in the SONPD.

Approach: We intend to estimate the incidence of influenza infection in the SONPD via health facility-based prospective surveillance.

Timeline: This cooperative agreement between CDC and the Noguchi Memorial Institute for Medical Research was awarded October 2012 and continues being funded. The first sample for this study was collected February 2013.

Progress and Findings: The study is effective and has been successful thus far. Participants are recruited from nine sites; seven in SONPD, one in Lower Manya District and one in North Tongu District with the total number of outpatient consultations also being collected from additional eight public health facilities in SONPD. Data and sample collection are ongoing, progressively being analyzed and yet to be published.

Conclusion: The results of this study will assist the Ministry of Health/Ghana Health Service and the global scientific community to generate effective policies based on reliable estimates of the burden of influenza and other respiratory infections. Furthermore, it will also be an essential step in establishing a strong platform for additional studies, which will help to better understand the epidemiology of influenza and other respiratory viruses not only in Ghana but in the African region.

Related Published Papers: Pending.

INDIA

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

This HHS/CDC cooperative agreement was established for five years (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 Aims and 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 two study areas (Ballabgarh and Vadu) 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, outpatient screening and enrollment was done to estimate the burden of non-hospitalized medically attended influenza disease.

Timeline: April 2009–September 2013.

Progress and Findings:

The proportion of patients with laboratory-confirmed influenza was higher at Vadu than Ballabgarh during all study years (21% vs. 5%, $p < 0.05$ in 2010; 18% vs. 5%, $p < 0.05$ in 2011; 23% vs. 5%, $p < 0.05$ in 2012).

- Annual acute medical illness hospitalization rates were similar at both sites (Vadu: 101.3 to 224.7 per 10,000; Ballabgarh 86.0 to 126.2 per 10,000), whereas annual influenza-associated hospitalization rates were 5–9 fold higher in Vadu (20.3–51.6 per 10,000) compared to Ballabgarh (4.4–6.3 per 10,000).

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 US.
- Age specific differences from site to site.

Related Published Papers:

Gupta V, Dawood F, Rai S, Broor S, Wigh R, Mishra AC, Lafond K, Mott J, Lal RB, Krishnan A. Validity of clinical case definitions for influenza surveillance among hospitalized patients: results from a rural community in North India. *Influenza Other Respi Viruses*. 2012 Jul 16. doi: 10.1111/j.1750-2659.2012.00401.x.

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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 Aims and Objectives: To estimate burden due to pneumonia, important viral (such as influenza, Respiratory Syncytial Virus) and bacterial causes (*H. Influenzae* and *Streptococcus Pneumoniae*) of acute respiratory infections and drug sensitivity patterns of the causing agents in a rural community.

Approach: Specimens collected from under 5 children of SARI cases from July 2009–June 2011 were tested for various pathogens using a real-time RT-PCR.

Timeline: July 2009–June 2011.

Progress and Findings: Analysis of 245 specimens revealed 127 (52% had presence of one of the respiratory virus, including high prevalence of RSV (20.3%), followed by Rhinovirus (17%) and PIV3 (4%) and influenza (7%).

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.

Related Published Paper: Broor S, Dawood F, Pandey BG, Saha S, Gupta V, Krishnan A, Rai S, Erdman D, Lal RB. Respiratory viruses are associated with a substantial proportion of hospitalizations among children aged <5 years in rural northern India. *Clinical Infectious Disease. Journal of Infection* (In press).

Epidemiological Study of Respiratory Pathogens in ARI among Children and Elderly in India

Acute respiratory infections (ARI) are a major cause of morbidity and mortality among children globally, with the greatest number of deaths occurring in developing countries. While estimates of total episodes of ARI and incidence of hospitalization due to pneumonia in India have been generated, no study has been carried out to date to systematically identify etiological agents in a population-based setting.

Study Aims and Objectives:

- Estimate the incidence of ALRIs among children (under 5 years) and elderly (over 60 years).
- Assess the relative burden of select viral and bacterial respiratory pathogens associated with ARIs.
- Characterize the clinical spectrum of ARI associated with identified bacterial and viral pathogens.
- Document the antimicrobial sensitivity pattern of major respiratory pathogens isolated from ARI patients.
- Quantify with a societal perspective the economic burden associated with morbidity due to ARI in under-fives and elderly.

Approach: A surveillance platform was initiated in August 2012 in four villages in Ballabgarh Block of Haryana State in North India, with a population of 2,754 children below 10 years. All children are screened weekly for presence of any respiratory symptom (cough, sore throat, ear ache/discharge, nasal discharge/congestion, shortness of breath, respiratory difficulty) or hospital admissions. World Health Organization (WHO) standard case definitions are used for ARI, including upper (AURI) and lower respiratory infections (ALRI), and pneumonia are used. Those identified by screening are assessed clinically by trained nurses. Records of those hospitalized are reviewed by medical officers. Throat and/or nasopharyngeal swab are collected from children meeting these case definitions, and tested for both bacterial and viral pathogens. Urine samples are also collected from pneumonia cases for pneumococcal antigen testing.

Timeline: August 2012–August 2016.

Progress and Findings: After initial pilot runs, the platform became fully functional on 14th August 2012. Until 28th March 2013 (90,031 child weeks of surveillance) 10,561 ARI cases have been reported, giving an incidence rate of 6.1 per child year. Four hundred thirty-four (434) cases of ALRI were diagnosed, giving an ALRI incidence rate of 0.25 per child year. Of the 429 cases tested for Influenza, 20 were positive (10 for H1N1pdm09; nine for Influenza B and one for H3). Of the 295 samples tested for bacteriology, 20 were positive for *S. pneumoniae*, 15 for *staph aureus* and three for *H. influenzae*. None of the 201 control specimens were positive for influenza and only one tested positive for *Strept. pneumoniae*. Tests for other viruses and urinary antigens are still to be carried out.

Conclusion: A fully functional population-based surveillance platform has been set up which is providing useful information on epidemiology and etiology of ARI in this cohort. This platform can serve as a potential vaccine trial site in future.

Related Published Paper: Nair H, Simões, EA, Rudan I, Gessner BD, Azziz-Baumgartner E, Zhang JS, Feikin DR, Mackenzie GA, Moisi JC, Roca A, Baggett HC, Zaman SM, Singleton RJ, Lucero MG, Chandran A, Gentile A, Cohen C, Krishnan A, Bhutta ZA, Arguedas A, Clara AW, Andrade AL, Ope M, Ruvinsky RO, Hortal M, McCracken JP, Madhi SA, Bruce N, Qazi SA, Morris SS, El Arifeen S, Weber MW, Scott JA, Brooks WA, Breiman RF, Campbell H; Severe Acute Lower Respiratory Infections Working Group. Global and regional burden of hospital admissions for severe acute lower respiratory infections in young children in 2010: a systematic analysis. *Lancet*. 381:1380–90, 2013.

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

Through collaboration between the All India Institute of Medical Sciences (AIIMS), CDC, University of Denver 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 Inactivated Polio Vaccine (IPV) (the control vaccine) and weekly household surveillance for febrile acute respiratory illness (FARI) is conducted among all household members.

Study Aims and 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 three 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 five 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–September 2014.

Progress and Findings:

- Enrollment, vaccination, and surveillance activities have been successfully implemented since October 2009 with high annual rates of vaccine acceptance (~90% of 3,700 eligible children) in every round, and exceptionally high rates of community acceptance for surveillance enrollment (90% of 18,000 persons consented to participate for weekly surveillance).
- Between November 2009–November 2012, 28,955 FARI cases have been sampled and influenza positivity was identified among 3,251 (11.2%) across all age groups.
- While predominant circulating strain in 2009 was A/H1N1pdm09, co-circulation of both influenza B and A/H1N1 pdm09 was observed in 2010. In 2011, A/H3N2 was predominant strain but in 2012, A/H1N1pdm09 re-emerged with no case of A/H3N2; Inf B co-circulated during both these years accounting for half of influenza positive cases.

- Influenza seasonality in Delhi coincides with rainy season in July, and evidence from the study was used to change the vaccination timing to pre-monsoon using SH strains from the earlier winter timing in November using NH strains.

Conclusion:

- Enrollment and immunization rates for Influenza vaccine are high, reflecting on high community acceptance.
- Likely no benefits of vaccination in first year (due to vaccine mismatch).
- 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 for first year estimated but further analysis needed.

Related Published Papers:

Sullender W, Fowler K, Anand K, Gupta V, Moulton L, Lafond K, Widdowson M-A, Lal RB and Broor S. Design and initiation of a study of the direct and indirect effects of influenza vaccine given to children in rural India. *Vaccine*, 30:5235–5239, 2012.

Fowler K, Broor S, Sullender W, Gupta V, Purakayastha DR, Widdowson MA, Lal RB, Krishnan A. Incidence rate of symptomatic 2009A/H1N1 and seasonal influenza infection in a rural Indian community. *Int J Infect Dis*. 2013 Sep 12. doi:pii: S1201–912(13)00265–8. 10.1016/j.ijid.2013.08.005. [Epub ahead of print]PubMed PMID:24103331.

Broor S, Sullender WS, Fowler K, Gupta V, Widdowson M-A, Krishnan A, Lal RB. Demographic Shift of Influenza A(H1N1)pdm09 during and after Pandemic, Rural India. *Emerg. Infect. Dis*. 18:1472–1475, 2012.

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 on the other hand 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 Aims and 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 viral and 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 hold 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) downregulates the PKR pathway via interaction with HSP-40 (PLoS One published).
- The Influenza A virus Neuraminidase protein via upregulation 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.

Related Published Papers:

Tripathi S, Cao W, Sharma K, Patel JR, Ranjan P, Katz JM, Cox NJ, Lal RB, Sambhara S, Lal SK. Influenza A virus Nucleoprotein induces cell death in human lung epithelial cells by targeting Clusterin. *Cell Death & Disease* 2013 Mar 28;4:e562. doi: 10.1038/cddis.2013.89.

Sharma K, Tripathi S, Ranjan P, Kumar P, Garten R, Deyde V, Katz JM, Cox NJ, Lal RB, Sambhara S, Lal SK. Influenza A virus nucleoprotein exploits Hsp40 to inhibit PKR activation and eIF2 α phosphorylation. *PLoS One*. 6:e20215. 2011.

Sharma S, Mayank A, Tripathi S, Patel JR, Gaur P, Katz JM, Cox NJ, Lal RB, Sambhara S, Lal SK. Influenza A virus Nucleocapsid protein interacts with a cytoskeleton scaffolding protein α Actinin-4 to regulate viral replication and transcription. *PLoS One*. 6:e20215. 2011.

Gaur P, Patel JR, Sharma S, Ranjan P, Gangappa S, Katz JM, Cox NJ, Lal RB, Sambhara S, Lal SK. Influenza A virus neuraminidase protein enhances cell survival through interaction with CEACAM6. *J. Biol. Chem*, 287(18):15109–17, 2012.

Severe Acute Respiratory Infection (SARI) among Children Less than Five Years of Age: Use of TAC Multiple Pathogen Detection Platform in International Influenza Program Sites (TAC-KID)

The study is a prospective case-control study aimed at understanding the etiology of severe respiratory disease among hospitalized children less than five years of age in a tertiary level hospital in Delhi, India.

Study Aims and Objectives:

- Estimate the prevalence of selected viral and bacterial respiratory pathogens among children less than five years of age hospitalized with SARI and among children without respiratory illness over a 12 month period.

- Describe the seasonality and compare the etiology of SARI among children less than five years of age.
- Identify risk factors for severe acute respiratory infection by etiology and compare by site among children less than five years of age.

Secondary Objective:

- To compare laboratory findings from TaqMan array cards to standard laboratory wet assay procedures.

Approach: The study will enroll case children less than five years of age who are hospitalized with SARI and control children who are visiting outpatient clinics but not experiencing an infectious illness. The proposed study will seek to enroll cases and controls over a 12 month period.

Timeline: July 2013–June 2014.

Progress and Findings: The study has just started enrolling patients.

This study is part of multi-site study; other sites are in Malawi, Peru and South Africa.

Cost of All-cause and Influenza-associated Acute Respiratory Infections in North India

Acute Respiratory Infections (ARI) are important causes of mortality and morbidity, especially among children. Data on the economic burden of ARI are important to guide policy decisions about options for ARI prevention, such as national recommendations for available vaccines against pneumococcus and influenza. However, the economic burden of ARIs is not well documented in India where a large proportion of the population incurs out of pocket expenditure for medical care.

Study Aims and Objectives:

- Evaluate costs to patients for medically-attended (outpatient and inpatient) episodes of ARI in select public and private hospitals in and around Delhi, India.

Approach: A cross-sectional survey was conducted among patients attending the outpatient departments or hospitalized at three public and eight private hospitals. Participants were asked about direct medical costs (costs of consultation, investigations, and medications), non-medical costs (travel, food, etc.) and indirect costs (loss of wages) for treatment of ARI episodes. Nasopharyngeal swabs were taken only from hospitalized children (<10yr) and elderly (>60yr) patients and tested for influenza viruses by RT-PCR. Data on the cost of outpatient services and hospital beds for public hospitals were obtained from the World Health Organization-Choosing Interventions that are Cost Effective (WHO-CHOICE, 2011) and these costs were added to costs reported by patients to calculate total direct medical costs of hospitalization; all costs were converted to 2013 US dollars using the Wholesale Price Index (WPI) of India.

Timeline: September 2012–March 2013.

Progress and Findings:

- A total of 523 patients were enrolled, including 309 outpatients and 214 hospitalized patients.
- The median direct and indirect costs to the patient per outpatient ARI episode were US\$ 3.8 (IQR 2.70–9.69) and US \$1.9(IQR 1.9–3.8) for public facilities and US \$9.5 (IQR 4.0–28.0) and US \$3.8 (IQR 1.9–3.8) for private facilities.

- Compared to hospitalizations at public facilities, total costs (medical and non-medical) were higher at private facilities (US \$117.6 IQR 70.9–283.5 versus US \$236.31, IQR 155.0–375.0); medical costs alone were also higher (US \$92.4, IQR 56.2–198.3 versus US \$203.5, IQR 140.4–336.8).
- In contrast, caregiver's median wage loss due to hospitalization was lower at private facilities (US \$19.1; IQR 15.2–25.2) than at public facilities (US \$28.8; IQR \$19.1–61.1), likely due to longer hospitalization.
- Of the 112 hospitalized patients, 6 (5.4%) had respiratory specimens positive for influenza viruses.

Conclusion: The economic impact of ARI episodes in India is substantial, with a single hospitalization costing more than the monthly per capita income (US \$114.5) in India. These results are from one site, and point to the need for generation of such estimates from other parts of the country.

Divergent Patterns of Circulating Influenza Viruses in Two Regions of Northern India: When Does Equator Not Define Hemisphere?

Distinct patterns of pandemic 2009A/H1N1 (pH1N1) influenza were observed recently in the two northern regions of India (500 miles apart), with Srinagar having peaks of influenza activity in the winter season (January–February 2009) and the Delhi region in monsoon times (July–August 2010).

Study Aims and Objectives:

- To reassess the seasonality of influenza in two cities in north India to and allow better recommendation of appropriate vaccination timing for these cities in India.

Approach: A total of 4,652 patients presenting with influenza-like illness (ILI) at the tertiary care centers of SKIMS, Srinagar (n=2,126) or AIIMS, New Delhi (n=2,526) were enrolled from January 2011 to December 2012. Nasopharyngeal swabs were tested by real time-PCR for seasonal and pandemic influenza viruses. Sequence analysis was carried out.

Progress and Findings:

- Influenza positivity was higher (375/2,126; 17.6%) in Srinagar, than in Delhi (239/2,526; 9.46%).
- Distinct seasonality and subtypes patterns were observed in these two cities: Srinagar had peaks in January–March with predominant strains being A/H1N1pdm09 in 2011 and A/H3N2 in 2012, followed by resurgence of A/H1N1pdm09 in November–December 2012.
- In contrast, Delhi has peak influenza circulation in July–September with co-circulation of A/H3N2 and influenza B in 2011 and A/H1N1pdm09 in 2012.
- For both years we observed that a co-circulating subtype in Delhi area precedes than that of Srinagar area, suggesting a remarkable difference in distribution of circulating influenza viruses at these two centers.
- In addition, influenza seasonality in Srinagar (34.090N) matched the pattern observed in temperate climate, whereas Delhi with monsoon in July–September and a latitude of 28.660N which is just 500kms from Srinagar revealed influenza during summer months (July–September).

Conclusion:

- Thus India, though physically located in northern hemisphere, has distinct seasonality related to the latitude location of the city.

- While cities with temperate seasonality will benefit with vaccination in October–November, cities with peaks in monsoon season in July–September will likely benefit with vaccination in May–June of every year.

Related Published Papers:

Koul P, Khan U, Bhat K, Saha S, Broor S, Lal RB, Chadha M. Recrudescence Wave of A/H1N1pdm09 Influenza Viruses in Winter 2012–2013 in Kashmir, India. *PLOS Currents Outbreaks*. 2013 Sep 26. Doi:10.1371/currents.outbreaks.f1241c3a2625fc7a81bf25eea81f66e6.

Broor S, Krishnan A, Roy DS, Dhakad S, Kaushik S, Mir MA, Singh Y, Moen A, Chadha M, Mishra AC, Lal RB. Dynamic patterns of circulating seasonal and pandemic 2009A (H1N1) influenza viruses from 2007–2010 in and around Delhi, India. *PLoS One*. 7:e29129, 2012.

Koul PA, Muneer MA, Bali NK, Chawla-Sarkar M, Sarkar M, Kaushik S, Khan UH, Ahmad F, Garten R, Lal RB, Broor S. Pandemic and seasonal influenza viruses among patients with Acute Respiratory Illness in Kashmir (India). *Influenza Other Respi Viruses*. 5(6):521–527, 2011.

KENYA

Ongoing 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 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 Aims and 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 antivirals or vaccines.

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 KEMRI/CDC laboratory in Nairobi for influenza and other viral pathogens using real time RT-PCR.

Timeline: Surveillance for respiratory illness in the two sites began in 2006. Scientific presentations and publications from this surveillance platform are described further below.

Progress and Findings: Influenza virus has been shown to circulate year-round with a peak in activity generally between July and October of every year. During peak season the percent of outpatient ILI and hospitalized SARI patients testing positive for influenza approaches what has been observed during

winter seasons in temperate climates. From January 2008 to December 2012 the average rate of influenza-associated hospitalization (inpatient SARI) was 3.6(95% CI 2.3–75.5) per 1,000 person-years in children <5 in Lwak. The average rate of influenza-associated medically-attended SARI (inpatient and outpatient) in children <5 was 13.6 (95% CI 10–18.5) per 1,000 person-years in Lwak and 20.2(95% CI 16.4–24.9) per 1,000 person-years in Kibera. The average rate of influenza-associated medically-attended ILI in children <5 was 67.5(95% CI 59.9–76.1) per 1,000 person-years in Lwak and 45.5(95% CI 39.3–52.6) per 1,000 person-years in Kibera. Few children who had laboratory-confirmed influenza were diagnosed with influenza by the treating clinician in the inpatient (0%) or outpatient (2.5%) settings. 55% of children in Lwak and 99% of children in Kibera meeting the SARI case definition were treated as outpatients. Outpatient influenza-associated SARI cases in Lwak and Kibera were as likely as hospitalized influenza-associated cases to be hypoxic ($p=0.48$) and to be diagnosed with pneumonia ($p=0.11$).

Conclusion: The burden of influenza-associated hospitalization in Kenyan children from 2008–2012 was 1.2–10 times higher than estimates of influenza-associated hospitalizations in the US States of Oregon and Michigan from 2008–2011. Many children who had an IMCI danger sign were not hospitalized and the SARI case definition applied was fairly stringent, which may indicate that the true burden of influenza-associated severe disease in Kenyan children is much higher than current estimates suggest. Few clinicians diagnosed children with influenza despite the presence of a global pandemic during the reporting period. Influenza-associated disease remains under-recognized in Kenyan children. In general at both sites, rates of influenza-associated illness are highest among children <2 years old and lowest among adults ≥ 50 years old.

Related Published Paper: Katz MA, Lebo E, Emukule G et al. Epidemiology, Seasonality, and Burden of Influenza and Influenza-Like Illness in Urban and Rural Kenya, 2007–2010. *J Infect Dis.* 2012 Dec 15;206 Suppl 1:S53–60. doi: 10.1093/infdis/jis530.

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

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

Study Aims and Objectives:

- Characterize etiologies of hospitalized respiratory illness at Siaya District Hospital.
- Monitor the impact of influenza on hospitalizations and deaths 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 KEMRI/CDC 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 RT-PCR. Testing using newly developed Taqman Array Card (TAC) multi-pathogen PCR technologies has recently started to test all specimens simultaneously for seven bacterial and 13 viral pathogens. Comprehensive clinical data are also collected, and asymptomatic controls are being recruited to evaluate the attributable fraction of illness associated with different pathogens.

Timeline: Surveillance is ongoing.

Progress and Findings: From August 2009 to July 2012, we enrolled 5,507 SARI patients and 1,632 ILI patients. Most (SARI=75%, ILI=77%) were children <5 years; median age was 1.6 years and 2.4 years for SARI and ILI patients, respectively. The respiratory viruses most commonly detected in hospitalizations using conventional PCR methods were AdV, PIV-3, RSV, HMPV and influenza A.

The average annual incidence per 1,000 persons of influenza-associated SARI was 4.8(95% CI 3.0–7.6) among children < 2 years; 1.4(95% CI 0.7–2.8) in children aged 2–5 years; and 0.3(95% CI 0.2–0.4) among persons ≥5 years. The incidence of influenza-associated medically attended ILI per 1,000 was 32.6(95% CI 19.2–55.4) among children < 2 years; 19.0(95% CI 11.3–31.8) in children aged 2–5 years; and 3.8(95% CI 2.6–5.7) among persons ≥5 years.

Rates per 1,000 persons of SARI associated with RSV were 10.7(95% CI 7.9–14.6) among children < 2 years; 2.0(95% CI 1.1–3.4) in children aged 2–5 years; and 0.1(95% CI 0.0–0.2) among persons ≥5 years. Rates of ILI associated with RSV were 29.4(95% CI 16.8–51.4) among children < 2 years; 21.6(95% CI 13.3–35.1) in children aged 2–5 years; and 0.8(95% CI 0.3–1.9) among persons ≥5 years. The case fatality proportions were 13/348 (4%) and 14/437 (3%) among SARI patients with laboratory confirmed influenza and RSV, respectively.

Taqman array analyses have found *Streptococcus pneumoniae*, Rhinoviruses, RSV, AdV, Enteroviruses, and Influenza viruses to be most commonly detected in hospitalized patients. Attributable fractions of detected pathogens associated with illness will be calculated with the recruitment of asymptomatic controls during the upcoming year.

Conclusion: Influenza and RSV produce a significant burden of disease in western Kenya. These estimated rates of the inpatient and outpatient burden of influenza in Kenya are higher than published direct estimates from the US. Our estimates of the incidence of hospitalized influenza-associated SARI are also consistent with other studies in western Kenya. Additional work will further estimate the burden of non-medically attended influenza and RSV in this context. During the upcoming year we will also evaluate the burden of multiple respiratory pathogens in the context of underlying HIV and TB.

Related Published Paper: Fuller JA, Summers A, Katz MA et al. Estimation of the National Disease Burden of Influenza-Associated Severe Acute Respiratory Illness in Kenya and Guatemala: A Novel Methodology. *PLoS One*. 2013;8(2):e56882. doi: 10.1371/journal.pone.0056882. Epub 2013 Feb 27.

Surveillance for Hospital-acquired Infections in Kenya

While healthcare-associated infections (HAIs) are an important cause of morbidity and mortality worldwide, the burden of HAIs has not been documented in Kenya. In 2010, the Kenyan Ministry of Health, the Kenya Medical Research Institute (KEMRI) and CDC, initiated surveillance for respiratory in an initiative to build infection control capacity. Surveillance for HAIs is conducted at three public hospitals— a national referral hospital, a provincial general hospital, and a district hospital.

Study Aims and Objectives:

- Monitor respiratory HAI among patients admitted to selected surveillance wards at three hospitals in Kenya.
- Assess the incidence of respiratory HAIs on surveillance wards.

- Describe the epidemiology of incident respiratory HAI on surveillance wards.
- Provide a platform for expanded HAI and anti-microbial resistance surveillance.

Approach: At each site, surveillance officers survey pediatric, adult general, surgical, and specialty wards for HAIs. Patients admitted to the hospital for more than three calendar days who develop new onset of fever or hypothermia ($\geq 38^{\circ}\text{C}$ or $< 35^{\circ}\text{C}$) or who developed new onset of cough or sore throat are considered to have suspected HAI. Suspected HAI cases are assessed for onset of clinical symptoms and signs by questionnaire and medical record review. Nasopharyngeal and oropharyngeal samples are then collected from these patients and sent to the CDC-Kenya laboratory in Nairobi, where they are tested by RT-PCR for influenza A & B, adenovirus, respiratory syncytial virus, human metapneumovirus, and parainfluenza virus 1, 2 and 3. Specimens positive for influenza A are subtyped by real time RT-PCR.

Timeline: Surveillance started in September 2009 and is ongoing.

Progress and Findings: From April 2010–September 2012, of the 379 cases of rHAI; 230 (60.7%) were males and 217 (57.3%) children < 18 years old. The incidence of rHAI was 9.2/10,000 patient-days. Incidence in ICUs (33.0) was significantly higher than in pediatric wards (8.4) and medical wards (7.1) ($p < 0.0001$ for both). Of the 140 cases with specimens tested, 45.7% had at least one virus detected.

Conclusion: This is the first systematically collected surveillance data to estimate the burden of healthcare-associated respiratory illness in East Africa. Our estimates of the incidence of HAIs in Kenya provide baseline estimates that have some consistency with respiratory HAI rates observed elsewhere. Infection control measures should be strengthened in Kenyan hospitals, and this platform will be expanded to monitor the burden of additional HAIs and anti-microbial resistant pathogens.

Observational Seasonal Influenza Vaccine Effectiveness Study

In recent years, surveillance has demonstrated a high burden of influenza throughout Africa. However, influenza vaccine is rarely used on most of the continent. Little is known about the effectiveness of the vaccine in Africa, where HIV, malnutrition, malaria and other comorbidities are prevalent. KEMRI/CDC, 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 Aims and 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.
- Acceptability of influenza vaccination among community residents.

Approach: The vaccine is offered on a voluntary basis to children aged 6 months to 10 years 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 on the benefits and availability of the influenza vaccine. After vaccination, the study

participants are followed using the routine IEIP surveillance which includes bi-weekly home visits where field workers ask household members questions about recent illnesses and deaths. The study participants also have 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 KEMRI/CDC laboratory using real time RT-PCR for influenza virus.

Timeline: Data from the past three years of influenza surveillance have shown that the influenza season in Kenya peaks from July to 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 has just completed its third and final year.

Progress and Findings: Of the approximately 10,000 eligible children in the two sites, 30%, 36%, and 38% were fully vaccinated in 2010, 2011, and 2012, respectively; 11%, 12%, and 13% were partially vaccinated. During the three 9-month follow-up periods, there were 144, 77, and 102 cases of influenza, respectively, among children aged 6 months–10 years. The median age of cases was 4.8 years and 149 (46%) were female. In the first two years, influenza A predominated (67% of cases in 2010–2011 and in 2011–2012), and influenza A comprised 50% of influenza cases in 2012–2013. In 2010–2011 and 2011–2012, most influenza A cases were influenza A(H1N1)pdm09, while in 2012–2013, influenza A(H3N2) predominated. We included 244 cases and 608 test-negative controls in our evaluation. VE among fully vaccinated children was 44% for the entire study period (95% confidence interval (CI) = 20–61%). Season-specific VE was 51% (95% CI = 16%–71%) during 2010–2011, 48% (95% CI = -16%–77%) during 2011–2012, and 26% (95% CI = -29%–60%) during 2012–2013. We cultured 48/144 specimens in 2010–2011 and 29/77 specimens in 2011–2012; all isolates were considered well-matched to vaccine strains. Strain information for 2012–2013 is not yet available.

Conclusion: During three years in an urban and rural community in Kenya, over one-third of eligible children were fully vaccinated through a campaign offering free trivalent inactivated influenza vaccine. Among children aged 6 months–10 years, fully vaccinated children were ~40% less likely to have an influenza-associated medically attended respiratory illness overall. VE appeared lower in 2012–2013; pending antigenic characterization data may help explain this finding.

Related Published Papers: In Progress.

Determinants of Influenza Vaccine Uptake in Kenya

The overall safety record of trivalent inactivated influenza vaccine in young children is excellent. The challenge with respect to use of the inactivated influenza vaccine in young children is not demonstrating safety and efficacy, but the practicalities of delivering two doses of the vaccine in the first year, followed by annual vaccination. We have undertaken research to evaluate the uptake of seasonal influenza vaccine in children of two Kenyan communities following a seasonal influenza vaccine effectiveness study. We also specifically have investigated the independent roles that mothers and fathers play in the decision to have children vaccinated for influenza.

Study Aims and Objectives:

- To evaluate the social, demographic and geographic determinants of seasonal influenza vaccine uptake in Kenya.
- To evaluate the independent role that fathers and mothers play in the decision to vaccinate children for influenza.

Approach: From 2010–2012 the Kenya Medical Research Institute/Centers for Disease Control and Prevention-Kenya (KEMRI/CDC) and the Kenya Ministry of Public Health and Sanitation implemented an observational seasonal influenza vaccine campaign, offering free vaccine to study residents aged 6 months–10 years old within two existing KEMRI/CDC population-based disease surveillance sites (population=53,000) in Kenya; Lwak, a rural community in western Kenya, and Kibera, an informal urban settlement in Nairobi. During this campaign we evaluated the social, demographic and geographic factors associated with influenza vaccine uptake among eligible children. In 2012, nurses at health facilities administered standardized questionnaires to the parents or caretakers of vaccinated children during the campaign from March–May. We also sampled approximately 500 households at each site and conducted structured interviews of male health care decision-makers/caretakers from July–August. Households were stratified into three categories: fully vaccinated against influenza (all children in the household received the recommended number of doses of influenza vaccine), partially vaccinated (one or more of the children in the household received a dose of vaccine, but not all children received all needed doses), and not vaccinated against influenza (no children in the household received vaccine).

Timeline: March 2010–July 2012.

Progress and Findings: We provided 27,602 doses of vaccine to 17,613 children in the two communities. Of the 1,074 sampled households, 456 (42.5%) were fully vaccinated against influenza, 405 (37.7%) were partially vaccinated and 213 (19.8%) were not vaccinated. Families living >5km from the facilities were significantly less likely to have their children vaccinated (aOR=0.69; 95% CI 0.53–0.90; p=0.006). Over 90% of children were brought by their mothers to the vaccination campaign. Households in which both fathers and mothers were away from home for a duration >3 months at the time of vaccination were less likely to vaccinate their children compared to households in which both parents were in the home during the vaccination campaign (aOR=0.29; 95%CI 0.09–0.88; p=0.03). However households where either the father (aOR=0.81; 95%CI 0.62–1.06; p=.12) or the mother (aOR=1.60; 95%CI 0.68–3.74; p=.28) was away during the vaccination campaign were more likely not to have their children vaccinated than households where both parents were present. Households with fathers who believed that influenza vaccines are important (aOR=5.10; 95%CI 1.63–15.97; p=0.01); mothers who believed that influenza vaccines are important (aOR=11.98; 95%CI 3.97–36.14; p<0.001); and where both parents believed that influenza vaccines are important (aOR=8.35; 95%CI 3.16–22.06; p<0.001) were each more likely to have their children vaccinated when compared to households where both parents did not believe that the vaccines were important. Finally, households where fathers joined in the decision to have children vaccinated were more likely to vaccinate their children than households where the father did not join the vaccination decision (aOR=3.01; 95%CI 1.62–5.62; p<0.001).

Conclusion: Future campaigns will need to consider ways to adapt to vaccination schedules to the needs of working parents, and community mobilization efforts may need to specifically target alternative family members or designated caretakers that may bring children for vaccination if working parents are unavailable. These findings support the notion that future influenza vaccination campaigns in Africa may need to consider opening additional vaccination centers if large portions of the targeted population will have to travel greater than five kilometers for vaccination. Mothers are highly visible parental caretakers at the time of vaccination, as 90% of vaccinated children were brought by their mothers for influenza vaccination in Kenya. However despite the visibility of mothers at the time of a child's vaccination, the fathers' role in vaccine decision-making should not be overlooked. Targeting fathers in sensitization activities during future campaigns may also help to increase vaccination coverage rates.

Related Published Paper: Otieno NA, Nyawanda BO, Audi A et al. Demographic, Socio-economic, and Geographic Determinants of Seasonal Influenza Vaccine Uptake in Rural Western Kenya, 2011. *Vaccine*. 2014 Jan 22. pii: S0264-410X(13)01498-9. doi: 10.1016/j.vaccine.2013.10.089. [Epub ahead of print].

Direct Economic Burden of Medically Attended Influenza in Western Kenya from the Parental Perspective, 2009–2011

The economic costs of influenza in tropical Africa remain under-explored. Calculating the economic burden of influenza is crucial to estimating the costs and benefits of vaccine implementation. We estimated the direct costs of medically-attended influenza disease in Kenya from the parental perspective.

Study Aims and Objectives:

- To estimate the direct economic impact of medical care for influenza on families in Western Kenya.

Approach: We analyzed medical records for 7,388 inpatients and outpatients at Lwak Mission Hospital (LMH) to identify persons with severe acute respiratory illness (SARI) or influenza-like illness (ILI) during January 1, 2009–December 31, 2011. We examined medical chart data for prior outpatient consultations and medications taken for the current illness, routine diagnostic tests, medications administered to patients during the current visit, and length of stay (for hospitalized patients). We determined costs for consultation, routine diagnostic tests, medications, and hospital admission from the medical facility catalogue of prices. Cost estimates for consultations prior to any hospital visit were taken from published literature from western Kenya. Influenza diagnostics are not routine and were not included in direct cost estimates.

Timeline: Medical records reviewed from 2009–2011.

Progress and Findings: The mean (SD) patient cost in Kenyan shillings (KSH) of influenza-associated outpatient ILI, outpatient SARI, and hospitalized SARI in children under age 5 was 1,254(1,129.1–1,378.5), 937(307.6–1,566.9), and 2,609(2,152.2–3,064.8), respectively. The total annual direct economic burden of influenza in children < 5 in Nyanza Province was estimated to be from 68.6 to 133.0 million KSH (US \$847,000–\$1,642,000) for outpatients (ILI and SARI); and 4.8 to 10.3 million KSH (US \$59,000–\$127,000) for inpatients.

The mean (SD) patient cost in KSH of influenza-associated outpatient ILI, outpatient SARI, and hospitalized SARI in persons aged 5 and older was 1,155(45.4–2,265.5), 1,997(1,937.9–2,055.5), and 3,036(2,681.3–3,389.9), respectively. The total annual direct economic burden of influenza in persons aged 5 and older in Nyanza Province was estimated to be from 243.8 to 335.4 million KSH (US \$3,010,000–\$4,141,000) for outpatients (ILI and SARI); and 2.8 to 7.0 million KSH (US \$35,000–\$86,000) for inpatients.

The mean direct costs per outpatient visit and hospitalization for influenza of 1,400 and 3,000 KSH are approximately 5% and 11% of average monthly income (28,000 KSH), respectively.

Conclusion: The direct costs of inpatient and outpatient care for influenza-associated illness are considerable. Ongoing work will now estimate the indirect costs of influenza, costs from the governmental perspective, and then the cost-effectiveness of influenza vaccination.

The Role of HIV in the Household Introduction and Transmission of Influenza in an Urban Setting, Nairobi, Kenya, 2008–2011

Little is known about how high HIV seroprevalence affects the transmission dynamics of influenza within household settings in tropical sub-Saharan Africa.

Study Aims and Objectives: We use household and clinic data collected at the IEIP research site in Kibera during 2008 through 2011 to describe

- the association between the HIV status of household members and their risk of introducing influenza to the home, and
- the association between the HIV status of index cases of influenza in the home and the subsequent risk of developing secondary influenza-like illness (ILI) among their household contacts.

Approach: We used respiratory illness data gathered from a population-based household and clinic surveillance system in Kibera urban informal settlement, Nairobi, Kenya to examine the association between the HIV status of household members and their risk of introducing influenza to the home. We also examined the association between the HIV status of laboratory-confirmed influenza index cases in homes and the risk of their household contacts developing influenza-like illness (ILI).

Timeline: Household and clinic data collected from 2009 through 2011.

Progress and Findings: In comparison to persons over age 18, index cases of influenza were more likely to be children aged <2 (aRR 4.78; 95%CI 3.18–7.17), 2–4 (aRR 3.35; 95%CI 2.20–5.09), and 5–17 years (aRR 2.56; 95%CI 1.78–3.67) when adjusted for HIV status and household size. HIV status was not associated with influenza index case status (aRR 1.34; 95%CI 0.68–2.66), when controlling for age group of the household member and household size. However, the risk of developing ILI among household contacts of HIV-positive index cases was more than twice the risk of developing ILI among household contacts of HIV-negative index cases, when adjusted for age group of the household-contacts (aRR 2.36; 95% CI 1.19–4.66).

Conclusion: These results suggest that while children may be most likely to bring influenza into the homes in Kibera, HIV-positive influenza index cases may enhance transmission of influenza within the home. HIV status should be considered in future studies evaluating the risk factors for influenza transmission. HIV control programs also have a possible additional benefit of reducing influenza transmission. Finally prioritizing vaccine availability for clinics treating HIV-positive individuals and encouraging such individuals to be vaccinated may be of possible value not only because HIV-positive individuals are at an elevated risk for severe clinical symptoms and mortality, but also because of the potential to reduce influenza transmission within the home.

Predicting Mortality among Hospitalized Children with Respiratory Illness in Western Kenya, 2009–2012

Pediatric respiratory disease is a major cause of morbidity and mortality in the developing world; 70% of global deaths from respiratory disease in children occur in Africa and South East Asia. In Kenya, approximately 16% of annual deaths in children < five years of age are attributed to acute respiratory infections (ARI). In most health facilities in Kenya, resources are limited; having a better understanding of which children hospitalized with respiratory illness are at greatest risk of severe outcomes could improve patient triage and inform resource allocation. We developed a modified Respiratory Index of Severity in Children (mRISC) scoring system using easy to monitor syndrome-based risk factors for in-hospital mortality in children aged less than five years that were hospitalized with a respiratory illness in Siaya District Hospital (SDH) in Western Kenya.

Study Aims and Objectives:

- To develop a clinical prediction tool with practical utility to rapidly identify children most at risk for fatal outcomes due to respiratory disease in Western Kenya.

Approach: We analyzed data from children <5 years old who were hospitalized with respiratory illness at Siaya District Hospital (SDH) from 2009–2012. We used a multivariable logistic regression model to identify patient characteristics predictive for in-hospital mortality. Model discrimination was evaluated using the concordance statistic. Using bootstrap samples, we re-estimated the coefficients and the optimism of the model. The mRISC score for each child was developed by adding up the points assigned to each factor associated with mortality based on the coefficients in the multivariable model.

Timeline: Influenza and respiratory disease surveillance data from Siaya District Hospital, 2009–2012.

Progress and Findings: We analyzed data from 3,581 children hospitalized with respiratory illness; including 218(6%) who died. Low weight-for-age [adjusted odds ratio (aOR)=2.1; 95% CI 1.3–3.2], very low weight-for-age (aOR=3.8; 95% CI 2.7–5.4), caretaker-reported history of unconsciousness (aOR=2.3; 95% CI 1.6–3.4), inability to drink or breastfeed (aOR=1.8; 95% CI 1.2–2.8), chest wall in-drawing (aOR=2.2; 95% CI 1.5–3.1), and being not fully alert on physical exam (aOR=8.0; 95% CI 5.1–12.6) were independently associated with in-hospital mortality. The positive predictive value for mortality increased with increasing mRISC scores.

Conclusion: This study shows that a clinical prediction tool, similar to the RISC score initially developed in South Africa, may also have practical utility to rapidly identify children most at risk for fatal outcomes due to respiratory disease in Western Kenya. As a complementary tool for use alongside the IMCI guidelines, the mRISC could help improve the clinical management and in-hospital triage of children admitted with respiratory illness.

Related Published Papers: In Progress.

Influenza in Pigs in Kenya

Influenza A H1N1pdm09 (H1N1pdm09) virus is a swine-origin virus that was first detected in humans in April 2009 and has since been detected in multiple animal species in countries across the world. Infections in animals have often been associated with contact with humans infected with the pH1N1 virus. There has been limited surveillance for influenza virus strains circulating in pigs in Kenya. We carried out surveillance in pigs at a local slaughterhouse located near Nairobi to determine prevalence and sero-prevalence of influenza A viruses.

Study Aims and Objectives:

- To determine prevalence and sero-prevalence of influenza A viruses in pigs in Kenya.

Approach: We collected nasal swabs and blood samples from pigs arriving at the slaughterhouse for an interval of 10 days during May of 2010, and then for five subsequent ten day periods in August and December of 2011 and April, August and December of 2012.

Timeline: Samples were collected in slaughterhouses between May 2010 and December 2012.

Progress and Findings: Between May 2010 and December 2012, blood and nasal swab specimens were collected from 978 pigs. In addition, 226 bronchiole swabs were collected. Of 609 pigs for which there was information on their source location, 503 (82%) were from Kiambu County in Central Kenya. The overall mean herd size of source farms was 119 pigs (SD=257) and the median pig herd size was 10 (range 1–500). Of 930/978 sera that were tested, 155 (16.7%) were positive for influenza A antibodies. Influenza A seroprevalence across the six sampling periods was 15.0%, 40.4%, 22.8%, 5.7%, 5.1%, 7.8% with the highest seroprevalence observed in pigs sampled in August 2011 and the lowest among pigs sampled in August 2012. HI assays were undertaken on 129/155 positive sera. Of these, 76 (59%) were reactive to the H1N1pdm09 antigens. Fifty-three (41%) were cross reactive to more than one of the test antigens, or to none of the antigens in the panel, and were termed as inconclusive. For the six sampling periods between May 2010 and April 2012 for which HI has been carried out, H1N1pdm09 was detected in each period and the overall H1N1pdm09 seroprevalence by sampling period ranged from 2.4% and 15.4%. Of 971 nasal swabs tested for influenza A, 5 (0.5%) were positive for influenza A. None of 226 bronchiole swabs tested were positive for influenza. Three virus isolates were obtained from nasal swabs collected in August of 2011 and all subtyped as influenza A H1N1pdm09.

Conclusion: Pigs play a role in the ecology of influenza virus in Kenya and continued surveillance in humans, pigs and other potential reservoirs of influenza viruses may be of value for identifying the risk to humans at the animal-human interface.

A Double-Blind, Randomized, Controlled Trial to Evaluate the Safety, Immunogenicity, and Efficacy of Trivalent Inactivated Influenza Vaccine, High-Dose Trivalent Inactivated Influenza Vaccine, and/or Intradermal Trivalent Inactivated Influenza Vaccine in HIV-Infected and HIV-Uninfected Pregnant Women in a Malaria-Endemic Area of Rural Western Kenya

Immunogenicity of flu vaccine in HIV-infected and HIV-uninfected pregnant women.

Study Aims and Objectives:

- To evaluate the immunogenicity of TIV, hdTIV and idTIV in HIV-infected and uninfected pregnant women.
- To evaluate the level of vaccine-induced influenza antibody transfer to infants of HIV-infected and uninfected pregnant women who receive TIV, hdTIV or idTIV.
- To evaluate the safety of TIV, hdTIV and idTIV in HIV-infected and HIV-uninfected pregnant women and fetus.

Approach: This trial will be conducted as a double-blind, randomized, controlled trial stratified by HIV status in up to 720 (960 if 4 arms) pregnant women in their second and third trimesters and their infants residing in health and demographic surveillance sites (HDSS) around Siaya District Hospital and Lwak Mission Hospital in Nyanza Province, Western Kenya.

Timeline: Trial enrollment will begin in the first quarter of 2014 and will continue for approximately one year.

Lao People's Democratic Republic (Lao PDR)

Feasibility Assessment in Measuring Impact of Seasonal Influenza Status on Birth Outcomes

Capturing pregnant women vaccinated in May–June 2013 and linking with birth outcome measures, with non-vaccinated new mothers' and infants as controls. Also, a 10-year historical review of birthing information in establishing trends and using for historical comparative purposes.

Approach: Collecting information of pregnant women at the time of vaccination and linking with birth outcomes. This work is being carried out at the Maternal and Child Hospital, and collection of birthing information (not practiced in Laos) will be expanded to the north and south of the country.

Timeline: One year.

Progress and Findings: Nine hundred (900) pregnant women have received seasonal influenza vaccine (May–June 2013). To date, 300 vaccinated and 300 (control) unvaccinated women have been interviewed post-delivery and outcome (birth weight measures) obtained. Finally, 10 years of MCH birthing information (~30,000 records) has been entered into a data management program.

LATIN AMERICA

Multi-centric Evaluation of Trivalent Seasonal Influenza Vaccine Effectiveness to Prevent Severe Acute Respiratory Infection among High Risk Groups Targeted for Vaccination (REVELAC-i)

Case test-negative control design.

Study Aims and Objectives:

- Explore the quality of sentinel surveillance data to support vaccine effectiveness estimates.
- Estimate influenza vaccine policies and utilization among target groups.
- Explore influenza and other respiratory vaccine utilization among target groups.
- Estimate trivalent seasonal influenza vaccine effectiveness to prevent severe acute respiratory infection among high risk groups targeted for vaccination.
- Model the impact of influenza vaccine among populations targeted for vaccination.

Approach: During April–December 2012 influenza season, we conducted a pilot test-negative case-control study in 18 influenza sentinel surveillance hospitals in Costa Rica, El Salvador and Panama to assess whether the quality of routinely collected surveillance data was sufficient to conduct a case-control study. The study population included children and elderly who were eligible for vaccination available free of charge. The outcome of interest was severe acute respiratory infections (SARI) associated with laboratory-confirmed influenza. All countries used the regional standard definition for SARI (CDC–PAHO 2006 protocol), consisting of fever and, cough or sore throat, and shortness of breath or difficulty breathing, in the absence of other diagnoses and requiring hospitalization. SARI patients had a respiratory sample (nose and throat swabs or aspirates) collected and tested for the presence of influenza viruses as part of influenza sentinel surveillance. An influenza case was a SARI patient with a positive RT-PCR result for any influenza virus. A control was a SARI patient with a negative RT-PCR result for influenza. For every case, we selected three controls from the same age group and epidemiological week (± 2 weeks).

Timeline: 2012–2014.

Progress and Findings: We identified 915 SARI patients that belonged to target vaccination groups and enrolled 260 influenza cases and 655 controls; 151 cases and 354 controls in Costa Rica, 78 cases and 234 controls in El Salvador and, 31 cases and 67 controls in Panama. Among these, 648 (71%) were children <12 years and 267 (29%) were elderly >60 years. Half (51%) were male patients and 915 (100%) had information on pre-existing medical conditions. The date of sample collection was available for 550 (60%) participants. Most (93%) had information about their 2012 influenza vaccine status. Only 280 (33%) had received an influenza vaccine. Only 86 (10%) had received the 2011 influenza vaccine. Similarly, 345 (38%) were vaccinated against pneumococcal disease. Data collection will continue during the 2014 austral influenza season.

Conclusion: Data collected routinely as part of SARI surveillance included most of the critical variables necessary for estimating vaccine effectiveness but strengthening the data collection and adopting electronic nominal vaccination records are likely to facilitate vaccine effectiveness estimates.

This project is occurring in Argentina, Brazil, Chile, Colombia, Costa Rica, El Salvador, Honduras, Panama, and Paraguay.

Evaluation of Pandemic Influenza Preparedness and Response in Central America

Self-assessment and analysis of surveillance data.

Study Aims and Objectives:

- Measure changes in pandemic preparedness in this region, and identify their related causes, using evaluations conducted between 2008 and 2012.

Approach: Eight Central American countries scored their pandemic preparedness across 12 capabilities in 2008, 2010 and 2012, using a standardized tool developed by CDC. Scores were calculated by country and capability and compared between evaluation years using the Student's t-test and Wilcoxon Rank Sum test, respectively. Virological data reported to WHO were used to assess changes in testing capacity between evaluation years. Linear regression was used to examine associations between scores, donor funding, technical assistance and WHO reporting.

Timeline: 2008–2014.

Progress and Findings: All countries improved their pandemic preparedness between 2008 and 2012 and seven made statistically significant gains ($p < 0.05$). Increases in median scores were observed for all 12 capabilities over the same period and were statistically significant for eight of these ($p < 0.05$): country planning, communications, routine influenza surveillance, national respiratory disease surveillance, outbreak response, resources for containment, community interventions and health sector response. We found a positive association between preparedness scores and cumulative funding between 2006 and 2011 ($R^2 = 0.5$, $p < 0.01$). The number of specimens reported to WHO from participating countries increased significantly from 5,551 (2008) to 18,172 (2012) ($p < 0.01$).

Conclusion: U.S. donor funding and technical assistance provided to the region is likely to have contributed to the improvements in pandemic preparedness we observed.

This project is occurring in Belize, Costa Rica, Dominican Republic, Honduras, Guatemala, El Salvador, and Panama.

Incidence of Influenza and Other Respiratory Viruses and Associated Economic Burden in Central America Cohorts and Population-based Surveillance Platforms

Community-based cohort.

Study Aims and Objectives:

- Estimate the incidence of influenza-like illness by etiology and age group.
- Explore risk factors associated with laboratory-confirmed influenza illness.
- Estimate the cost associated with health seeking for influenza-like illness.

Approach: Patients presenting at three tertiary hospitals (Santa Rosa, Quetzaltenango, and Guatemala City) with symptoms of respiratory disease were invited to undergo screening. Those meeting enrollment criteria and provided written informed consent were asked to provide nasopharyngeal and oropharyngeal swabs and urine samples. Trained staff administered a face-to-face interview to collected patient demographic,

clinical, and risk factor information using a personal digital device (PDA). A chest X-ray and blood culture were obtained. Laboratory confirmation was by real-time reverse transcriptase polymerase chain reaction. Unadjusted annual incidence rates of hospitalized influenza were calculated for Santa Rosa and Quetzaltenango using the denominator population from Guatemala's National Institute for Statistics (INE) data. We calculated age-specific hospitalized influenza incidence of patients hospitalized with influenza and estimated 95% confidence intervals (CI) using the Poisson distribution. Adjustment for health care utilization was based on surveys conducted prior to surveillance system implementation.

Timeline: 2008–2014.

Progress and Findings: During May 2008–July 2012, we identified 446 hospitalized influenza patients, 362 (81%) had influenza A and 84 (18%) had influenza B. The median age of case-patients was 2.4 years (interquartile range: 0.7–32.3). Median length of hospitalization was five days (range: 0–77). Eighty (17.9%) were admitted to the ICU, 28 (6.2%) died; overall, 88 (19.7%) experienced either ICU admission or death. Children aged <6 months comprised 19% of cases, 22% of those admitted to the ICU, and 7% of the deaths. Other deaths occurred in 11 (6%) children aged 7–60 months, 6 (6%) persons aged 5–50 years, and 9 (11%) patients aged >50 years. Women of child-bearing age comprised 6% of cases (2 admitted to ICU; 1 death). The annual incidence of hospitalized laboratory-confirmed influenza in Santa Rosa and Quetzaltenango was 19.7/100,000 overall and 85.4/100,000 for children aged <5 years. In general, Santa Rosa department had higher incidence of hospitalizations with influenza for those aged <25 years. Quetzaltenango department had higher rates for all older age groups except for those aged >65 years. Costa Rica data analyses are ongoing.

Conclusion: Influenza is an important cause of hospitalization in Guatemala, especially among children aged <5 years, who comprised 59% of all hospitalizations for influenza.

This project is occurring in Costa Rica and Guatemala.

Estimating the Incidence of Influenza-associated Hospitalizations and In-hospital Decedents

Leveraging influenza-like illness and severe acute respiratory infection sentinel site data to assess burden through multiplier and linear regression models.

Study Aims and Objectives:

- Assess burden of severe influenza illness among health seekers.

Approach: We quantified the number of persons nationally hospitalized with severe acute respiratory infection (SARI) (or their ICD-10 code proxies J9–18) and who died in the hospital. We calculated the proportion of nose and throat specimens that nationally tested positive for influenza through immunofluorescence or reverse transcription polymerase chain reaction. To estimate how many case-patients would have tested positive for influenza if all had been sampled, we multiplied the number of SARI cases by the proportion testing positive for influenza each month. In Argentina, we also used Serfling regression models to estimate the excess case-patients during influenza periods. We divided the influenza-associated number of severe acute respiratory infections by the national or sentinel site census to estimate influenza-associated rates. We corrected for health utilization (e.g. El Salvador, Honduras, and Panama) when appropriate.

Timeline: 2008–2014.

Progress and Findings: We identified 29,870 SARI case-patients during 2009–2012. Twenty percent (3,352) of 16,597 case-patients with respiratory specimens tested positive for influenza. We estimated that 6,634 (95% confidence interval [CI] 5,134–8,163) influenza hospitalizations and 787 (95% CI 468–1,125) deaths occurred during the study period. These represented 1.4 influenza-associated hospitalizations per 1,000 person-years (py) and four influenza-associated deaths per 100,000py. Deaths occurred primarily among persons aged >60 years where influenza-associated mortality was a mean of 29/100,000py vs. 1/100,000py among younger persons ($p=0.01$).

Conclusion: There was a significant influenza-associated hospitalization and mortality rates particularly among persons aged ≥ 60 years.

Related Published Papers:

Azziz-Baumgartner E, Cabrera AM, Chang L et al. Mortality, severe acute respiratory infection, and influenza like-illness associated with Influenza A(H1N1)pdm09 in Argentina, 2009. PLoS ONE 2012 Available at <http://dx.plos.org/10.1371/journal.pone.0047540>.

Clara A, Armero J, Rodriguez D, et al. Influenza-associated severe pneumonia rates in children younger than 5 years in El Salvador, 2008–2010. Bulletin of the World Health Organization 2012;90:756–763. doi: 10.2471/BLT.11.098202.

This project is occurring in Argentina, Belize, Colombia, Costa Rica, El Salvador, Honduras, Guatemala, Panama, Paraguay, and Peru.

Influenza-associated Mortality in the PAHO Region

Linear modeling of influenza-associated mortality rates by age group.

Study Aims and Objectives:

- Estimate the incidence of influenza-associated mortality rates.

Approach: We identified hospitalized persons and deaths in persons diagnosed with pneumonia and influenza (P&I, ICD-10 codes J10–J18) and respiratory and circulatory illness (R&C, codes I00–I99 and J00–J99). We defined the influenza season as the months when the proportion of samples that tested positive for influenza exceeded the annual median. We used hospitalizations and deaths during the influenza off-season to estimate, using linear regression, and the number of excess deaths that occurred during the influenza season. To explore whether excess mortality varied by sex and age group, we used Poisson regression of the influenza-associated rates.

Timeline: Used 2001–2013 surveillance data.

Progress and Findings: Influenza-associated mortality rates were similar to those reported in countries with published rates. For example, in Argentina, during 2002–2009, 2,411 P&I and 8,527 R&C mean excess deaths occurred annually from May–October. If all of these excess deaths were associated with influenza, the influenza-associated mortality rate was 6/100,000 person-years (95% CI 4–8/100,000 person-years for P&I and 21/100,000 person-years (95% CI 12–31/100,000 person-years) for R&C. During 2005–2008, we identified an average of 7,868 P&I excess hospitalizations and 22,994 R&C hospitalizations per year, resulting in an influenza-associated hospitalization rate of 2/10,000 person-years (95% CI 1–3/10,000 person-years) for P&I and 6/10,000 person-years (95% CI 3–8/10,000 person-years) for R&C.

Conclusion: Our findings suggest that annual rates of influenza-associated hospitalizations and were substantial particularly among older adults.

Related Published Paper: Marinho de Souza MF, Widdowson MA, Alencar AP et al. Trends in mortality from respiratory disease in Latin America since 1998 and the impact of the 2009 influenza pandemic. *Bull World Health Organ* 2013;91:525–532. DOI: <http://dx.doi.org/10.2471/BLT.12.116871>.

This project is occurring in Argentina, Brazil, and other PAHO member countries.

Costs of Severe Acute Respiratory Infections and Influenza-associated Hospitalizations in Central America

Investigators identify severe acute respiratory infection case-patients and use questionnaires and administrative data to estimate direct and costs associated with illness.

Study Aims and Objectives:

- Estimate the direct, indirect, and provider costs associated with severe acute respiratory infection and hospitalized influenza-illness.

Approach: We retrospectively estimated the costs of hospital treatment among a random sample of severe acute respiratory case-patients (operationalized as those diagnosed with ICD–10 codes J9–18 admitted during 2009–2011 and treated in teaching hospitals. The average costs were expressed in 2011 international dollars (I\$). Costs were determined through the review of administrative records and billing data. In a second phase of the project, investigators sought to obtain the costs associated specifically with laboratory-confirmed influenza case-patient hospitalization.

Timeline: 2009–2013.

Progress and Findings: We reviewed 671 medical records: 337 in Guatemala, 184 in Honduras, and 150 in Nicaragua. The average cost per SARI hospitalization was I\$1,435. On average, cost at general hospitalization among children was I\$725 in Guatemala (95% CI I\$548–903), I\$1,142 in Nicaragua (95% CI I\$953–1,332) and I\$1,143 in Honduras (95% CI I\$991–1,296). In adults, the average cost at general hospitalization was I\$2,942 in Guatemala (95% CI I\$2,500–3,383) vs. I\$1,969 in Honduras (95% CI I\$1,554–2,383). Data analyses in Costa Rica and El Salvador are ongoing.

Conclusion: Influenza hospitalization is an important financial burden of health care systems in Central America.

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

Panama and El Salvador Children’s Oseltamivir Study (PECOS)

Randomized controlled trial.

Study Aims and Objectives: The primary objective of this study is to evaluate the efficacy of oseltamivir phosphate treatment initiated at the time of hospital admission to reduce disease severity among children aged <10 years hospitalized with influenza-associated respiratory illness. Additional objectives are to: 1) evaluate the tolerability of oseltamivir phosphate treatment, 2) evaluate the effect of oseltamivir treatment on viral clearance and development of oseltamivir-resistant influenza virus during and after treatment in children hospitalized with influenza, 3) estimate the direct and indirect costs of all-cause respiratory illness

and influenza-associated respiratory illness requiring hospitalization, and 4) evaluate the effect of empiric oseltamivir treatment during the influenza season on these costs.

Approach: This study is a multi-site randomized, double-blinded, placebo-controlled clinical trial conducted at five tertiary care hospitals in Panama and El Salvador. Children hospitalized <7 days after symptom onset with cough or sore throat plus tachypnea were eligible. Participants were randomized 1:1 to receive empiric standard dose oseltamivir suspension or placebo twice daily for 10 doses, and then had respiratory specimens tested for influenza viruses. During hospitalizations, participants underwent standardized twice daily physical exams to assess key clinical outcomes which included duration of increased work of breathing and duration of hypoxia. Participants' guardians were interviewed about costs associated with the illness episode at the time of hospital discharge and again 7–9 days after hospital discharge.

Timeline: 2012–2014.

Progress and Findings: During September–October 2012 and April–August 2013, 551 participants were enrolled and randomized, of whom 327 (59%) were male and 292 (53%) were aged <1 year. Twenty-six (5%) of participants had laboratory-confirmed influenza.

This project is occurring in El Salvador and Panama.



← PECOS investigators from CDC, the Universidad del Valle de Guatemala (UVG), and Hospital del Niño (HDN) and study monitors at a meeting to discuss study progress in Panama City, Panama. From left to right, Jorge Jara (UVG), Salvador Rivera (UVG), Fatimah Dawood (CDC), Patricia Durango (UVG), Angie Donado (HDN), Ilenia Forero (HDN), Mariana Rodríguez (HDN), Dora Estripeaut (HDN), Onix de Suman (HDN),



← Dr. Cesar Ortiz draws up study medication for a newly enrolled PECOS participant at Hospital San Juan de Dios de Santa Ana, Santa Ana, El Salvador.



↑ Dr. Leonidas Benavides administers a questionnaire about costs of hospitalization to a parent of a PECOS participant at Hospital Materno Infantil José Domingo de Obaldía, David, Panama.

Timing of Influenza Activity, Predominant Strains, and Match to Available Vaccine Formulations in the Americas

Analyses of virology surveillance data in the Americas.

Study Aims and Objectives:

- Describe the timing of influenza activity in tropical countries before and after the pandemic.
- Assess which antigenic characteristics seemed predominant annually during the past decade.
- Determine which vaccine formulations were best matched to identified strains.
- Explore ecological associations between the timing of influenza activity and climate parameters.

Approach: We obtained the monthly number of samples which tested positive for influenza from the World Health Organization. We defined epidemics as months when the proportion of samples that tested positive for influenza exceeded the annual median. We also obtained antigenic characterization data from the CDC. We defined influenza strains as predominant during each season if they comprised the largest proportion of positive samples.

Timeline: Used data from 2002–2013.

Progress and Findings: South America reported 690,015, Central America 58,542, and North America 3,773,340 samples to the World Health Organization. Southern influenza seasons started on average in May and Central America seasons in June. South America submitted 2,625, Central America 1,225, and North America 21,333 samples for antigenic characterization. Preliminary findings suggest that southern strains were predominant in five (71%) of seven subsequent Central American and six (67%) of nine North American seasons. Central American strains most often matched the southern hemisphere vaccine formulation (57% of years).

Conclusion: Strains identified in South America typically became predominant in subsequent Central and North America seasons. Central America should consider vaccinating with the southern hemisphere formulation.

This project is occurring in Belize, Costa Rica, Honduras, Guatemala, El Salvador, and Panama.

Demographics and Clinical Characteristics of Influenza A(H1N1)pdm09 Deaths

Analysis of surveillance data.

Study Aims and Objectives:

- Explored the demographics and clinical characteristics persons who died with influenza A (H1N1) pdm09 infection during 2009–2010.

Approach: We identified influenza-associated deaths by hospital-based surveillance of severe acute respiratory infection (SARI) in Argentina and seven Central America countries (Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama and Dominican Republic). 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 (H1N1)pdm09 by real time polymerase chain reaction 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: 2009–2013.

Progress and Findings: Our case-series suggest that decedents in the Latin America were similar to those described by other case-series throughout the world. For example, during May 2009 to June 2010 Central America staff identified 186 cases with influenza A (H1N1)pdm09 decedents. The median age was 31 years and 48% were aged 15–44 years. One-hundred and three (55%) were female of which, 21 (20%) were pregnant, and seven (7%) were postpartum. Among 113 cases (61%) that had a pre-existing medical condition, 27 (24%) had obesity, 23 (20%) had diabetes, 21 (19%) had asthma, 15 (13%) had other chronic metabolic diseases, 11 (10%) had chronic obstructive pulmonary disease, 11 (10%) had seizure disorder, and seven (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.

Conclusion: The pandemic affected the young and those with pre-existing medical conditions. Most patients belatedly sought health care after oseltamivir could provide much benefit. Based on these results, it may be useful to review the indications in targets groups and availability of oseltamivir in the country.

Related Published Paper: Balanzat AM, Hertlein C, Apezteguia C et al (2012) An Analysis of 332 Fatalities Infected with Pandemic 2009 Influenza A (H1N1) in Argentina. PLoS ONE 7(4): e33670. DOI: 10.1371/journal.pone.0033670.

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

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 were analyzed at the NIC for influenza detection and characterization, but also for the detection of other respiratory viruses, using a multiplex real-time PCR implemented at the NIC.

Study Aims and 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 17 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: This study began in November 2010 ended in August 2013.

Progress and Findings: From November 2010 to August 2013, 930 SARI cases were recruited, including 32 cases of aggravation and 37 deaths. Most patients included were less than five years old (>75%). NIC tested all 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 25.4% (236/930) were negative for all respiratory viruses tested. RSV was the most common virus detected with 37.3% of positivity rate, followed by influenza A (18.6%), HRV (13.7%) and adenovirus (8.3%).

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).

Seasonal Pattern of Influenza in Antananarivo, Madagascar, from 2002 to 2012

In settings where seasonal fluctuations in climate factors are limited, it has been proposed that non-environmental factors can dictate the seasonality of influenza, perhaps via population fluxes with regions that experience seasonal outbreaks. Madagascar is a particularly interesting case study because it is an island comprising a range of climatic zones, which presumably experiences relatively limited external virus seeding relative to the African continent. In this study, we aim to disentangle the contribution of environmental forcing and population travel on influenza transmission in Madagascar. We assess the association between influenza activity and local climate in Antananarivo (Madagascar's capital) and compare influenza activity patterns in countries in close proximity or with high connectivity to Madagascar.

Study Aims and Objectives:

- Describe influenza activities in Antananarivo, Madagascar, from 2002 to 2012.
- Describe the travel patterns in Madagascar from 2002 to 2012.
- Study temporal comparison with influenza circulation from other countries.
- Assess climatology effects on influenza detection in Antananarivo, Madagascar, from 2002 to 2012.

Approach: Influenza virus activity was based on laboratory testing of respiratory specimens contributed by Influenza Surveillance Sentinel Network participating to ILI surveillance in Antananarivo, Madagascar. We compiled the weekly numbers of influenza A/H3, A/H1, A/H1pdm and B positive specimens for the period 2002 to 2012. We obtained similar data from WHO FluNet to study viral activity patterns in countries in the Sub-Saharan African region and those contributing most foreign travelers to Madagascar (France, Germany). Weekly climate indicators were compiled for the study period.

Spearman cross-correlation and hierarchical clustering were used to assess statistical association between time series.

Timeline: We analyzed influenza detection in Antananarivo from 2002 to 2012 and compared it to the patterns of travels, to other countries detection and to climatologic factors during the same period.

Progress and Findings: Viral detection in Antananarivo did not reveal a clear seasonal pattern. Overall, the pattern of circulation of seasonal strains in this locality led viral activity in the Northern Hemisphere winter (in particular, France), and presented poor temporal matching with viral activity in other countries from the African continent. Influenza detection was not associated with any climatologic signal in Antananarivo.

Conclusion: Our analyses indicate that the timing of circulation of influenza strains in Antananarivo is not driven by influenza circulation from countries which contribute the most to volume of travelers to Madagascar, nor does it fall in months with most intense incoming travelers to the island. Therefore, the intriguingly irregular circulation of influenza in Madagascar may obey unidentified internal factors that are highly stochastic, rather than “echo” patterns from more connected places.

Related Published Paper: Influenza Seasonality in Madagascar: The Mysterious African Free-runner. (Article in preparation).

Outcome Risk Factors during Respiratory Infections in a Pediatric Ward in Antananarivo, Madagascar 2010–2012

Acute respiratory infections are a leading cause of infectious disease-related morbidity, hospitalization, and mortality among children worldwide, particularly in developing countries. In these low-income countries, most patients with acute respiratory infection (ARI), mild or severe, are still treated empirically. The aim of the study was to evaluate the risk factors in relation with the evolution and the outcome of respiratory illnesses in patients under 5 years old.

Study Aims and Objectives:

- Evaluate the risk factors in relation with the evolution and the outcome of respiratory illnesses in patients aged under 5 years old.

Approach: In the context of SARI surveillance implemented in Madagascar, we collected demographic, socio-economic, clinical, epidemiological data and samples for laboratory analysis from patients less than five years suffering from respiratory infections and hospitalized in a pediatric ward in Antananarivo. Deaths, clinical aggravation, hospitalization time more than 10 days were considered as worse outcomes. The impact of the co-infections has been also studied using multivariate analysis.

Timeline: We conducted a prospective study in a pediatric ward in Antananarivo from November 2010 to July 2012.

Progress and Findings: From November 2010 to July 2012, a total of 290 patients were enrolled. Co-infection was found in 192 patients (70%). The co-infections were more frequent in children under 36 months, with statically difference for the groups [19–24 months] (OR: 8.0). Sixty nine percent (230/290) of patients fully recovered without complication during hospitalization; 60 children had a worsening during hospitalization. Nine patients (3%) died. Risk factors significantly associated with worse evolution during hospitalization were admission for age group less than 6 months (OR= 5.3), comorbidities (OR= 4.6) and low household incomes (OR= 4.1).

Conclusion: Numerous respiratory infection hospitalized cases were due to viral and bacterial pathogens also found in community setting. Some of these etiologies could be prevented by vaccine (e.g. influenza, pneumococcus, haemophilus) to reduce the burden of respiratory diseases on childhood morbidity and mortality in low income countries.

Related Published Paper: Rajatonirina S, Razanajatovo NH, Ratsima E et al. Outcome risk factors during respiratory infections in a paediatric ward in Antananarivo, Madagascar 2010–2012. PLoS One. 2013 Sep 12;8(9):e72839. doi: 10.1371/journal.pone.0072839. eCollection 2013.

MALAWI

Enhanced Surveillance for Influenza in an African Population with a High Burden of HIV, Malaria and Malnutrition

Hospital-based surveillance for respiratory illness at Queen Elizabeth's Central Hospital.

Study Aims and Objectives:

- Provide robust surveillance for Severe Acute Respiratory Infection (SARI) in the context of HIV, malaria and malnutrition amongst adults, pregnant women, and children presenting to Queen Elizabeth Central Hospital, Blantyre, Malawi. Surveillance will include viral and bacterial respiratory pathogens and assess implications of co-infection.
- Assess the severity (attendee hospitalisation rate, high-dependency care admission rate) and outcome (hospital attendee mortality) of laboratory proven influenza and examine the influence of HIV, malnutrition and malaria on influenza presentation and outcome.
- Document the frequency of secondary invasive bacterial infections in adults and children with influenza-associated SARI and examine the influence of HIV, malnutrition and malaria.
- Evaluate the utility of the TAC diagnostic platform on samples from SARI patients and non-SARI controls.

Approach: All patients presenting to pediatric and adult admission and emergency units are screened for fever and respiratory symptoms. Each day the first four subjects meeting the SARI case definition are enrolled in the pediatric unit and four are enrolled from the adult unit. During the pandemic period up to 10 children/adults meeting the case definition were sampled. Nasal aspirates are collected for influenza testing by rRT-PCR.

Timeline: Initiated in 2011.

Progress and Findings: Over 1,500 case-patients were enrolled in SARI surveillance in 2011. Influenza was in circulation from January to September 2011 with peak percent positive in March and April. Adults with SARI were more likely to be HIV-infected than adults with ILI ($p < 0.01$).

Conclusion: Influenza contributed substantially to the burden of acute respiratory illness among children and adults seeking hospital care in Malawi.

Related Published Papers: In preparation.



A photo of the Queen Elizabeth Central Hospital in Blantyre, Malawi. It is the largest hospital in Malawi. →

The Association between Malaria, Influenza and Lower Respiratory Tract Infection in Malawian Infants

This work will aim to assess the contribution of influenza virus infection to pneumonia and severe malaria in Malawian children <1 year of age presenting to a large Central Hospital in Malawi. Specifically we will investigate whether influenza infection is associated with an increased risk of having World Health Organization (WHO) defined radiological pneumonia (WHO-RP) and non-WHO defined radiological pneumonia in hospitalized Malawian infants with clinical LRTI.

Study Aims and Objectives:

- Determine the proportion of infants with WHO defined radiological pneumonia (WHO-RP) and non-WHO defined radiological pneumonia (Other-RP) who are co-infected with influenza.
- Determine the proportion of clinical pneumonia co-infected with influenza.
- Determine the proportion of severe hypoxaemic pneumonia co-infected with influenza.
- Determine the prevalence of antibodies to seasonal and pandemic influenza virus amongst infants presenting with pneumonia, with and without concurrent influenza infection.
- Determine the potential modifying effect of malaria on the association between influenza virus and pneumonia.

Approach: Infants meeting the WHO-RP and clinical pneumonia case definitions will have nasal aspirates collected for influenza virus testing by rRT-PCR.

Timeline: Under IRB review, anticipated start date October 2013.

Progress and Findings: Under IRB review.

The Prevalence of Serum Antibodies to Seasonal and Pandemic Influenza Virus in Pregnant Women and their Infants

Assess the prevalence of serum antibodies to influenza in pregnant women in the third trimester and transplacental transfer to their infants in a population with a high burden of HIV, malaria and malnutrition.

Study Aims and Objectives:

- Determine the prevalence of influenza antibodies amongst mothers and their new born infants
- Assess the influence of maternal HIV and placental malaria on influenza antibody transfer.

Approach: Women will be consented and enrolled in this study when they present at the labor ward for delivery. A peripheral blood sample will be obtained from the mother to document the presence of circulating influenza antibodies and this will be compared to antibody titers found in a cord blood sample from the infant. The potential impact of HIV and/or placental malaria on antibody titers will be assessed controlling for gestational age of the infant estimated by Ballard exam.

Timeline: Subject recruitment began in June 2013.

Progress and Findings: Nearly 200 women have been enrolled to date, another 100 will be enrolled.

The Influence of Influenza and HIV on Clinical Severity and Outcomes of Respiratory Illness in Adults

This work will include a case control study of adult inpatient SARI subjects and outpatient ILI subjects to assess the influence of HIV infection and influenza on clinical severity in hospital-attended cases of respiratory illness. It will also assess the role of influenza, other bacterial and respiratory pathogens, HIV and other clinical factors on outcomes of in-patient pneumonia cases.

Study Aims and Objectives:

- Assess the impact of HIV on clinical severity of influenza infections.
- Assess the role of influenza and other pathogens on outcomes of pneumonia hospitalizations.

Approach: Additional clinical data are abstracted from HIV-infected SARI case-patients. Patients are also followed-up at home four weeks post-discharge to assess outcomes.

Timeline: Initiated in 2013, enrollment ongoing.

Progress and Findings: Pending.

Nosocomial Transmission of Influenza and RSV in a High-dependency Unit for Infants <6 Months of Age

This work will evaluate new onset respiratory illness in infants <6 months of age hospitalized three or more days in the high-dependency nursery at Queen Elizabeth's Hospital, a large referral hospital in Blantyre.

Study Aims and Objectives:

- Determine the frequency of nosocomial influenza and RSV acquisition in the high-dependency nursery.
- Determine whether physical location in the unit, respiratory symptoms in mothers, or other factors affect nosocomial transmission of influenza and RSV.

Approach: Caregivers of infants admitted to the high-dependency nursery will be asked to participate in the study. Infants <6 months old with new onset respiratory illness hospitalized three or more days in the high-dependency nursery will be considered possible cases of nosocomial transmission. Nasal aspirates will be obtained from enrolled infants on admission, at three days, at seven days and then weekly until hospital discharge.

Timeline: Study began enrollment in September 2012.

Progress and Findings: There has been little influenza circulation in the community this year and therefore little nosocomial transmission of influenza. However, several cases of RSV have been detected.

Conclusion: Pending.

NICARAGUA

Nicaraguan Influenza Birth Cohort Study

Birth Cohort Study.

Study Aims and Objectives: Estimate influenza-associated illness rates among young children.

Approach: We plan to enroll infants into the study for three years, with 250 infants recruited each year and followed until two years of age, for a total sample size of 750 infants. Approximately 21 newborns, aged four weeks or less, are enrolled in the study each month to maintain the age structure. Families of infants are contacted on a weekly basis by study personnel, and data on daily symptoms are collected using diary cards. Children are provided with all primary medical care through the study, and 189 variables are collected at each medical visit. All participants presenting with fever or reported fever are tested for influenza by RT-PCR. Respiratory samples are collected at the participant's home in the event that the family does not bring the infant in to the health center for a consultation. Yearly blood samples are collected beginning at 6 months of age to enable detection of asymptomatic influenza infections and to evaluate nutritional status. Socio-economic, household risk factor, and breastfeeding data are collected at enrollment and on a yearly basis.

Timeline: 2011–2013.

Progress and Findings: As of August 15, 2013, 528 infants have been enrolled, with a median age at enrollment of 14 days. At enrollment, a majority of infants were breastfed (95.3%); however, only 48.9% were exclusively breastfed. The participants attended more than 6,222 medical visits at the study health center. Among the 528 infants who participated in the study, the incidence of influenza was 33.9 cases per 100 child-years (95% CI: 29.0, 39.6). A majority of influenza infections occurred in infants aged 6 months and older. A total of 142 participants were transferred to the hospital. Forty-seven percent of the transfers to the hospital were for respiratory illness; one infant was transferred for bronchial hyperactivity and 52 for pneumonia. Three infants died of pneumonia.

Conclusion: Infants in Nicaragua experience a high incidence of influenza with a majority of cases occurring in infants aged 6 months and older.

NEW ZEALAND

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

In September 2011, the Institute of Environmental Science and Research (ESR) in New Zealand was awarded a five-year research cooperative agreement with CDC, 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 Aims and Objectives: The SHIVERS study has nine objectives.

- Determine the incidence and prevalence of severe acute respiratory infections.
- Assess influenza vaccine effectiveness.

- Study the interaction between influenza virus and other pathogens.
- Ascertain the causes of respiratory mortality.
- Determine the incidence and prevalence of non-severe respiratory illness.
- Conduct an influenza seroprevalence study.
- Determine influenza risk factors.
- Study the immune response to influenza.
- Determine the health care and societal economic burden and vaccine cost-effectiveness of influenza.

Approach: A multi-centre and multi-disciplinary collaboration between ESR, Auckland District Health Boards (ADHB), Counties Manukau District Health Board (CMDHB), University of Otago, University of Auckland, the WHO Collaborating Centre at St. Jude Children’s Hospital in Memphis, USA, and the U.S. Centers for Disease Control and Prevention, to set up hospital and general practitioner-based surveillance in the Auckland area.

Timeline: Hospital-based surveillance for SARI was established in year one. Year two of the study focused on establishing influenza surveillance through primary health care providers and developing robust estimates of vaccine effectiveness.

Progress and Findings: Since 30 April 2012, four hospitals serving ADHB and CMDHB have enrolled and tested 2,550 SARI cases, of which 453 (18%) tested positive for influenza. Infants under one year experienced the highest rate of influenza hospitalization (412/100,000) followed by persons 80 years and older (223/100,000). Rates among Pacific People and Maori were 136 and 70/100,000 respectively compared to 38/100,000 among those of European descent.

Conclusion: The SHIVERS study is expected to answer many questions related to the epidemiology of influenza in a southern hemisphere setting at a time when influenza is not circulating in the northern hemisphere through enhanced real-time surveillance in hospitals and primary health care providers

PERU

Influenza Seasonality, Incidence and Economic Burden in Four Ecologically Diverse Regions of Peru

Population-based surveillance.

Study Aims and Objectives:

- Describe the variation in incidence rates, health seeking behavior, and seasonal pattern of influenza illness in four different regions of Peru.
- Estimate the direct and indirect costs of influenza episodes according to the health care utilization per episode from June 2009 to December 2010 in Peru.
- Explore between influenza illness, pre-existing conditions, and vaccine use.

Approach: Since 2009 NAMRU–6 has conducted active community-based household surveillance in four ecologically distinct regions of Peru: coastal desert (Lima), dry forest (Tumbes), highlands (Cuzco) and

rainforest (Puerto Maldonado). Approximately 7,200 people in 1,500 randomly selected households are visited three times per week. Nasopharyngeal swabs are collected from persons with influenza-like illness (ILI) and tested for influenza virus by RT-PCR.

Timeline: January 2009–September 2014.

Progress and Findings: After 15,583 person-years (PY) of follow-up (the overall influenza incidence was approximately 102/1,000 PY (95% CI: 97–107). Of these, 46/1,000 PY (95% CI 43–50) sought care, 0.8/1,000 PY (95% CI: 0.5–1.4) required hospitalization, and one died. Overall, the highest total cost was observed in the hospitalized category, US\$263 (median 171; IQR 145) and the lowest in the self-treated category, US\$19 (median 13; IQR 21). Overall, 92% of study participants took some medication for their ILI, of which 13% were prescribed by a physician.

Conclusion:

- Laboratory confirmed influenza illness burden in Peru is considerable particularly in children less than 11 years of age.
- Influenza poses a significant economic burden to the ill person and their households.
- Prescription drugs, including antibiotics, are frequently taken by persons with ILI.

Intra-household Backyard Influenza Cross-species Transmission Dynamics in Semi-rural Communities in Peru

Population-based surveillance.

Study Aims and Objectives:

- To assess the prevalence of antibody to pandemic H1N1 influenza virus (pH1N1) and to detect active infection in swine from community backyard farms in Tumbes, Peru, before, during, and after the pandemic.

Approach: We conducted surveillance for influenza among backyard swine in Tumbes, Peru, from 2009 to 2011. Sera, tracheal swabs, and lung samples were collected in March 2009 (pre-pandemic), October 2009 (human pandemic peak), April 2010 and October 2011 (post pandemic). The hemagglutination inhibition test to detect antibodies against pH1N1 virus was performed on sera. Tracheal swabs and lung tissue were cultured in SPF embryonated chicken eggs and positive specimens tested and sequenced by rRT-PCR.

Timeline: January 2009–September 2013.

Progress and Findings: We collected 1,303 sera samples, 923 tracheal swabs, and 962 lung samples. None of the 310 animals sampled during pre-pandemic period were positive by PCR or HI. Antibody prevalence to pH1N1 during the pandemic was 8% (27/321), with virus isolation from three tracheal swabs (1%) and one lung sample (< 1%). Samples from the two post-pandemic periods showed an antibody prevalence of 24% (79/328) and 1% (4/343), respectively. No virus was isolated from the post-pandemic period. Characterization of pH1N1 isolates in pigs confirmed a close phylogenetic relationship with human isolates circulating at the time in Tumbes.

Conclusion:

- Infection with pH1N1 in backyard swine occurred frequently during and after the pandemic period in Tumbes.

- Continuous surveillance at different production levels (i.e. pig farms and slaughterhouses) is necessary in order to monitor zoonotic and interspecies transmission of pH1N1 and swine influenza viruses in Peru.

Related Published Paper: Manuscript currently under development: Cross-species transmission of pandemic influenza (pH1N1) virus from humans to backyard pig farms in semirural communities in Tumbes, Peru.

Influenza Virus Surveillance in Swine Populations of Peru

Population-based surveillance.

Study Aims and Objectives:

- To determine the different strains of influenza (swine, avian and human) circulating swine raised in the Department of Lima and surrounding areas of the central coast of Peru.

Approach: Surveillance was conducted at a central slaughterhouse in Lima in which animals are brought from Lima and the surrounding region. Upon arrival at the facility, animals were inspected for health status and kept in groups according to the seller for a maximum of 14 hours. Blood and nasal and tracheal swab specimens were collected at the time of slaughter. Serum was tested for IgG antibody to influenza A virus by ELISA (IDEXX Laboratories, Maine, USA) and the swab samples by rRT-PCR using the CDC Flu A assay to detect universal influenza A virus. PCR positive samples were further analyzed with subtype-specific primers.

Timeline: January 2009–September 2013.

Progress and Findings: From December 2011 to May 2012, 963 adult pigs were sampled, with a prevalence of IgG antibody of 60% (573/958) and 4% (42/963) of animals positive by PCR. Subtype identifications from the 42 PCR positive animals were 25 (60%) pandemic H1N1, 8 (19%) H3, 6 (14%) seasonal H1, and 4 (10%) unsubtypeable, on which sequencing is presently underway. H1 pandemic/H3 co-infection was noted in 3 (7%) samples.

Conclusion: Pigs in the study area are frequently infected with influenza A viruses, primarily human subtypes, providing ample opportunity for coinfections and reassortment.

Related Published Paper: Manuscript currently under development: Influenza A Virus in Swine in Peru.

SENEGAL

Safety, Immunogenicity, and Effectiveness of Influenza Vaccines among Children in Senegal

These research activities encompass three separate but related clinical trials to evaluate the safety and immunogenicity of inactivated, adjuvanted, and live-attenuated influenza vaccines, as well as the effectiveness of live-attenuated and inactivated vaccines among children in Senegal.

Study Aims and Objectives:

- Evaluate the direct and indirect effectiveness of inactivated influenza vaccine (IIV) in reducing influenza among vaccinated children and their communities, as compared to inactivated polio vaccine (IPV).

- Evaluate age-specific post-vaccination immune responses to IIV among a subset of vaccinated children.
- Estimate the immunogenicity of MF59-adjuvanted IIV by age group, and compare these findings with immunogenicity of unadjuvanted IIV.
- Estimate the efficacy of live-attenuated influenza vaccine (LAIV) in reducing influenza among LAIV-vaccinated children, as compared to those receiving placebo.
- Describe the safety profiles of IIV, MF59-adjuvanted IIV, and LAIV in this population.

Approach: This research is conducted through a partnership with PATH, the Institut de Recherche pour le Développement (IRD), and Institute Pasteur Dakar. Study One is a Phase IV village-randomized trial to evaluate of the direct and indirect effectiveness of IIV. In this study, ~3,500 children aged 6 months to 10 years received either IIV or IPV control, with a small subset of vaccinated children providing additional immunogenicity and safety data following vaccination. Study Two is a Phase 2b trial to evaluate the immunogenicity of MF59-adjuvanted IIV. For Study Two, ~300 children 6 months through 5 years of age were randomized to receive either MF59-adjuvanted IIV, IIV, or placebo, and followed for up to four months for safety outcomes. Study Three, a Phase III trial to evaluate the efficacy of LAIV, randomized ~1,700 healthy children 2 years through 5 years of age to receive LAIV or intranasal placebo. Laboratory-confirmed influenza outcomes among vaccinated children (for Study One and Study Three) and consenting unvaccinated persons (for Study One only) are collected by a combined approach of active and passive influenza surveillance by routine visits to study households and village health posts.

Timeline: August 2008–July 2014.

Progress and Findings: Three vaccination rounds with IIV and IPV control took place each spring from 2009 to 2011, with full vaccination of between 7,600 and 9,500 eligible children annually. Surveillance activities for IIV direct and indirect effectiveness (Study One) continued until the end of 2012. During this time, over 20,000 febrile respiratory episodes were identified and tested for influenza, with influenza A(H1N1)pdm first detected in February 2010. Vaccination activities for Study Two and Study Three were carried out in spring 2013, with follow-up and laboratory testing ongoing.

Conclusion: Influenza viruses are a major cause of febrile respiratory illness in this population. Preliminary year one findings of IIV effectiveness indicate that although vaccine was not matched to the drifted influenza A(H3N2) virus that circulated widely in the population, significant reduction in laboratory-confirmed influenza was still measured among vaccinated children. Moreover, significant benefit was measured at the level of the entire community, including unvaccinated children too young to receive vaccine and unvaccinated adults.

SOUTH AFRICA

Prospective Cohort Study of Influenza Viral Shedding in HIV Infected and Uninfected Adults

This study aims to assist with the description of influenza viral shedding in a high HIV prevalence setting. The results will assist with policy recommendations on the use of antiviral drugs in HIV infected and other persons at risk for severe illness or increased transmission due to prolonged shedding.

Study Aims and Objectives:

- To determine the time period (range and median) in days that HIV-infected and -uninfected adults continue to shed influenza virus after influenza symptom onset using quantitative reverse transcriptase (RT)-PCR and viral culture.
- To compare the intensity of influenza virus shedding (viral load) amongst HIV-infected and – uninfected adults.
- To correlate the duration of influenza virus shedding detected by RT-PCR and viral culture with influenza signs and symptoms.
- To determine amongst HIV-infected adults whether individuals with more severe immunosuppression (based on CD4 count) shed influenza virus for longer and with greater intensity than individuals with higher CD4 counts.
- To assess factors that may affect influenza virus shedding dynamics.

Approach: Patients who are enrolled into the influenza-like illness surveillance programme at public health clinics are tested for influenza by rapid test and enrolled into the shedding study if they test positive for influenza. Respiratory samples are taken for qualitative and quantitative viral testing. Patients are then followed up at regular interval until 28 days post presentation. Respiratory samples are collected at each visit. HIV status is also determined at enrollment to ensure enrolment of HIV-infected and HIV-uninfected patients.

Timeline: The influenza shedding study started in the 2012 influenza season and is ongoing through the 2013 season.

Progress and Findings: Thirty seven patients have been enrolled. Eleven patients completed follow up in the 2012 influenza season. The median age of patients was 13 years (range 3–38 years) and 73% (8/11) were male. One patient was later hospitalized with severe acute respiratory illness. Eight patients had A(H3N2) and three had influenza B. One child had prolonged viral shedding of A(H3N2) of 14 days duration.

Conclusion: With the small number of patients who have complete data, it is difficult to draw conclusions.

Household Transmission Study of Influenza Virus from HIV-infected and— uninfected Index Cases, South Africa

Understanding the transmission dynamics of influenza virus in settings with high HIV-prevalence is important to be able to properly advise and implement appropriate public health measures for influenza control. In this study, we aim to characterize the transmission dynamics of influenza virus from HIV-infected and— uninfected index cases to household contacts.

Study Aims and Objectives:

- To determine the secondary infection risk and associated risk factors in household contacts of HIV-infected and -uninfected index cases in South Africa.

Approach: Participants will be recruited from the ongoing influenza-like illness (ILI) surveillance program. Potential participants with ILI will be screened at primary health care facilities in Pietermaritzburg and Klerksdorp. Patients meeting the enrollment criteria of a positive rapid influenza test and agreement to HIV testing will be enrolled as index cases following informed consent. A home visit will then be scheduled

to enroll household contacts of those with a positive rapid influenza test for follow-up. Household contacts will be offered HIV testing at the initial visit. Nasopharyngeal swabs and questionnaires will be administered at follow-up visits to household contacts.

Timeline: Patients will be enrolled through the 2013 and 2014 influenza seasons.

Progress and Findings: Thirty-two index patients and 66 household members have been enrolled.

Conclusion: This study will add to the burden and risk factor influenza data that will inform vaccine programmes and resource allocation at policy level.

Estimating the Economic Burden of Respiratory Illness Associated with Influenza and Respiratory Syncytial Virus in South Africa

This study aims to describe the patient and hospital/clinic costs associated with influenza-like illness and severe influenza-related severe acute respiratory illness (SARI) in order to inform policy on influenza prevention (vaccine), influenza treatment (antiviral) and resource allocation. The study enrolls patients enrolled into the SARI surveillance programme and collects data on the cost of hospitalization including direct costs (e.g., medication, procedures) and indirect cost (e.g., staff, administration) related to admission. The patients cost in terms of transport, payment for medical care and loss of income are collected by interview.

Study Aims and Objectives:

- To estimate the annual cost of hospitalized severe acute respiratory illness and outpatient influenza-like illness (ILI) of any aetiology in South Africa.
- To estimate the annual cost of hospitalized severe acute respiratory illness and outpatient influenza-like illness (ILI) associated with influenza in South Africa.
- To estimate the annual cost of hospitalized severe acute respiratory illness and outpatient influenza-like illness (ILI) associated with respiratory syncytial virus in South Africa.

Approach: Patients are enrolled in age groups each week at all SARI and ILI surveillance sites. Data on hospital/clinical costs will be sourced from each hospital/clinic and from published health care costs which are available at district level. Hospital day bed costs and length of hospital stay will provide the base for the costing model. Additional costs of procedures, laboratory costs and medication will be added to the costing model for each patient. Initially a model will be built for all cause SARI and then costs for pathogen specific admissions will be constructed.

Timeline: Enrollment started in March 2013 and will continue through March 2014.

Progress and Findings: The target sample size of 144 patients is nearly completed, however there are challenges in recruiting certain age groups (young adults and the elderly) so enrollment will continue through 2013. Site visits to collect hospital cost will start in September 2013, with the aim to start data analysis early in 2014.

Conclusion: Costing of influenza will inform policy on resource allocation and is an important component of a national influenza policy.

Development of a Severe Respiratory Disease Clinical Score— South Africa

Respiratory diseases are a common cause of morbidity and mortality worldwide. In South Africa, respiratory diseases are the second most common cause of death. Health care providers often use pneumonia severity prediction tools to quickly identify patients at highest risk of severe outcomes, such as death or Intensive Care Unit (ICU) stay, early in their presentation. Such tools help to identify which patients may require additional interventions and can help distribute scarce health care resources. Although many pneumonia severity prediction tools exist, most have been developed and tested in North America and Europe in populations that may differ from those in South Africa. The ability of existing tools to accurately predict severity in patients with severe respiratory disease in South Africa is unknown.

Study Aims and Objectives:

- To validate existing pneumonia severity prediction tools in inpatient adult populations (≥ 18 years) at three sites in South Africa.
- To develop a new respiratory severity prediction tool for adults including HIV-specific variables, such as CD4 count, and compare its performance to existing severity scores.

Approach: We will use data collected as part of ongoing surveillance for severe acute respiratory illness (SARI). All adult patients enrolled in SARI will be included in the analysis population. Patients from June 2010–June 2012 will be included. Some variables important in forming a respiratory illness severity prediction tool which are not included in the SARI database will be abstracted from patient charts.

Timeline: Complete by July 2014.

Progress and Findings: Data extraction is completed. Data analysis is to begin soon.

Conclusion: The goal of this study is to help health care providers accurately triage patients at greatest risk of severe outcomes of respiratory illness in settings which may have limited resources and a high prevalence of HIV or tuberculosis.

Surveillance for Outpatient Influenza-Like Illness and Asymptomatic Respiratory Virus Colonization in South Africa

We make use of the structure provided by the public health care influenza-like illness surveillance platform to enroll patients asymptomatic for respiratory illness.

Study Aims and Objectives:

- To describe the burden and aetiology of outpatient ILI in children and adults in selected sites in South Africa, in HIV-infected and HIV-uninfected populations.
- To determine the prevalence of selected respiratory viruses in a subset of asymptomatic children and adults, i.e., colonization, in selected sites in South Africa.
- To determine the relative contribution of selected respiratory pathogens to severe respiratory disease in a setting with a high prevalence of HIV by comparing to individuals with ILI or who are asymptomatic.
- To determine risk factors for development of severe disease due to influenza and other respiratory viruses by comparing asymptomatic illness to mild and severe disease.

- To identify the strains of influenza and the genotypes of rhinovirus, enterovirus, adenovirus, and respiratory syncytial virus responsible for asymptomatic and mild respiratory infections among humans.
- To characterize and determine cytokine profiles associated with asymptomatic and mild respiratory syncytial virus, rhinovirus, and other respiratory viruses, among individuals with and without HIV co-infection.

Approach: Patient attending primary health clinic for non-respiratory ailments with no respiratory symptoms in the preceding 14 days are systematically enrolled by age group and HIV status. Meaning that patients enrolled represent all age groups and are equally enrolled by HIV status. Respiratory samples in the form of nasal pharyngeal aspirates or throat swabs are collected and tested at NICD for 13 respiratory viruses and for *Streptococcus pneumoniae*, *Bordetella pertussis*, *Haemophilus influenzae* type B, atypical bacterial causes of pneumonia (*Legionella* species, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*).

Timeline: Started enrolling in March 2013 and enrollment is ongoing.

Progress and Findings: Up to June 2013, 544 healthy controls have been enrolled. As compared to healthy controls patient with influenza were eight times for likely to have ILI (Adjusted relative risk ratio (aRRR) 8.2 (95% confidence interval CI 4.3–15.7) and three times more likely to have SARI (aRRR 3.4 95%CI 1.8–6.7). Similarly patient with RSV (ILI:aRRR 1.8(95% CI 1.1–2.8 and SARI aRRR2.5 (95% CI 1.6–3.8)) and hMPV (ILI: aRRR10.9 (95% CI 2.6–45.2) and SARI 6.2(95%CI 3.0–45) were associated with ILI and SARI. Although rhinovirus (ILI: aRRR 1.6 (95%CI 1.2–2.1) and SARI (aRRR1.4 95% CI 1.1–1.9) was associated with ILI and SARI the measure of effect was small and so the clinical significance is marginal. Comparing clinical factors associated with SARI as compared to ILI, HIV infection (odds ratio (OR) 2.2 (95% CI 1.1–4.5) and extremes of age (age<5 years OR 3.9; 95% CI:1.7–9.0) and age 65 years and older, OR 9.7 (95% CI 2.3–41.7) were associated with severe infection.

Conclusion: This study will allow us to describe the respiratory viruses associated with ILI and SARI in a high HIV prevalence setting.

Human Infections with Avian Influenza H7N1 and H5N2 Strains during Outbreaks in Ostriches

Several avian influenza outbreaks have been reported in ostriches over the last 10 years in South Africa, the most recent in the Western Cape being highly pathogenic Influenza A H5N2 in 2004 and 2011, low pathogenic (LPAI) H7N1 in 2012 and H7N7 in 2013. No evidence exist of H5N2 and H7N1 strains causing severe disease in humans although a serosurvey in 2004 documented serological evidence of H5N2 infection in 3 of 129 highly -exposed persons. Recent emergence of LPAI H7N9 causing severe disease in humans in China and the continued threat of highly pathogenic (HPAI) H5N1 in Asia and other areas raise the question as to the risk of infection and possible symptoms due to other LPAI H7 or HPAI H5 strains in humans.

Study Aims and Objectives:

- To conduct a serosurvey of at-risk persons during the 2011 and 2012 outbreaks and track positive cases retrospectively to identify symptoms.

Approach: In August 2011 and August 2012, sera were collected from 207 and 66 people respectively with a history of direct contact with ostriches infected with AI H5N2 (2011) or H7N1 (2012) either through handling or slaughtering of infected birds. Sera were also collected from 38 state veterinarians from across the country at an annual congress in 2012 a proportion that had been involved in the AI outbreaks.

Questionnaires including demographic, occupational characteristics and clinical illness history data were administered during the 2011 outbreak while an abbreviated questionnaire and retrospective follow up of seropositive people occurred in 2012. Hemagglutination inhibition assays (HAI) reference antigens as well as outbreak-specific inactivated were run at the National Influenza Centre, NICD against H5 and H7 AI and human influenza specific control antisera and serum samples from study participants.

Timeline: The serosurvey was conducted at two time points. Neutralization confirmation is expected by November 2013.

Progress and Findings: Of 207 veterinarians, ostrich farmers, farm workers and abattoir workers exposed to avian influenza H5N2, and 66 exposed to H7N1 in the 2011 and 2012 outbreaks three people with HAI antibody titers greater than 1:40 to influenza H5N2 and one person with H7N1 antibodies were identified. For H5N2 this included a veterinarian who was actively involved in post-mortem investigations of ostriches, a farm worker and an abattoir worker. The H7N1 positive person was an abattoir worker. Reported symptoms included conjunctivitis and influenza-like illness for two of the H5N2-seropositive cases, whilst the third H5N2 seropositive case and the single H7N1 seropositive case reported no symptoms.

Conclusion: A low risk exists for human infection with Influenza A H5N2 (1.4%) and H7N1 (1.6%) during outbreaks amongst ostriches suggesting a need for increased biosecurity and surveillance of humans in contact with ostriches during avian influenza outbreaks, even if the strains are LPAI or birds are asymptomatic.

Pneumocystis jirovecii (PCP), tuberculosis (TB), *Streptococcus pneumoniae*, *Bordetella pertussis*, *Haemophilus influenzae* type B and Atypical Bacterial Causes of Pneumonia (*Legionella* species, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*) in Hospitalized Patients with Severe Acute Respiratory Infections (SARI)

This study makes use of the SARI programme structure to take additional samples from patients admitted with a longer duration of symptoms (14 days) and suspected TB. In addition to the upper respiratory tract samples taken as part of SARI, an induced sputum sample is collected.

Study Aims and Objectives:

- To estimate the incidence of and proportion of patients with PCP, TB, *Streptococcus pneumoniae*, *Bordetella pertussis*, *Haemophilus influenzae* type B and atypical bacterial causes of pneumonia (*Legionella* species, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* in HIV-infected and HIV-uninfected patients admitted with severe acute respiratory illness (SARI).
- To estimate the extent of respiratory virus co-infection with these cause of pneumonia and describe how these viral co-infection relate to patient morbidity and outcome.

Approach: Samples collected as part of the SARI surveillance will be tested for the additional pathogens. A modified case definition to include a longer duration of symptoms and a physician diagnosis of suspected TB will be used to enroll patients at two sites. Additional specimens will be collected to enhance detection of PCP and TB; these include induced sputum and oropharyngeal mouth rinse. TB samples will be tested by Gene Xpert and followed up by culture. PCP samples are to be tested by PCR.

Timeline: Recruitment started in 2012 and continues through 2013.

Progress and Findings: From June 2013 to date the following tests have been conducted. A total of 1,469 oral washes have been tested for PCP and 5% (74) were positive, 810 induced sputa samples were tested and 11% (88) were positive for PCP. Of the 2,085 nasopharyngeal samples that were tested 7% (146) were positive for PCP DNA. To date 1,231/1,984 (61%) of enrolled patients have been tested for TB and 22% (271) were positive for TB. The detection rate for atypical bacteria was higher on induced sputa (N=728): with a 1% (9) detection rate for *Mycoplasma pneumoniae*, 0.3% (2) for *Chlamydia pneumoniae* and 3% (19) for Legionella species.

Conclusion: PCP and TB infection is commonly associated with SARI in our setting. Validation of testing methods for PCP is not complete but it appears induced sputa has a higher detection rate for PCP. Although the detection rate for atypical bacteria is low these pathogens are more commonly detected in the 15–45 age group, the age group with the highest HIV prevalence.

Health care Utilization Surveys— Soweto, Gauteng Province, and Klerksdorp, Northwest Province

Understanding patterns of health care utilization in South Africa for common illnesses and conditions such as respiratory disease including influenza and febrile illness such as enteric fever is important to be able to interpret sentinel surveillance data and estimate the true disease burden at the community level. In this study, we aim to characterize the health care utilization behaviors related to influenza-like illness, pneumonia, diarrhea, meningitis, febrile illness and death in a peri-urban site by conducting a cross-sectional survey.

Study Aims and Objectives:

- Estimate the catchment population, the proportion of people who seek medical care at sentinel surveillance sites for ILI, pneumonia and febrile illness, and characterize the health care utilization behaviors related to these illnesses; the catchment population will be used as denominator for incidence calculations and to adjust for residents not seeking care.
- Characterize health care utilization behaviors related to other conditions such as diarrhea, meningitis, and chronic respiratory illness.

Approach: We conducted a cross-sectional survey of representative samples of households throughout the catchment area of the Klerksdorp-Tshepong hospital complex and Chris Hani Baragwanath Academic Hospital and their surrounding regions. Sampled households were visited and administered a questionnaire on demographics and socio-economic factors, medical conditions, ILI in the last 30 days, pneumonia in the past year, diarrhea in the last 14 days, meningitis in the past year, and deaths in the last year. In addition, we collected GPS (global positioning system) coordinates of the interviewed household. For each disease syndrome, we collected information on the type of health care services sought and the services obtained.

Timeline: The survey is complete; analysis is ongoing.

Progress and Findings: Eighty six percent of target households were enrolled (1,426/1,653) in Klerksdorp and 71% (970/2,396) in Soweto. More than 50% of households had a monthly income of less than USD \$200. Most people were able to identify the symptoms of influenza-like illness, however the majority thought that influenza is caused by cold weather. A little more than half the respondents knew that there is a vaccine for influenza (821/1,426, 58% in Klerksdorp and 598/970, 62% in Soweto). The majority of respondents (1,202/1,426, 84% in Klerksdorp and 728/970, 75% in Soweto) said they would access a vaccine at a public health care clinic.

Conclusion: Early results suggest that people in the population access health care at primary health care clinics and public hospitals. This will allow us to estimate the population served by the hospital and facilitate models to estimate incidence of influenza and other respiratory illness in the community.

THAILAND

Randomized Controlled Trial of 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 Aims and Objectives:

Primary Objective

- To assess humoral antibody responses to intramuscular versus intradermal TIV in HIV-infected MSM prior to vaccination and at one month, six months, and 12 months post vaccination. We will further characterize humoral antibody responses by low versus high CD4 cell count.

Secondary Objectives

- 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 one week, one month and six months post-vaccination. We will further characterize cell-mediated immune responses by low versus high CD4 cell count.

Approach: Randomized controlled trial.

Timeline: November 2011–September 2013.

Progress and Findings: We enrolled and vaccinated 480 participants (400 were HIV positive and 80 were HIV negative). Of the 480, 476 (99%) returned for the one month visit and 461 (96%) for the six month visit. The 12 month visits will be complete in September 2013. Investigators remain blinded and are drafting the analytic plan.

Conclusion: None yet.

Pediatric Respiratory Infections Cohort Evaluation (PRICE)

This is a longitudinal study to follow healthy and high-risk children aged 0–36 months for two years. At medically-attended respiratory events, swabs were tested for influenza and RSV by PCR; direct and indirect costs and outcomes were recorded.

Study Aims and 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 nutritional and inflammatory markers particular Vitamin D with the incidence, duration and severity of influenza 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 and RSV infections.

Approach: Prospective, observational cohort study.

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

Progress and Findings: We enrolled 466 high-risk and 619 healthy children. We will enroll until we get 500 high-risk children. Preliminary analysis is underway.

Conclusion:

- Compared to high-risk children, healthy children had higher incidences of influenza and RSV-associated ARI and incurred similar costs for outpatient influenza and RSV and hospitalized influenza.
- Influenza-associated ARI prevention should target both healthy and high-risk young children.

Influenza Vaccine Knowledge, Attitudes, and Practices in Pregnant Women and Their Providers in Thailand: A Vaccination Program Evaluation

To evaluate Thailand's current influenza vaccination program and develop additional strategies to address barriers to influenza vaccination, we conducted a knowledge, attitudes, and practices (KAP) survey of pregnant women and their health care providers to identify barriers to influenza vaccination and characterize perceptions of influenza and influenza vaccination during pregnancy.

Study Aims and Objectives:

Primary Objective

- To ascertain knowledge, attitudes, and practices related to influenza vaccination of pregnant women, including barriers to vaccination, among both pregnant women and their health care providers in Thailand.



A research nurse at Queen Sirikit National Institute of Child Health interviews a mother and child as part of prospective study on influenza in young children in Bangkok, Thailand.

Secondary Objective

- To identify predictors of willingness to receive influenza vaccine during pregnancy among pregnant women.

Approach: Survey.

Timeline: December 2012–June 2013.

Progress and Findings:

- Pregnant women: 967 (90%) of 1,072 pregnant women surveyed completed the questionnaire.
- Physicians: 643 (57%) of 1,134 completed the questionnaire.
- Analysis is ongoing.

Conclusion:

- Over half of Thai pregnant women were willing to get an influenza vaccine; however, influenza vaccine uptake was low.
- Subsidizing vaccine and encouraging health care providers of pregnant women to support influenza vaccination recommendations may be effective strategies to improve influenza vaccine uptake in Thai pregnant women.
- To improve influenza vaccination coverage among pregnant women, strategies are needed to increase vaccine availability and free vaccine services in ANCs in Thailand.
- Efforts to increase health care provider awareness of Ministry of Public Health recommendations for influenza vaccination are warranted and additional targeted outreach to physicians providing care to pregnant women should be considered.

Influenza Associated Mortality in Thailand, 2006–2011

We analyzed weekly mortality and viral data from 2006–2011 to estimate deaths attributable to influenza in Thailand.

Study Aims and Objectives:

- Estimate influenza-associated deaths in Thailand.

Approach: Negative binomial regression model.

Timeline: November 2012–June 2013.

Progress and Findings: Analysis ongoing.

Conclusion: Influenza-associated mortality in Thailand is much greater than previously appreciated.

UNITED STATES OF AMERICA (ATLANTA, GA)

Severe Acute Respiratory Infection (SARI) among Children less than Five Years of Age: Use of TAC Multiple Pathogen Detection Platform in International Influenza Program Sites (TAC-KID)

The TAC-KID study is a prospective case-control study aimed at understanding the etiology of severe respiratory disease among hospitalized children less than five years of age in four countries. The study will enroll case children less than five years of age who are hospitalized meeting the WHO case definition for severe acute respiratory infections and control children who visiting outpatient clinics but not experiencing an infectious illness over a 12-month period. Epidemiologic data including demographics, risk factors, clinical course of illness, treatment, and vaccination history will be obtained. Both nasopharyngeal and oropharyngeal swabs will be collected from case and control children and will be tested using the TaqMan array technology. The Taqman® Array Card (TAC, Life Technologies, Carlsbad, CA), is a multiple pathogen detection tool that uses a solid-phase quantitative polymerase chain reaction assay technology. This platform allows for the rapid simultaneous detection of multiple pathogens (approximately 34) from up to six individual clinical specimens in a single TaqMan array card and requires a relatively low volume for testing.

Study Aims and Objectives:

- To estimate the prevalence of selected viral and bacterial respiratory pathogens among children less than five years of age hospitalized with SARI (first hospitalization for SARI episode) in four countries during a 12 month period.
- To estimate the prevalence of selected viral and bacterial pathogens among children without respiratory illness in four countries over a 12 month period.
- To determine the proportion of SARI cases caused by select pathogens by comparing the prevalence of select viral and bacterial respiratory pathogens among children with SARI and children without SARI.
- To describe the seasonality and compare the etiology of SARI among children less than five years of age at four international sites (Peru, Malawi, South Africa, and India).
- To identify risk factors for severe acute respiratory infection by etiology and compare by site among children less than five years of age. To describe clinical episodes attributable to specific etiologies and understand asymptomatic carriage.

Approach: To establish severe acute respiratory infection surveillance at sites to enroll children into the TAC-KID study and to obtain non-infectious controls from clinics at the same facilities.

Timeline: Study will be conducted for 12 months at each of the four sites, August 2013–December 2014.

Progress and Findings: Study was initiated in India in August 2013 and is expected to begin in all other sites by November 2013.

This project is occurring in India, Malawi, Peru and South Africa.



A surveillance team member at Edendale Hospital in Pietermaritzburg, South Africa interacts with a patient's mom to complete the severe acute respiratory infection and TAC-KID study questionnaire.



The TAC-KID project officer at Kalawati Hospital in New Delhi, India is asking the mother of a child admitted to the hospital a screening questionnaire to determine enrollment eligibility while other mothers in this busy hospital ward look on.

Global Seasonal Influenza Mortality Estimation

The World Health Organization (WHO) estimates that 250,000–500,000 seasonal influenza deaths occur globally each year. However, this estimate does not account for differences between countries in influenza virus circulation, population structure, underlying health status of populations, and access to life-saving interventions or care. Our goal is to develop a model to estimate global mortality due to seasonal influenza that attempts to account for differences in population age structure, risk of influenza-associated death, and variation in mortality between influenza seasons.

Study Aims and Objectives:

- To estimate global mortality due to seasonal influenza using excess mortality data by country and region.
- To obtain estimates of respiratory and circulatory excess mortality by year and age group from a set of diverse countries to calculate base respiratory and circulatory excess mortality rate estimates.
- To apply these estimates to populations of countries where such estimates cannot be obtained.
- To develop a method to account for variation between countries in risk of influenza-associated death due to respiratory illness and use this method to adjust the base respiratory mortality rate as needed to calculate influenza-associated respiratory deaths for each country and/or region.
- To develop a method to account for variation between countries in risk of influenza-associated deaths due to circulatory etiologies to apply to the base circulatory mortality rate to calculate influenza-associated circulatory deaths for each country and/or region.

Approach: We will use vital records and viral surveillance data from a diverse group of countries from different regions of the world to create a base influenza mortality rate estimate and develop methods to extrapolate the base estimate to other countries around the world that lack such data. After building our base estimate(s), we will develop an extrapolation approach to account for variation in risk of influenza-associated deaths across countries to develop a robust, globally representative estimate of annual seasonal influenza mortality.

Timeline: Study is ongoing.

Progress and Findings: We have established collaborations with approximately 25 countries around the world to participate in this work. We have both analyzed individual country data and received mortality estimates from countries to incorporate into our model to estimate global seasonal influenza mortality. We have established a collaboration with the World Health Organization Global Burden of Disease group to develop adjustment factors for respiratory and circulatory mortality.

Global Respiratory Influenza Proportional Positive (GRIPP)

Many countries worldwide now conduct hospital-based surveillance for influenza. Yet these data are rarely used to determine absolute burden. This study aims to collect the influenza percent positive among children and adults hospitalized for respiratory disease to get to a global disease burden estimate using published estimates of lower respiratory tract disease.

Study Aims and Objectives:

- To estimate global burden of pediatric hospitalizations due to seasonal influenza using percent positive by country and region for both pandemic and seasonal influenza.
- To estimate global burden of adult hospitalizations due to seasonal influenza using percent positive by country and region for both pandemic and seasonal influenza.
- To understand the percent of all respiratory hospitalizations positive for influenza before and after the pandemic of 2009.

Approach: We extract data from published papers and use and viral hospitalized surveillance data from a diverse group of countries from different regions of the world. We then conduct a meta-analysis to come up with a best point estimate of the percent positive by age and region and if pandemic year or not. We then apply this percent positive to the envelope of adult and [pediatric hospitalization globally.

Timeline: Study is ongoing.

Progress and Findings: We have completed the study for children and are now completing the final data extraction and analysis for adults.

VIETNAM

SARI and ILI in the North of Vietnam: Burden, Economic Impact, and Health Care Utilization

The study consists of two components: a hospital-based study of severe acute respiratory infection (SARI) patients to estimate the burden of SARI at three hospitals in Thai Binh province (North Vietnam), and a community-based household survey on health care utilization among people who experienced influenza-like illness (ILI) and SARI.

Study Aims and Objectives:

- To determine the burden of influenza-related SARI in hospitalized patients in one district hospital and in two provincial hospitals in Thai Binh province.

- To describe clinical, virological and epidemiological characteristics of influenza-related hospitalized SARI cases.
- To describe health care utilization of SARI hospitalized patients admitted to hospitals.
- To describe the economic impacts to patients of influenza-related SARI.
- To determine the incidence of self-reported ILI/SARI and the health seeking behaviors of people in the community when they have self-reported ILI/SARI.

Approach: A descriptive study has been conducted to describe epidemiological, virological and clinical features at the district and provincial hospital levels in Thai Binh province, North Vietnam. Adults and children with SARI admitted to Kien Xuong District Hospital, to Thai Binh Pediatric Hospital and to Thai Binh Provincial Hospital have been enrolled into the study. Epidemiological, virological, clinical, economic, and health-seeking information from these patients are collected to estimate the disease burden of seasonal influenza virus in hospitalized SARI in the province. In addition, health-care utilization information regarding ILI/SARI episodes was obtained from a household survey in Thai Binh city (urban area) and in Kien Xuong district (rural area).

Timeline: The hospital SARI surveillance began January 2013 and will last for 24 months. The household survey was conducted in May–June 2013.

Progress and Findings: Within the household health utilization survey (HUS), a total of 2,100 households were visited, including 630 households (2,094 members) from Thai Binh city and 1,470 households (4,666 members) from Kien Xuong district. Of the 6,760 total household members, 704 (10.4%) self-reported ILI and SARI and were individually interviewed for their health seeking behavior. There was a significant difference of reported SARI and ILI prevalence between the urban and rural areas ($p < 0.05$), and the majority of sick people sought some health care services from the public sector. Under the burden of SARI component of the study, as of 10 August 2013, 704 SARI patients are enrolled from the three participating. Of 682 SARI samples tested, 117 (17%) were positive for influenza, of which 89 (76%) were influenza A/H1N1pdm09, 15 (13%) were influenza A/H3, 10 (8%) were influenza B, 2 (2%) were co-infected with influenza A/H1N1pdm09 and influenza B, and 1 (1%) was influenza A/H3 and influenza B co-infection.

Conclusion: Preliminary analyses indicate a significant difference in the prevalence of self-reported ILI/SARI between Thai Binh city and Kien Xuong district. Most sick people sought health care, often from the public sector. Interim results from the burden of disease component show that influenza viruses contribute to the burden of acute respiratory disease in hospitals in Vietnam. Full analysis of the entire 24 month time period is pending.

Animal-Human Interface Longitudinal Study to Identify Influenza Viruses Infecting Humans and Animals over Time in Vietnam

The study proposes to determine the level of influenza virus, and to identify potential risk factors for developing influenza, in people, pigs, and poultry over time, through 12 months of follow-up of influenza-like illness (ILI) in selected households in the rural areas of Vietnam. If any people or animals are confirmed with influenza infection by RT-PCR test of swab samples, other people and animals in the same household will be tested to determine if any of them also have or had influenza. In addition, baseline samples from people, pigs, and poultry will be taken at the beginning and end of the study to assess the types, prevalence, and burden of influenza viruses in these species in the areas.

Study Aims and Objectives:

- To identify and characterize over time and place, the circulating influenza viruses in people, pigs, and poultry living in close proximity in Vietnam.
- To determine the variables associated with cross-species influenza virus transmission events over time.
- To determine the phylogenetic relationships and genomic characteristics of human and animal influenza virus isolates over time in Vietnam.
- To correlate influenza virus strains from this study with strains identified through existing influenza surveillance systems in Vietnam.

Approach: The study has been initiated in Binh Minh commune, Kien Xuong district, Thai Binh province (North Vietnam) and Lac Tan commune, Tan Tru district, Long An province (South Vietnam), with participating households that raise both pigs and poultry. Baseline blood samples were collected from humans, swine and poultry of study households at the beginning and in 12 months later. During the 12 months syndromic surveillance, all enrolled households are observed for ILI events through frequent visits. Once a person or animal in one of the households develops an ILI, humans in the same household will have throat swabs and blood samples taken and tested for influenza by RT-PCR, as part of the ILI virologic and serologic surveillance. If tests are positive, humans and animals in the same household will be followed for two weeks and will have blood samples and throat swabs taken to detect influenza infection. An epidemiological surveillance will be also run in parallel with the other surveillance components, where epidemiological and environmental information are collected from baseline, ILI cases, and their households, to identify the risk factors for influenza virus transmission and other variables relating to the animal-human interface.

Timeline: The first baseline serological sampling was conducted in February 2013, followed by 12 months of surveillance. The second baseline sampling will be done at the end of the follow-up study.

Progress and Findings: In Thai Binh province, 150 households (409 participants) and in Long An province, 119 households (444 participants) were enrolled for the syndromic and ILI virologic and serologic surveillance. During the syndromic surveillance, a total of 79 ILI human cases, 20 ILI pig cases and 11 ILI poultry cases were identified in both study sites. Swab and blood samples have been collected from these cases for influenza testing. Of the human ILI tested samples, 12 (15%) were positive for influenza, of which 10 (83%) were influenza A(H1N1)pdm09 and 2 (17%) were influenza B. None of the animal ILI tested samples were positive for influenza. Among the households with human ILI cases, three asymptomatic pigs with influenza A were identified in the same household where a human case of influenza A(H1N1)pdm previously identified.

Conclusion: Preliminary findings suggest that multiple strains of seasonal influenza viruses circulate in humans, creating the potential for transmission of virus among human and animal populations in rural communities of Vietnam.

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

This study proposed to determine the level of influenza virus, and to identify potential risk factors for developing influenza, in people, pigs, and poultry in a rural Mekong River Delta area of southern Vietnam. Through a syndromic surveillance, study investigators determined if any people, pigs, or poultry in the household have flu-like symptoms, and subsequently sampled and tested for influenza. Other people and

animals in the household were investigated to determine if any of them also have or had influenza. In addition, the investigators took baseline blood samples from people, pigs, and poultry to assess the types, prevalence, and burden of influenza viruses in these species.

Study Aims and Objectives:

- To identify the co-circulation of animal and human influenza viruses in selected rural communities.
- To determine the dynamics of animal-human interface transmission of influenza viruses in selected rural communities.
- To determine the phylogenetic relationships and genomic characteristics of virus isolates.

Approach: The study was conducted in households that raise both pigs and poultry in Lac Tan commune, Tan Tru district, Long An province (south Vietnam). In between two baseline serologic samplings, the syndromic and ILI virologic and serologic surveillance was implemented, with frequent visits (once every two days) to the enrolled participants' households for ILI event monitoring. Any person or animal in one of the households that developed an ILI will have throat/nasal swabs and blood samples taken and tested for influenza by RT-PCR. If tests were positive, humans and animals in the same household were followed up for two weeks and had blood samples and swabs taken to detect influenza infection. In addition, baseline samples were collected from all species from the study households at the beginning and end of the study.

Timeline: The field study was implemented from February to May 2013.

Progress and Findings: A serological sampling was conducted in eligible study households in Lac Tan commune at the beginning of the study (February 2013) and three months later (May 2013). In the first baseline sampling, blood specimens were collected from 444 human participants, 301 pigs and 1,396 poultry from 119 households. In the second baseline sampling, 409/444 people participated, and 207 pigs and 1,511 poultry from the same study households were sampled. During three months of syndromic surveillance, seven human ILI cases were identified, with swab and blood samples collected for influenza testing. One of seven specimens (14%) was positive for influenza B. No ILI cases were identified among the animal population, or from the subjects living in the same households with the human ILI cases. Epidemiological and environmental information was collected from the ILI cases to identify the risk factors for influenza virus transmission and other variables relating to the animal-human interface. Data analyses are ongoing.

Conclusion: Preliminary conclusions suggest that in a rural community over a short period of time, events of ILI are minimal, limiting potential for exchange of influenza viruses between animals and humans, when living in close proximity.

Animal Human Interface Study: Serological and Genetic Analyses of Influenza Virus Isolates Collected from Humans and Animals in Vietnam

Using virus isolates collected from previous AHI studies (Pilot Extension North, Pilot Extension South, and Longitudinal) in Vietnam, serological analyses will be performed to identify influenza virus types and phylogenetic analyses of virus isolates, including virus clade identification, characterization of select virus isolate genes, and full genome sequencing of select influenza virus isolates. This data will assist in the determination of potential evolutionary patterns and species characteristics of influenza virus isolates in people, pigs, and poultry living in close proximity in select rural communities in Vietnam.

Study Aims and Objectives:

- To perform serologic analyses of human and animal virus isolate samples to detect antibodies to influenza virus infection;
- To determine the genomic characteristics and phylogenetic relationships of human and animal influenza virus isolates over time in Vietnam;
- To characterize over time and place, the evolution of circulating influenza viruses in people, pigs, and poultry living in close proximity in Vietnam.

Approach: Serological tests for the measurement of influenza A-specific antibody include the hemagglutination inhibition (HI) test and the microneutralization (MN) assay. Of these, the MN assay is the recommended test for the measurement of highly pathogenic avian influenza specific antibody. Isolates will be further characterized by genomic sequencing, bioinformatics, and phylogenetic analyses. The eight genes, HA, NA, PB1, PB1, PA, NS, M and NP of the influenza virus will be sequenced. Animal serum samples will be screened by ELISA assay and reconfirmed by HI test for different antigens.

Timeline: February 2013–February 2014.

Progress and Findings: Serum samples were collected from a total of 1,262 people, 703 pigs and 1,511 poultry from study sites in Thai Binh (North Vietnam) and Long An (South Vietnam) in the AHI Pilot Extension South and AHI Longitudinal studies. HI tests have been completed on 30% of the collected human serum samples, and MI assay is being performed for confirmatory results. Approximate 1,000 animal serum samples are being tested by ELISA and HI against the antigens of influenza A (H1N1pdm; H3; H5 – clade 1, clade 2.3.4, and clade 2.3.2.1; H7; and H9).

Conclusion: Study in progress.

Co-Evolution of Human and Animal Influenza A Viruses in Vietnam

This study is to better understand the co-evolution of influenza viruses in animals and humans in Vietnam, including HPAI A(H5N1) viruses, to better understand their potential threats to human and animal health. A principal focus will be to better understand how influenza viruses transmit across species, and their pandemic potential, by evaluating novel human and animal influenza viruses, and comparing identical subtypes of influenza viruses in humans and animals, both geographically and temporally.

Study Aims and Objectives:

- To understand the phylogenetic evolution and co-evolution of human and animal influenza viruses, especially as related to adaption mechanisms of A(H5N1) influenza virus from animals to humans by conducting molecular virology analysis.
- To identify amino acid changes that may be critical for acquisition of high virulence and reduced susceptibility to antiviral drugs, by conducting bio-informatic analysis of the virus isolates.

Approach: Human and avian influenza isolates that are matched by time of and location of collection were selected for phylogenetic characterization. For human H5N1 isolates, at least two poultry H5N1 isolates were selected and then matched with the human isolate. The poultry virus isolates that were closer both temporally and geographically to a human isolate were prioritized for selection. Standard methods for phylogenetic characterization of human and avian influenza viruses, including RNA extraction, PCR analysis, antigenic characterization, nucleotide sequencing and phylogenetic analysis, were performed.

Bioinformatics were used as means for detecting amino acid changes related to mutations associated with replication, virulence, susceptibility to antivirals, and vaccine match. Viruses were monitored especially for evidence of emerging genotypes arising from mutations or re-assortment that could pose an increased risk for animal-to-human transmission and for epidemic or pandemic spread.

Timeline: The analyses were performed in January–August 2013.

Progress and Findings: Avian influenza A(H5N1) isolates from 30 human samples and 59 avian samples were sequenced for all genes using Sanger sequencing (ABI 3130). Phylogenetic trees were analyzed.

Conclusion: Genotypes Z and V strains have a continued predominance of the A(H5N1) virus in Vietnam. All of the human viruses isolated between 2003 and 2012 belonged to clades 1 and 2.3.4, while animal viruses clustered into clades 1, 2.3.2, and 2.3.4. Mutations related to resistant or reduced susceptibility or resistance to oseltamivir were found in some isolates in 2005–2008.

Related Published Paper: ISIRV Conference in Hanoi, Vietnam in October 2012, poster “Co-evolution of human and animal of Highly Pathogenic Avian Influenza A/H5N1 in Vietnam”.

Cross-sectional Study of Influenza in Humans and Swine at Slaughterhouses in Select Areas in Vietnam

A repeated cross-sectional study at swine slaughterhouses will be conducted in nine select provinces in Vietnam to determine the prevalence and sub-types of influenza A viruses. At three month intervals over a 12-month period, live pigs at slaughterhouses will have collected blood and nasal swab specimens for evidence of influenza virus. Pigs identified as previously exposed to influenza virus will be traced back to their sources, and potentially identified for additional studies. Slaughterhouse workers and matched human controls will be also sampled, once every six months occurring at the same time as the animal sampling. The sampled slaughterhouse workers and human control subjects will be interviewed through structured questionnaires to identify potential risk factors for the exposure to and transmission of influenza viruses at slaughterhouses.

Study Aims and Objectives:

- To estimate the prevalence of influenza A virus infection in pigs and in humans who are currently working at the slaughterhouses and slaughter points, and in matched human controls living in the same areas.
- To determine the potential exposure of the swine slaughter workers to influenza viruses, and to assess the potential transmission that may have occurred between humans and pigs.
- To identify risk factors of influenza virus exposure and transmission at slaughterhouses and slaughter points in three main regions of Vietnam.

Approach: The study will be conducted in nine provinces in Vietnam, including Bac Ninh, Hai Phong, Nam Dinh (North), Quang Tri, Thua Thien Hue, Quang Nam (Central), Binh Duong, Tien Giang and Vinh Long (South). A cross-sectional sampling of humans and pigs will be conducted in the selected slaughterhouses, once every three months over a 12 month period. During each round of animal sample collection, 10 live pigs in each slaughterhouse will be sampled for blood and nasal swab specimens. At the second and fourth (six months later) rounds of animal sample collection, two workers from each slaughterhouse and two matched control subjects living in the same area of the slaughterhouse will have throat swabs

and blood taken. The collected specimens will be tested for influenza A by RT-PCR (for swabs) and hemagglutinin inhibition (for sera). Trace-back information from slaughterhouse owners regarding the sources and quantity of pigs present for slaughter will be conducted. Risk factors for potential exposure and transmission of influenza viruses are obtained from structured questionnaire interviews of slaughterhouse workers and the controls.

Timeline: Field study is proposed to start in September 2013 and end in September 2014 with animal sampling in every three months. The first human sampling will be conducted in December 2013, in concurrence with the second animal sampling.

Progress and Findings: To prepare for the field activities, three training seminars were organized in North, Central, and South Vietnam for 65 animal health field staff and 37 human health field staff. The training seminars introduced the study and the implementation plan for discussion, and provided guidance for data collection as well as sample collection, storage and transportation.

Conclusion: Study in progress.