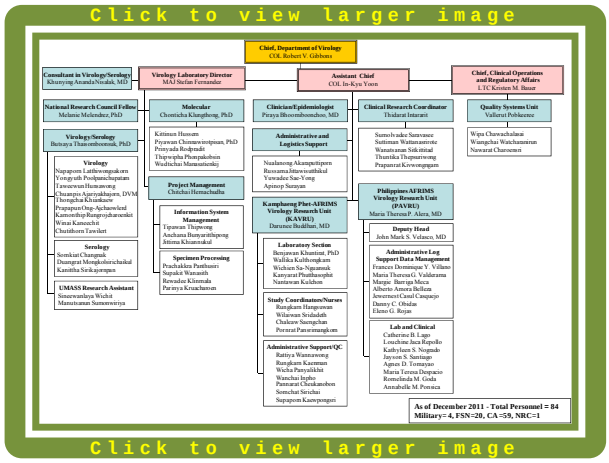
DEPARTMENT OF VIROLOGY

DEPARTMENT MISSION

To develop and evaluate products, and collect epidemiologic data to protect the Soldier and citizen from infectious diseases.

PERSONNEL



IN-HOUSE TRAINING PROGRAMS AND OUTSIDE TRAINING OF PERSONNEL

In-House Training of Personnel:

- AFRIMS Annual Occupational Health, Safety and Biosurety Program
- AFRIMS' SPSS Using - Problems and Solution
- AT Level 1 Awareness Training (AT/FP)
- Basic Life Support and Automated External Defibrillator Training
- Basic Good Clinical Practices
- Basic Life Support for Healthcare Providers Course
- Biological Safety Manual (version 2)
- Biosafety in Microbiological and Biomedical Laboratories (BMBL): 5th edition
- Bioterrorism Awareness-Level Training (online)
- BSL-3 Laboratory Manual (version 2)
- BSL-3 Meeting Minutes: Lessons Learned from Previous Operations
- Business Office and Agreements Overview
- CITI Training
- DoD Government Purchase Card Refresher Training
- Cold Chain Management Awareness Course (CCM Training)
- Diseases from Flooding and Flooding Preparation
- DoD Information Assurance Awareness
- EEO & POSH (Equal Employment Opportunity and Prevention of Sexual Harassment)
- Ethics Training
- English Language Program
- Freezerworks Unlimited Procedures

- Geographic Information System (GIS) in Statistical Aspect
- GFEBS Program
- Management of Investigational Products during Clinical Trails Version 4.0
- Management of Samples for Clinical Trails Version 3.0
- Cold Chain Monitoring for Products and Specimens for Investigational Sites Version 2.0
- HSPP Training for New Investigators
- Human Participant Protection
- Intermediate SPSS
- Introduction to Geographic Information System (GIS) in Public Health
- Level B Training SERE 100 Code of Conduct
- Mandatory Command/QAU SOPs
- Mandatory Departmental SOPs
- MMB-ZA-000-01 PMCs on Generic Freezer and Refrigerator
- MMB-ZA-002-00 Temperature Mapping for Cooling System
- MMB-ZG-000-00 Environment Check
- MMB-ZI-000-01 Electronic Safety Test
- MS Excel 2007 for Medical Research Training
- Nanoparametric (distribution-free, rank) Statistics
- Odds Ratio and Logistic Regression
- Overview of Acquisition Ethics (CLM003)
- Physical Security Plan
- Pre-OCONUS Travel File (PRO-File)
- Primary Hazard Training
- Sponsored Research Programs (How can WRAIR Business Office Help Find Outside Research)
- SPSS-Essential
- Standard Course in Clinical Trials
- Statistical Refresher on Geometric Mean
- Strengthening Military Biomedical Research in the AFP
- Strengthening Public Private Partnership
- USFK Theater Specific Required Training

Outside Training of Personnel:

- 2011 Disease Surveillance Workshop
- 33rd Annual Convention Infectious Diseases Apocalypse Seamless Take-over
- 3rd Clinical-Physiologic Conference
- 3rd Vaccinology Course
- 58th Anniversary Celebration and Annual Scientific Meeting of the Philippines
- Association of Military Surgeons Inc.
- Advanced Voucher Examination
- Antrimicrobial Resistance (Part 1)
- Avian Influenza
- CAJE (Computer-Aids Job Evaluation) briefing
- Certificate Course in Emerging Infectious Disease Epidemiology
- Clinical Trial Forum Thailand 2011
- Clinical Epidemiology Course
- ITKS Implementing and Managing Microsoft Windows

- ITKS Microsoft SharePoint Foundation 2010
- Leading Stragtegic Execution
- Pandemic Influenza Overview (Pan FLU Preparedness for LHDs, Session 1)
- Performance Management Workshop for Supervisors of LE Staff
- Recognition and Management of Bioterrorist Agents: An Overview (online)
- Refresher E2 Training
- Reportable Disease Surveillance
- Smallpox: Biosecurity Threat and Preparedness Country Workshop
- Syndromic Surveillance 101: Module 1 - 4

AWARDS

Non-applicable

ACCOMPLISHMENTS

1. Long-Term Follow-Up Study of Enrollees in the Dengue Hemorrhagic Fever Project III: Continued Prospective Observational Studies of Children with Suspected Dengue

Ninety-seven (97) subjects who were previously enrolled in the study entitled "The Dengue Hemorrhagic Fever Project III: Continued Prospective Observational Studies of Children with Suspected Dengue" were originally eligible for enrollment. Following the IRB approval process 53 subjects remained eligible (42 subjects fell out of the 3-year follow-up period due to the length of the approval process and two subjects were lost to follow-up due to relocation). The project was implemented June 2010. From then to November 2010, 53 subjects were enrolled.

Fifty-three subjects have completed all clinical activities. Clinical activities were completed in October 2011. All specimens have been collected and distributed to the appropriate laboratories to complete protocol defined testing. Data analysis and laboratory testing are underway. At the time of this report there are no results to summarize.

Future Plans: Complete laboratory testing as outlined in the protocol, analyze and report the data generated.

2. The Dengue Hemorrhagic Fever Project IV: Continued Prospective Observational Studies of Children with Suspected Dengue

Enrollment of subjects started in August 2010. During 2010, 55 subjects were enrolled. Follow-up visits (4-5 days after discharge) were completed for subjects that enrolled in 2010. During year 2011, between July and November, 51 subjects were enrolled.

Specimens were collected and archived at AFRIMS. Laboratory testing is ongoing. Data will be transferred to appropriate collaborators for data analysis. In process of analyze and interpret data that were collected during this reporting period.

Future Plans: Continue to follow-up study subjects that were enrolled in Year 2010 and 2011. Prepare study staff and study equipment and materials for the 2012 dengue season. Continue season 2 data analysis and interpretation. Continue assess lessons learned and adjust study methodologies accordingly. In process of submission protocol amendment to changes investigators, study procedures and correct errors.

3. Japanese Encephalitis Surveillance in Nepal

In this reporting period, there were no samples sent to AFRIMS for testing. The study was extended to continuation of testing samples and to identify other etiologies of encephalitis in JE negative specimens, data analysis, final study report and manuscripts.

Future Plans: Laboratory testing to identify other etiologies of encephalitis in JE-negative specimens, data analysis is ongoing.

4. Influenza Surveillance in Southeast Asia

AFRIMS works in close collaboration with the U.S. and Thai CDCs, the Thai MoPH, public health officials in Nepal, Bhutan and the Philippines, and with NAMRU-2. The influenza surveillance is divided into individual country projects each for Thailand, Nepal, U.S. Embassies in the region, Bhutan and the Philippines. Provision of staff, equipment, infrastructure development, and training is well underway at all sites. AFRIMS has set up training on molecular diagnostics in Kathmandu, Kamphaeng Phet, and in the Philippines. The infrastructure of a dedicated respiratory pathogens laboratory is already completed in these sites. This has allowed immediate processing of influenza samples, and will help ensure on-time reporting. BSL-3 laboratory is certified at AFRIMS. The virology respiratory laboratory will be equipped with a real-time PCR machine, serology set up, viral isolation, computers for data entry, and capabilities for specimen storage and archiving.

Respiratory specimens are collected from sites in Thailand, Nepal, the Philippines, Bhutan and U.S. Embassies in SE Asia with plans for expansion to Cambodia and Vietnam, through other AFRIMS departments and definitive test results are shared with the Ministries of Health and WHO Flu Net. This surveillance data gathered may contribute toward the annual re-formulation of the influenza vaccine as well as early detection of novel influenza strains or existing subtypes with pandemic potential which can increase the lead time for implementation of control and prevention measures.

The results during the reporting period are shown below.

Table 1 Real-time PCR results of samples collected in 2011

Collection Sites		Flu A/ H3	Flu A/ SW H1	Flu A/ SW H1 + Flu B	Flu A/ SW Origin	Flu A/ unsub-type	Flu B	Neg	Pending	Total
Bhutan		4	44	0	3	0	5	192	52	300
Nepal		14	0	0	0	0	11	24	0	49
Philippines		17	13	1	0	1	46	161	0	239
Thailand	Kamphaeng Phet Hospital	5	0	0	0	0	0	18	0	23
	Phramongkutklao Hospital	132	17	0	0	1	73	613	0	836
US Embassy	Dhaka, Bangladesh	0	0	0	0	0	0	2	0	2
	Rangoon, Burma	1	0	0	0	0	0	2	0	3
	Beijing, China	0	3	0	0	0	0	0	0	3
	Cairo, Egypt	1	0	0	0	0	0	3	0	4
	New Delhi, India	4	0	0	0	0	2	28	0	34
	Jakarta, Indonesia	1	1	0	0	0	0	1	0	3
	Vientiane, Laos	1	0	0	0	0	0	2	0	3
	Manila, Philippines	0	0	0	0	0	4	6	0	10
	Bangkok, Thailand	6	1	0	0	0	1	36	0	44
	Chiangmai, Thailand	0	0	0	0	0	0	1	0	1

4.1 Influenza Surveillance in the Philippines

From 1 Jan to 31 December 2011, 152 ILI study samples were collected from sentinel sites in Cebu City with 42/152 (28%) positive for influenza infection. Majority 28/42 (67%) were from the 0-4 age group with male to female ratio of 0.8. Breakdown of samples positive for influenza as follows: 18/42 (43%) positive for influenza B, 13/ 42 (31%) positive for influenza A/H3, 9/42 (21%) positive for influenza A/swH1, 1/42 (2%) positive for influenza A/ unsubtypable and 1/42 (2%) influenza A/swH1 and influenza B.

During the same period, a total of 87 ILI service and study samples were collected from the Armed Forces of the Philippines Medical Center (AFPMC) with male to female ratio of 1.3. Thirty-six (41%) out of eighty seven ILI samples were positive for influenza virus by RT-PCR testing. Influenza PCR results are as follows: 28/36 (78%) influenza B, 4/36 (11%) were influenza A/H3, 4/36 (11%) were influenza A/swH1, and 51/87 (59%) were negative. Majority 34/87 (39%) were from the 0-4 year old age group. The AFPMC-AFRIMS Collaborative Molecular laboratory was inaugurated on 30 March 2011 by the Philippines Secretary of Defense and the U.S. Ambassador to the Philippines together with representatives from AFRIMS, central GEIS/ AFHSC, Team Chief of the Biosecurity Engagement Program, U.S. State Department, Deputy Chief of JUSMAGPHIL, AFP Chief of Staff representative, J staffs, AFP Surgeon General, Army, Navy and Air Force Command surgeons. The objectives of the establishment of this lab are to: 1) develop a diagnostic molecular laboratory to support influenza surveillance at AFPMC and other military hospitals with potentially expansion in the future to include ability to detect other emerging infectious disease threats, and 2) provide a platform for ongoing training of AFP personnel with regards to biosafety, biosecurity, and biosurety and to enhance their capability to perform future medical research. Influenza RT-PCR testing on samples gathered from the AFP is currently being done at the laboratory. A total of 168 medical personnel from the medical, nurse corps and civilian staff from the AFP site/s in Mindanao, and various medical departments of AFPMC were trained in influenza specimen collection, filling up of forms and administration of the influenza rapid test kit.

4.2 Influenza Surveillance in Thailand

From 1 Jan to 31 December 2011, 836 ILI study samples were collected from study sites in Phramongkutklao Hospital and the result show as in Table 1.

The new protocol “Sentinel Human Surveillance for Influenza in Asia, Site Specific Annex: Thailand” is expected to start subject enrollment in March 2012. The study will included the study sites at the Kwai River Christian Hospital (KRCH) in Sangkhlaburi, Thailand and Kamphaeng Phet Provincial Hospital (KPPH) in lower North of Thailand.

A maximum of 3,000 subjects will be enrolled per year from both study sites in Thailand. The duration of this study is planned for 10 years.

The new protocol “Sentinel Human Surveillance for Influenza Surveillance in Royal Thai Army Recruit” has been submitted to WRAIR IRB for review. This protocol will enroll approximately 1,200 new soldiers from the training center in barrack, Bangkok, Thailand. The study will describe prospective surveillance for influenza and other viral respiratory pathogens in RTA troops, Bangkok, Thailand. The newly enlisted Royal Thai Army (RTA) male soldiers recruit begin during May and November of each year.

4.3 Influenza Surveillance in Nepal

The influenza study in Nepal had completed 5 years and now the study is closed since the laboratory testing and data analysis was completed.

The new influenza surveillance in Nepal that is a part of protocol "Sentinel Human Surveillance for Influenza in Asia" has already approved by both local and WRAIR IRBs. The sentinel surveillance sites started the subject enrollment and study activities in December 2011.

AFRIMS and its field site in Nepal (WARUN) has been working with the National Public Health Laboratory to establish themselves as a NIC with the CDC. AFRIMS has supplied the NPHL with influenza probes and primers during the CDC panel testing. In addition, results from AFRIMS studies will be shared with the NPHL to be entered into FLUNET. AFRIMS will also help NPHL establish its viral isolation laboratory FLUNET. AFRIMS will also help NPHL establish its viral isolation laboratory

During 2011, 49 ILI study samples were collected from sentinel sites in Nepal with the results summarized in Table 1.

4.4 Influenza Surveillance in Bhutan

The protocol "Sentinel Human Surveillance for Influenza in Bhutan" was implemented to collect ILI specimens at nine sentinel sites from patients who meet the inclusion criteria. The study sites started enrolling subjects on 31 August 2011. From study start until 31 December 2011, 300 ILI study samples were collected from these sites in Bhutan; results are summarized in Table 1 above.

4.5 U.S. Embassies

AFRIMS is undertaking influenza surveillance with regional U.S. Embassy Medical Units. There are 20 medical units in 16 countries participating in this surveillance program.

ILI specimens were collected from 10 U.S. Embassy Medical Units in the region during 2011. A total of 107 specimens were submitted from U.S. Embassy Medical Units. All specimens have been tested using influenza real-time PCR with the result as in Table 1.

Future Plans:

1) To do an influenza surveillance study in Royal Thai Army troops living in barracks.
2) To start influenza surveillance in Lao PDR and Mongolia.
3) To expand influenza surveillance sites in Nepal, Bhutan and the Philippines.
4) To enhance local influenza surveillance, other respiratory pathogen causing disease and diagnostic capabilities.
5) To perform influenza gene sequencing and conduct phylogenetic studies.

5. Collaborative Electronic Disease Surveillance Project in the Philippines

The SMS Fever Surveillance system was implemented in Cebu City Health Department System. This comprises a total of 84 BHCs in Cebu City. In 2011, the surveillance system was based on data collected from seventy-five out of 84 BHCs. All BHCs located in the low lands urban area have participated in the surveillance. Around 90% of BHCs in semi-rural mountain barangays have participated. The current surveillance is representative of the city's population of about 822,628 people. Valid text on the first sending is 82%. Data, collected on a daily basis between July, 2010 and December 29, 2011 from the 75 BHCs, corresponds to 7,694 fever cases. Data was transmitted within 24 hours in 62% of cases, 48 hours in 8% of cases and 72 hours in 4% of cases.

As intervention, several trainings and workshops were conducted together with the CHD to midwives responsible for sending SMS or texting. The users received an auto-reply when an error has occurred in the text data format which prompts users to resend it in the correct

format. Likewise, users who sent incomplete data received an auto-reply to send back text message containing the missing data. The individuals who repeatedly sent error text messages were given additional training through either face-to-face coaching or through phone call support.

5.1 Clinical Data Entry System (CDES) in Cebu City Health Centers

Based on pilot testing of CDES at three Cebu City BHCs, it was determined that local acceptance of the system was inadequate due to frequently required changes in local reporting requirements. PAVRU, therefore, developed a new clinical data entry system to address the issue of frequent changes in reporting requirements by the national health authorities. The new clinical data system, termed "elogbooks," allows users to easily add, delete and edit patient logbook columns. The process of looking for a patient is made simpler by creating a search field. The database restricts entry of any data from unregistered clients. Previous data is read-only. "ELogbooks" was piloted at one BHC with favorable feedback. CHD is currently considering plans to deploy "elogbooks" to the main BHCs within its health center system. A more recent national development may affect the utility of distributing "elogbooks" to the Cebu City BHCs. The Philippines National Epidemiology Center (NEC) has decided to implement an electronic data entry system at major BHCs nationally. Cebu City has been identified as an early test site for this implementation. The relative advantages of "elogbooks" versus the new national system will need to be evaluated to determine the continued utility of "elogbooks" implementation locally.

5.2 EDE Integration into Philippines National Surveillance Programs

EDE was presented to the National Epidemiology Center (NEC) director and his staff to include the OIC of the Public Health Surveillance and Informatics Division and data managers of the Philippine Integrated Disease Surveillance and Response (PIDSR) and Vaccine Preventable Diseases (VPD) sections. The NEC director and his staff were supportive of the EDE software and gave permission for EDE to be installed at the Regional Epidemiology Surveillance Units (RESUs) to analyze PIDSR data at the regional level. EDE subsequently was presented during the national Program Implementation Review (PIR) meeting of RESU officers from different regions of the Philippines, RESU of Region 6, and at the ASEAN Regional Forum Workshop on Disease Surveillance and Detection: Enhancing Public and Veterinary Health Networks to Combat Infectious Disease and Bioterrorism. With regards to applications of EDE on analysis of Philippines national dengue data, retrospective analysis done 30 days prior to an official report at the municipality, provincial or regional level to the Epidemic Surveillance and Response Division of NEC covering the period July-August 2011, showed that EDE Alerts were triggered as early as 30 days (median of 27.5 and with range of 14-30 days) prior to the date of the official report. Based on analysis of data in a selected morbidity reporting week, for municipalities whose reported clinically suspect dengue cases were detected by the NEC system to be above the alert or epidemic threshold and with at least one day in the previous week with a case count of 5 or more cases, the median of the number of days which were associated with EDE alerts one week before the actual detection by NEC was 5 out of the 7 days. Alerts generated though were not consistent for municipalities with low case counts (i.e., 5 or less cases for all 7 prior days). Analysis of currently available data seem to show promising applications of EDE in early warning alert capability of impending increases in dengue cases in the Philippines though when case counts are low, "alert" results may not be very reliable. Validity of alerts generated by EDE for early detection of dengue outbreaks as well as the possible effects of seasonality, weekly trends or holidays is being investigated.

Future Plans: Assessments will be made about the ability of the SMS/EDE system to detect spikes in febrile illnesses in Cebu City, along with the actions taken by CCHD to respond to these spikes. The spikes detected by the SMS fever surveillance system will be compared in real time with the results from the existing AFHSC-GEIS supported laboratory-based sentinel influenza surveillance within CCHD to rapidly determine if city-wide fever outbreaks are most likely due to influenza. In addition, the SMS reporting system using real time aggregate data will be used to monitor other illness syndromes such as diarrheal disease and to assess the efficacy of CCHD public health initiatives such as public health education and vaccination campaigns. EDE will be rolled out to other regions in the Philippines. This will enable the RESUs to detect outbreaks in their jurisdiction and will increase the lead time in detection of outbreaks.

6. Prospective Studies of Avian Influenza Transmission in Cambodia and Thailand

6.1 Human Cohort Subject Enrollment

In Thailand, 800 cohort subjects were enrolled in the prospective study of avian influenza transmission. The enrollment was completed in October 2008 and baseline blood samples were obtained on all subjects. Twelve-month annual follow-up blood draws and questionnaires were initiated on the cohort subjects in April 2009 and completed in September 2009. Twenty-four-month annual follow-up on the cohort subjects was initiated in May 2010 and completed in December 2010. The 24-month samples were the final annual follow-up blood draw that was done in the cohort. The active surveillance of the cohort including the weekly follow-up and ILI case investigation was discontinued at the end of May 2011.

The blood samples were sent to the laboratories at the University of Iowa and University of Florida (Dr. Greg Gray) and the Thai NIH for influenza serological testing with testing of the 24-month samples anticipated for completion by the 4th quarter of FY11. The respiratory samples collected during the ILI investigations were sent to the Thai NIH for virus isolation, characterization, and sequencing.

At the completion of the active surveillance, 60 cohort subjects had been withdrawn from the study. Twenty-five subjects were withdrawn due to death, ten due to inability to comply with the study protocol, 24 due to permanent move out of the study area, and one due to development of an exclusion criterion. None of the reasons for withdrawal were caused by participation in the study. Forty-six replacement subjects were enrolled from the same villages as the withdrawn subjects during prior quarters.

6.2 Human Influenza-Like Illness Investigations

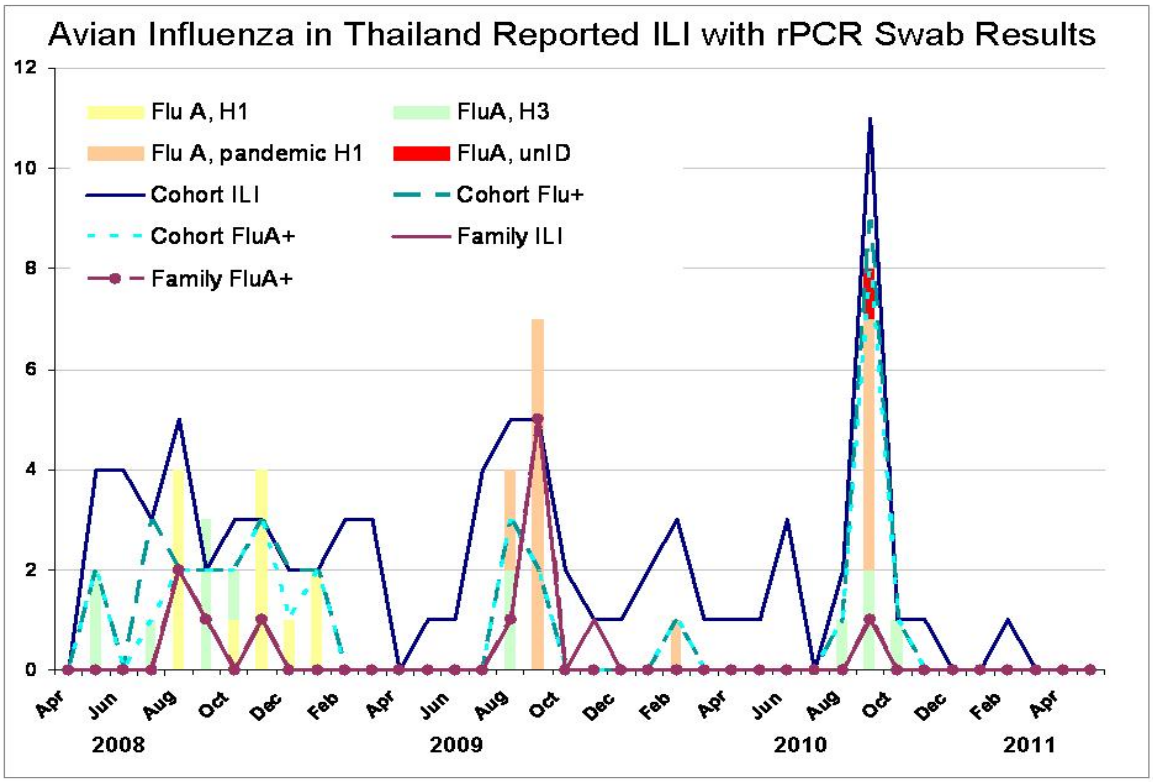
At the completion of the active surveillance (Figure 1), 80 ILI investigations in the human cohort were conducted. Thirty-five ILI cases were found to be positive for influenza virus by RT-PCR testing; the remaining 45 were negative for influenza virus. Of the 35 cases, 30 were positive for influenza A (seasonal H1=9, H3=11, pandemic H1=9, unidentified subtype=1), four were positive for influenza B, and one was positive for both influenza A (H3) and B. In addition, one cohort subject developed ILI during the 60-day follow-up period, but was negative for influenza virus.

6.3 Family Study of Influenza Transmission

At the completion of the active surveillance (Figure 1), 30 family transmission investigations have been conducted. Eighty-three household contact subjects were enrolled and underwent further testing. Eleven of these subjects had ILI symptoms and all 11 were PCR

positive for influenza A virus (seasonal H1=3, H3=2, pandemic H1=6) with the same subtype as the respective index case from the human cohort study. In addition, one case contact subject developed ILI during the 60-day follow-up period, but was negative for influenza virus.

Figure 1 Avian Influenza in Thailand Reported ILI with Real-Time RT-PCR Swab Results



6.4 Serological Analysis

Enrollment sera from 800 cohort subjects were evaluated for antibodies against influenza H1 and H3 viruses by hemagglutination inhibition (HI) assay and against avian influenza viruses by microneutralization (MN) assay. The viruses used in serological studies are shown in Table 1.

An HI titer of ≥80 and a MN titer of ≥10 were considered as evidence of respective infection. Analysis of the baseline blood samples showed that nearly 5% of the cohort had elevated titers against the A/Hong Kong/1073/1999 (H9N2) low pathogenic avian influenza virus, but exposure to poultry was not significantly associated with seropositivity. Elevated antibody titers against two circulating H5N1 highly pathogenic avian influenza viruses, A/Thailand/676/ 2005 (H5N1) and A/Thailand/384/2006 (H5N1) were also detected in this cohort. The data suggest that these viruses were at one time present in the study villages. Approximately 6% of the cohort had elevated antibody titers against A/Thailand/676/ 2005 (H5N1); however, poultry exposure was not significantly associated with seropositivity. For the cohort subjects (3.5%) with elevated antibody titers against A/Thailand/384/2006 (H5N1), exposure to poultry was associated with sero-positivity, but this was not significant after controlling for potential confounders. Interestingly, not having an indoor water source was a significant risk factor for exposure to either A/Thailand/ 676/2005 (H5N1) or A/Thailand/384/2006 (H5N1) indicating that these viruses may also be

contaminating environmental sources. The results from this study suggest serological evidence for previous infections with both low pathogenic and highly pathogenic avian influenza viruses among a cohort of which two-thirds reported exposure to poultry.

Preliminary result of the 12-month blood samples to monitor for changes in influenza antibody titers is shown in Table 2. A change in the antibody titer against H3N2 virus was observed. In 2008, approximately 68% of the cohort subjects showed positive HI titer, whereas in 2009, around 18% were positive. As demonstrated in the table, 11% (88/804) of cohort subjects showed positive HI titer against pandemic (pdm) H1N1. However, only 3 out of 27 cohort subjects who underwent ILI investigations were found to have pandemic H1N1 infection. This suggested that asymptomatic pandemic H1N1 infection occurs among the cohort subjects. Analysis of the 24-month serum is ongoing. Additional viruses - A/Sw/Gent/7625/99 (H1N2), A/Sw/Lutol/3/00 (H1N1) avian-like, A/Sw/Flanders/1/1998 (H3N2) - were included in the analysis.

6.5 Virus Culture and Characterization

Following the enrollment phase, the 800 cohort subjects were monitored on a weekly basis for influenza-like illness (ILI). Respiratory (nasal and throat swab) specimens were collected from subjects whose symptoms met the ILI case definition and tested for influenza viruses. A total of 31 RT-PCR influenza A positive respiratory samples collected during the study period were subsequently cultured. Of these, two respiratory samples were culture negative; the remaining 29 samples were culture positive.

Household transmission studies were initiated in the household of the cohort subject infected with influenza A. Respiratory (nasal and throat swab) specimens were collected from 11 contact subjects whose symptoms met the ILI case definition and tested for influenza viruses. All 11 samples positive for influenza A by RT-PCR were also culture positive.

Further characterization of these 40 isolates (29 isolates from cohort subjects + 11 isolates from contact subjects) was conducted by indirect immunofluorescence assay (IFA) or RT-PCR, HI and sequencing. Preliminary result as shown in Table 3 indicated that change in influenza virus strains occurred. In 2008, seasonal H1 and H3 isolates found among the cohort and contact subjects were related to A/Brisbane/59/2007-like virus (H1N1) and to A/Brisbane/10/2007-like virus (H3N2), respectively. Early in 2009, two cases of seasonal H1 related to A/Brisbane/59/2007-like virus (H1N1) were detected. Since August 2009, the seasonal H1 virus has been replaced by pandemic H1N1 virus [related to A/California/7/2009-like virus (H1N1)]. The seasonal H3 virus strains detected in 2009 and 2010 were related to A/Perth/16/2009-like virus (H3N2). Interestingly, changes in the nucleotide sequence of the hemagglutinin gene of the seasonal H1 viruses were also observed. All of the 2008 seasonal H1 isolates were related to A/South Dakota/6/2007-like virus whereas the 2009 H1 isolates were more related to A/Brisbane/59/2007-like virus (H1N1). However, based on the nucleotide sequence of the neuraminidase gene, all 12 of the sequenced H1isolates in 2008 and 2009 showed the H274Y mutation (oseltamivir resistance) whereas one of the eight H3 isolates showed the H274Y mutation. More intriguingly, the pandemic H1N1 virus found in the cohort changed from oseltamavir-sensitive to oseltamivir-resistant in one month. Later in 2010, the mutation in the HA gene (E374K) was also observed in the pandemic H1N1 isolates.

Table 1 Viruses used in serological studies. Unless otherwise indicated serologic study was performed using the microneutralization technique.

Avian viruses	Swine viruses
A/Duck/Alberta/35/76(H1N1)	A/Swine/Wisconsin/238/97(H1N1)*
A/Env/Hong Kong/MPU3156/2005(H2N2)	(Classical North American H1N1 strain)
A/Duck/Czech Republic/1/56(H4N6)	A/Swine/Minnesota/593/99(H3N2)*
A/Migratory duck/Hong Kong/MPS180/2003(H4N6)	(North American lineage strain)
A/Chukkar/Minnesota/14591-7/98(H5N2)	
A/Teal/Hong Kong/w312/97(H6N1)	
A/Turkey/Massachusetts/3740/65(H6N2)	**Human viruses**
A/Turkey/Virginia/4529/2002(H7N2)	A/New Caledonia/20/99(H1N1)*
A/Env/Hong Kong/MPB127/2005(H7N7)	A/Brisbane/59/2007(H1N1)*
A/Turkey/Ontario/6118/68(H8N4)	A/Panama/2007/99(H3N2)*
A/Migratory duck/Hong Kong/MP2553/2004(H8N4)	A/Brisbane/10/2007(H3N2)*
A/Turkey/Minnesota/38391(H9N2)	A/Thailand/384/2006(H5N1)† ‡
A/Migratory duck/Hong Kong/MPD268/2007(H10N4)	A/Thailand/676/2005(H5N1)† ‡
A/Chicken/Germany/49(H10N7)	A/Hong Kong/1073/99(H9N2)†
A/Duck/Memphis/546/74(H11N9)	A/Mexico/4108/2009(pandemic H1N1)*
A/Duck/Alberta/60/76(H12N5)	

*Virus studied with hemagglutination inhibition assay
†virus of avian origin
‡highly pathogenic virus, Clade 1

Table 2 Analysis of enrollment and 12-month serum

Virus	2008 (%)	2009 (%)
Human H1N1 Brisbane	4.1	5.1
Human pdm H1N1 California	N/A	10.9
Human H3N2 Brisbane	67.6	17.7
Avian H5N1 A/Thailand/384/2006	3.5	1.6

Future Plans: The laboratory testing of the 24-month sera and data analysis of the results will be continued.

7. Prospective Study of Influenza a Transmission from and to Household Animals in Thailand

At the completion of the active surveillance in the human cohort (the end of May 2009), 15 households participated in animal contact studies. A total of 67 animals (5 cats, 32 chickens, 7 cows, 12 dogs, 2 geese, and 9 rodents) were sampled. Respiratory or cloacal samples were collected for virus detection and isolation, and serum specimens for detection of antibody to influenza viruses. All the samples were sent to the Thai National Institute of Animal Health (NIAH) laboratory for testing.

Virus culture from respiratory and cloacal swab samples collected from animals from the households of influenza A-positive cohort subjects was conducted by Thai NIAH laboratory using egg inoculation technique. It was found that all 67 samples (33 respiratory samples + 34 cloacal samples) were culture negative as determined by hemagglutination assay. Serological

testing from animal blood specimens has been completed by Thai NIAH. To find evidence of avian influenza infection among household animals, serum neutralization (SN) test was conducted on mammal serum samples; whereas hemagglutination inhibition (HI) test was conducted on non-mammal serum samples. A titer of ≥16 against avian influenza H5N1 virus was considered as evidence of avian influenza infection. It was found that all samples tested showed no evidence of previous avian influenza infection. To investigate a possibility of human influenza virus transmission to household animals, the HI test against human influenza viruses (pdmH1N1 and H3N2) were conducted. An HI titer of ≥40 against human influenza was considered as evidence of human influenza virus infection. It was found that one dog showed evidence of pandemic H1N1 infection (an HI titer = 1:80). However, the virus was not detected from the dog's respiratory sample.

Future Plans: At the end of May 2011, the human cohort ceased to undergo active surveillance or annual follow up. Human cohort cases will no longer serve as index cases for household investigations. Instead, a new human use protocol is currently under development that will enroll sentinel patients presenting to the Kamphaeng Phet provincial hospital with influenza A infection who will then serve as index cases for household human and animal investigations. This change will increase the number of household human and animal investigations that can be conducted.

8. Dengue Virus Circulation, Evolution, Virus-Vector, and Virus-Host Interaction in Kamphaeng Phet Province, Thailand

8.1 Hospital-Based Study: Two hundred three in-patients at the Kamphaeng Phet Provincial Hospital with PCR-proven dengue virus infections were screened, briefed, and enrolled as index cases. Of the 203 subjects, 104 (51.2%) were male and 99 (48.8%) were female. The subject ages were 2 years 3 months old to 64 years old. Of the 203 index case subjects enrolled, 59 were DENV-1, 104 were DENV-2, 33 were DENV-3 virus, and 7 were DENV-4 virus types. Serology classified the assays as 4 acute primary dengue infection, 3 recent secondary dengue infections, 161 acute secondary dengue/flavivirus infections, and 8 were without serologic diagnosis due to availability of only a single specimen (unpaired); 27 serological result is pending.

8.2 Cluster Investigations: Total of 203 cluster investigations were initiated until 31 December 2011, corresponding to the number of enrolled index cases. The study team GPS mapped 10,310 houses, 1,783 eligible contacts were briefed, 889 subjects consented and were enrolled. Of the 889 subjects who were enrolled, 394 (44.3%) were male and 495 (55.7%) were female. The subject ages were 7 months old to 94 years old. Of the 889 contacts enrolled during cluster investigations, 25 were PCR-proven DENV-1, 45 were DENV-2, 25 were DENV-3, 5 were DENV-4 serotypes and 789 were negative. Thirty-one contact enrollee reported fever before the convalescent blood collection (between the acute and scheduled convalescent blood draw), a second acute blood sample was collected, of these 4 were DENV-1 PCR+, 10 were DENV-2, 3 were DENV-3, and 14 were negative. Serologic characterizations included 22 acute primary dengue infections, 8 recent secondary dengue infections, 145 acute secondary dengue infections, 1 acute dengue infection, 2 JE virus infection and 564 without evidence of a recent flavivirus infection; there was no serologic diagnosis available for 34 subjects due to single specimen, and 113 are pending for EIA and HAI assay completion.

8.3 Vector Studies: Investigators collected 6,813 *Aedes.* mosquitoes during the 203 cluster investigations. PCR of 5,951 samples on the female mosquitoes (the sex which transmits dengue) has been completed, of these 2 mosquitoes were DENV-1 PCR+, 31 were positive for DENV-2, 14 were positive for DENV-3 and 5,904 tested negative.

Future Plans: Continue CY2012 enrollment and submit for the grant renewal.

9. Kamphaeng Phet Prospective Dengue Surveillance Study

The Visit 2 (Terminal Visit) was scheduled during January to April 2011, there were 1,859 (98.6%) subjects who completed the study activities (Visit 1 and 2), 963 (51.8%) were male and 896 (48.2%) were female. Total dropout rate was 1.4%

Surveillance data: There were 166 subjects who had acute illnesses (from May 2010 to April 2011); 7 of these 166 subjects had 2 acute illness episodes; therefore, a total of 173 acute illnesses were investigated. Eighty-eight (53%) of these 166 subjects were male and 78 (47%) were female. There were 29 (18%) between 9 months- 2 years of age, 17 (10%) between 3-4 years old, 25 (15%) between 5-6 years old, and 95 (57%) between 7-11 years. Of 173 acute illness that were investigated, 11 cases had positive DENV PCR testing (10 were acute secondary dengue infection and 1 was acute primary dengue infection), 1 additional case had positive DENV EIA testing (acute secondary dengue infection), and 1 case had positive JEV EIA. These are classified by age group below:

Laboratory result	Age Group			
	9 mo- 2 years	3-4 years	5-6 years	7-11 years
DENV-1 (acute secondary dengue infection)	0	0	0	1
DENV-1 (acute primary dengue infection)	0	0	0	1
DENV-2 (acute secondary dengue infection)	0	0	2	4
DENV-3 (acute secondary dengue infection)	0	0	2	1
DENV-4	0	0	0	0
Acute secondary dengue infection	0	0	0	1
JEV infection	0	0	0	1
No evidence of recent flavivirus infection	29	19	22	87
Single specimen	1	0	0	2
Total	**30**	**19**	**26**	**98**

For influenza laboratory testing, 95 cases had positive Influenza PCR testing as classified by age group below:

Laboratory result	Age Group			
	9 mo- 2 years	3-4 years	5-6 years	7-11 years
Influenza A (pandemicH1)	3	3	7	21
Influenza A (pandemicH1 + H3)	1	0	0	3
Influenza A (H3)	2	1	3	9
Influenza A (Unidentified subtype)	1	0	0	1
Influenza B	1	2	2	31
Influenza A (pandemicH1) + Influenza B	0	0	0	1
Influenza A (Unidentified subtype) + Influenza B	0	0	1	2
Negative for Influenza by PCR	22	13	9	30
Total	**30**	**19**	**26**	**98**

Serological testing for influenza is pending. There were no dengue and influenza co-infection in any of the acute illnesses.

Future Plans: Contact with subjects has been completed. Laboratory testing and data analysis will continue.

10. Prospective Surveillance of Febrile Illness for Dengue-Endemic Areas in Asia (Kamphaeng Phet and Cebu Study Sites) (CYD34).

USAMRMC has great interest in Sanofi Pasteur's yellow fever-dengue vaccine (CYD) as a potential vaccine for U.S. military personnel and has adopted CYD into USAMMDA's Advance Development Program. The USAMRMC field site involved are KAVRU and PAVRU, both managed by the Department of Virology, USAMC-AFRIMS. CYD34 is a 1-year dengue surveillance study intended to evaluate the feasibility of clinical sites participates in Phase 3 clinical trial (CYD14). CYD34 was created in an effort to test the regulatory review processed in various Southeast Asian countries and to assess the field sites' ability to safety enroll subjects in a timely manner, complete phlebotomy and sample management, and follow subjects for dengue like illnesses.

Kamphaeng Phet Site: Enrollment was completed on 7 September 2010; 150 subjects were enrolled. A final analysis has not been completed.

Cebu Site: Enrollment started on 03 September 2010 and ended on 23 September 2010. A total of 150 subjects were enrolled. A final analysis has not been completed.

Future Plans: The final clinical visit occurred on 29 April 2011 and 10 May 2011 at Kamphaeng Phet and Cebu sites, respectively. The study at both sites is now closed. Investigators will complete laboratory testing to fulfill the study objectives and endpoints. Data analysis will continue and be finalized.

11. Efficacy and Safety of a Novel Tetravalent Dengue Vaccine in Healthy Children Aged 2 to 14 Years in Asia

The protocol aims to assess the efficacy of CYD dengue vaccine after 3 vaccinations at 0, 6, and 12 months in preventing symptomatic virologically-confirmed dengue cases in children aged 2 to 14 years.

Kamphaeng Phet Site: Subject enrollment started on 13 October 2011; 599 subjects were screened of which 585 subjects were enrolled.

Cebu Site: Subject enrollment started on 26 August 2011. There were 757 subjects who were screened of which 660 subjects were enrolled and vaccinated.

Future Plans: Subject enrollment and follow-up will continue as outlined in the approved protocol. Detection of suspected dengue cases is ongoing.

12. Pathological and Immunological Events in Fatal Dengue Based on Autopsy Evaluations

This study is to define the pathology associated with fatal dengue using up-to-date histological techniques to analyze tissue samples obtained from fatal dengue cases in both children and adults and to identify the cellular targets of dengue virus *in vivo* and to identify molecular changes of the endothelium and serosal epithelium in anatomical locations where plasma leakage occurs.

Post-mortem examination will be performed on deceased dengue patients by designated licensed pathologists at Vicente Sotto Memorial Medical Center (VSMMC). Tissue samples will be collected from vital organs. For tissue sample processing and storage, samples will be divided into two parts and processed

The study summary has been granted approval by the Ethics Review Committee of VSMMC.

Future Plans: The study will be implemented in March 2012.

13. Prospective Cohort Study of Influenza and Dengue Infection in Children and Adults, Cebu City, Philippines

To prospectively monitor a cohort of subjects (>6 months old) in Cebu City, Philippines, in order to determine age-specific incidence and clinical spectrum of influenza infection, relationship of pre-existing influenza A antibodies to incidence and disease severity of influenza A infection, transmission dynamics among household contacts, and degree and duration of respiratory viral shedding during the course of influenza A transmission.

A total of 1,000 age-stratified subjects will be enrolled and will undergo active fever surveillance. Blood samples will be collected at enrollment and at 12-month intervals for influenza antibody testing. Collection of respiratory sample, acute and convalescent blood sample will be done to measure the antibody response to influenza virus. Household contacts of cohort subjects with acute febrile illness will be asked to participate in a household contact investigation. They will also undergo clinical evaluation and baseline respiratory and blood samples, and follow-up blood samples. Serum aliquots will be frozen until ready for serological or molecular tests at AFRIMS, Bangkok, Thailand.

Protocol has been granted approval by local IRB. Study will be initiated on February 2012.

Future Plans: Study duration will be from 2 to 5 years. Initial cohort subjects will be followed by study staff for 2 to 5 years after enrollment.

14. Title of Research Project: Immunological Correlates of Clinical Outcomes in a Tetravalent Dengue Vaccine Cohort - An Ancillary Study (Cebu City, Philippines)

The overall purpose of this ancillary study is to identify immunologic correlates of clinical outcomes in participants in the parent CYD vaccine efficacy trial.

The study population will be composed of male and female Filipino children between the ages of 2 and 14 years living in Guadalupe, Cebu City, Cebu, Philippines. Up to 934 subjects will be enrolled in the parent trial. Therefore, up to 934 subjects will be screened and enrolled for the ancillary study. Visits related to this immunology ancillary study correspond to visits specified by the parent vaccine trial; two additional visits will be required for this ancillary study beyond what is required for all CYD14 subjects at 28 days after the first and second vaccine doses. The number of additional visits for this ancillary study is the same as the number of additional visits for the Sanofi sub-study.

In this reporting period, a total of 117 subjects have been enrolled into the study.

Future Plans: The current protocol will be amended and will be re-submitted to IRBs for review and approval.

AFRIMS PUBLICATIONS 2011

1. **Akala HM; Eyase L; Cheruiyot AC; Omondi AA; OgutuWaters NC; Johnson JD; Polhemus ME; Schnabel DC; Walsh DS.** Antimalarial drug sensitivity profile of western Kenya *Plasmodium falciparum* field isolates determined by a SYBR Green I *in vitro* assay and molecular analysis. **Am J Trop Med Hyg 2011 Jul; 85(1):34-41.**
2. **Aldstadt J; Koenraadt CJ; Fansiri T; Kijchalao U; Richardson J; Jones JW; Scott TW.** Ecological modeling of *Aedes aegypti* (L.) pupal production in rural Kamphaeng Phet, Thailand. **PLoS Negl Trop Dis 2011; 5(1):e940.**