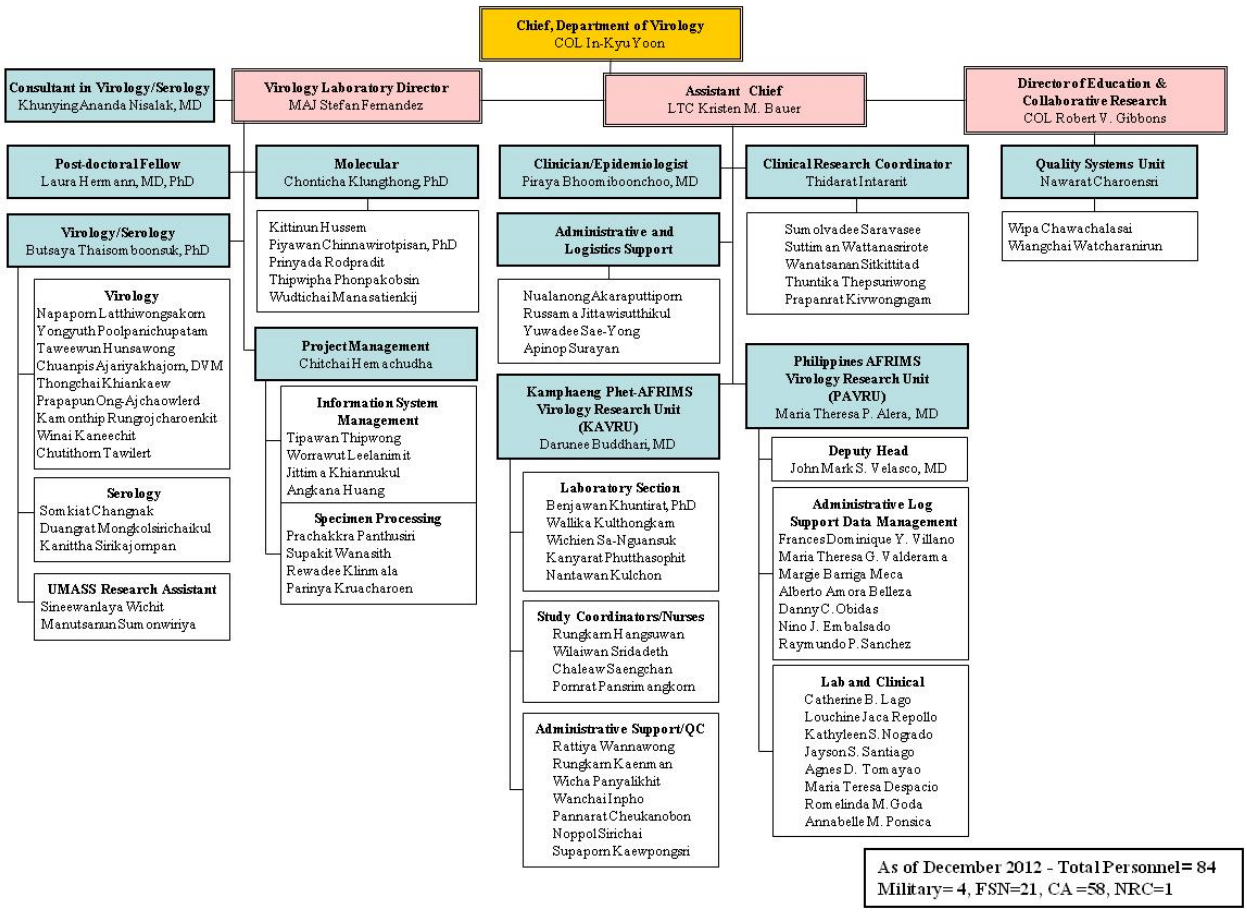
DEPARTMENT OF VIROLOGY

DEPARTMENT MISSION

To develop and evaluate products, and collect epidemiologic data to protect the soldiers 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 Programs
- 3 M Breath Easy Turbo PAPR Assembly
- Advanced Cardiovascular Life Support
- Animal Biosafety Level-3 (ABSL-3) Hazard Training
- AT Level 1 Awareness Trainng (AT/FP)
- Basic GCP
- Basic Life Support for Healthcare Providers Course
- Biological Safety Manual (version 2)
- Biosafety in Microbiological and Biomedical Laboratories (BMBL): 5th edition
- Biosurety Training for BPRP Personnel (Refresher)
- BSL-3 Laboratory Manaul (version 2)

- BSL-3 Meeting Minutes: Lessons Learned from Previous Operations
- CITI Training
- Clinical Investigator Training: Conducting Clinical Trials with Compliance
- Competency Assessment of Lab Personnel on Qualitative RT-PCR Assay for Influenza Viruses
- Competency Assessment of Lab Personnel on RT-PCR for Dengue Virus Genome
- CQA Auditor Training: Conducting Effective GCP Audits
- Do Functional Antibodies against Blood Stages of *Plasmodium falciparum* Play a Role in a Protection Model
- DoD Information Assurance Awareness
- EEO & POSH (Equal Employment Opportunity and Prevention of Sexual Harassment)
- Emergency Preparedness Response Course (EPRC) - Basic Awareness Course
- ESP/EAP: Email Writing (Section 002/2011)
- Ethics Training
- Fire Alarm Training at Freezer Farm
- Freezer Farm's FM 200 Fire Suppression System Training
- GFEBS Required Courses
- Good Clinical Practices
- Good Laboratory Practice Regulations
- Hazardous Transportation
- ILIMS Training
- Implementation of the New Guide (2011)
- Implementing DoDI 3216.02 and AR 70-25 Revision
- Introduction to GIS Using ArcGIS
- Introduction to Remote Sensing Using ArcGIS
- Level B Training SERE 100 Code of Conduct
- Physical Security Plan
- Pre-OCONUS Travel File (PRO-File)
- Primary Hazard Training
- Refresher GCP
- Respirator Fit Test
- Sample Prep (TruSeq RNA)
- Sexual Harassment/Assault Response Prevention Training
- Survival, Evasion, Resistance, and Escape (SERE) 100.1
- The Human Care and Use of Lab Animals
- Updates Regarding New Regulations/Policies/SOPs
- Weapons of Mass Destruction Preparedness
- WNSF-Personally Identifiable Information (PII) Course
- WNSF-Phishing Awareness Course
- WNSF-Portable Electronic Devices and Removable Storage Media
- WNSF-Safe Home Computing Course
- WNSF-Thumb Drive Awareness Virtual Training
- English Language Program
- Mandatory Command/QAU SOPs
- Mandatory Departmental SOPs

Outside Training of Personnel

- 3rd Clinical Trial Forum Thailand 2012
- 9th PSMID Midyear Convention
- A Practical Short Course on Infectious Disease Modeling

- Advanced Leadership
- American Society of Tropical Medicine and Hygiene Meeting
- An International Course on Human Subject Protection
- Asia Pacific Military Medicine Conference
- Biorisk Conference
- Emerging and Infectious Disease Research
- Freely-Available Tool for Alert and Early Warning Detection of Disease Surveillance
- International Congress on Infectious Diseases
- International Symposium on Respiratory Viral Infection
- Joint International Tropical Medicine Meeting
- KPH Infectious Collaboration Meeting 2012
- Mission Classification System (MCLASS)
- National Dengue Summit
- PA248 Basic Supervisory Skills Workshop
- State-of-the-Art Conference
- Transport of Biomedical Material (TMB) Course
- Virology Association Conferences

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 (53) 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 under-way. 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

There are 228 enrollees in the study: 55, 51, and 122 cases were enrolled in 2010, 2011, and 2012 respectively. Clinical, serologic and molecular diagnostic studies have been completed in enrollees recruited in 2010-2011. There were 60 DF cases and 36 DHF cases in the first 2 years of the study. There were 10 non-dengue cases. The clinical classification, virologic and serologic characterization of the patients enrolled in 2012 is underway. Infecting viruses were identified by PCR in 84 cases enrolled in 2010-2011 (88%): DENV1 19 cases, DENV2 39 cases, DENV3 20 cases, and DENV4 2 cases. The majority of the cases (88%) had a secondary dengue infection. Ten primary dengue infection cases consisted of 6 cases of DENV1 and 4 cases of DENV3 infection.

Plasma viral RNA levels and NS-1 levels were measured. Quantitative RT-PCR revealed similar viral RNA levels cases classified as DF or DHF. Consistent with the viral RNA levels, plasma NS-1 levels in DF and DHF cases were not different. The NS-1 levels appeared to be serotype-dependent as the NS-1 levels were higher in cases with DENV3 infection. The NS-1 levels were not different between cases with either a primary or secondary DENV infection.

Evaluations of cardiac functions by serial echocardiogram revealed evidence of volume contraction in DHF cases. Preliminary analyses revealed a decrease in left ventricular filling and a subtle decrease in ejection fraction in DHF compared to DF cases. Serial ultrasonographic studies confirmed the pattern of plasma leakage in DHF cases as previously reported.

Clinical classification, molecular and immunologic studies are underway for 2,012 enrollees. With the additional data we plan to further analyze the relations between indicators of viral load (viral RNA levels, NS-1 levels) and dengue disease severity, and further characterize the changes in cardiac functions in dengue cases.

Studies on adaptive immunity continue to characterize the T and B cell responses in dengue. The studies showed extensive cross-reactive responses in both B and T cell compartments. Further studies to characterize responses at clonal levels are underway.

Future Plans: Prepare study staff and study equipment and materials for the 2013 dengue season. Continue season 3 data analysis and interpretation.

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

A total of 1,239 specimens were collected from Thailand, Nepal, Philippines, Bhutan, and regional U.S. Embassies during this final quarter of CY2012 with influenza positive rate of 35.1%, a decline from the previous quarter (54.5%). Broken down into months, the percentage gradually fell in October to 45.4% and dropped to 19.8% in November before an incline in December to 29.4%. The rising percentage has regularly occurred in previous years during December (Figure 1). Overall, influenza B remains the predominant influenza type reported in the region followed by influenza A pandemic H1 and influenza A/H3. There were 22 hospitalized influenza cases with age ranging from 0.6-75.8 years (mean=23.2, median=11.4), and no fatal cases reported during this quarter.

From 1 October 1 through 31 December of 2012, a total of 29 influenza samples were tested by pyrosequencing including 13 influenza A/H3 and 16 pandemic 2009 influenza A/H1. During this quarter we did not complete any influenza B pyrosequencing analysis. All of the influenza A/H3 and pandemic 2009 influenza A/H1 showed resistance to M2 blockers (amantadine and rimantadine) and susceptibility to NA inhibitors (oseltamivir) that are common characteristics of these two subtypes.

The effects from efforts undertaken by the Department of Virology to improve the efficiency and timeliness of influenza testing and reporting had taken effect. Bottlenecks in the surveillance process were regularly examined and accounted for. During this quarter, the clinical and laboratory data entry lag at the field laboratories and subsequent data transmission to AFRIMS in Bangkok, Thailand reduced this process to a span of only one to two weeks with the aid of web-based clinical and laboratory data entry interface developed. Weekly data status reports instituted within the Department of Virology, AFRIMS, Bangkok is in use as a tool to

promptly identify delays and outliers providing immediate feedback to sites if any, as appropriate. All sites, including Bhutan, have reached timeliness PCR reporting of within 3-weeks of collection for 80% of the collection sites.

Thailand

Sentinel influenza surveillance sites in Thailand coordinated by Department of Virology include Kamphaeng Phet Provincial Public Hospital (KPPPH), Kwai River Christian Hospital (KRCH) in Sangkhlaburi, Phramongkutklao (PMK) Hospital and Queen Sirikit Institute of Child Health (QSNICH) in Bangkok. Outbreak service samples from the Royal Thai Army (RTA) and Ministry of Public Health (MoPH) were also tested. In the final quarter of CY2012, 451 respiratory samples were collected in Thailand from those sites and submitted to the Department of Virology. Influenza B continued to predominate in this quarter followed by influenza A pandemic H1 (see Table 1 for further details).

Philippines

In the final quarter of CY2012, there were 239 ILI samples sent from sentinel sites in Cebu City and the Armed Forces of the Philippines Medical Center (AFPMC) in Manila. All were tested using influenza real-time PCR and results shown in Table 1. Redistribution of sentinel sites to be more representative of the overall city was approved by the WRAIR IRB and the local Filipino IRB and was active this quarter. Data system issues which occurred during last quarter were resolved. A malfunctioning PCR machine at the site laboratory caused temporary reporting delays in this quarter. Corrective actions are currently taking place, including the acquisition of a new apparatus.

Nepal

In the final quarter of CY2012, 325 samples were collected from sites in Nepal. Influenza B predominated followed by influenza A pandemic H1 without any A H3 as detailed in Table 1. Excluding samples sent from NIC of Nepal (which were samples collected from outbreaks within the country and were already tested once in the NIC before transferring to laboratory in WARUN), 93.2% of the samples were tested and reported within 3 weeks of collection of which 9 of 14 that were late were only 1-2 days over the allotted time.

Bhutan

In the final quarter of CY2012, 111 samples were collected from sites in Bhutan (see Table 1 for details). As a result of the 'Influenza Testing Improvement Session' held in Bhutan during the last quarter of 2012, timeliness in reporting of influenza in Bhutan has notably increased. Nevertheless, the Department of Virology, AFRIMS continues to provide 100% confirmatory testing for PCR testing done at the PHL and will gradually reduce this percentage as testing on-site continues to improve. Annual training on all study processes including but not limited to consenting, sample collection, sample transportation, data collection, data entry, data transfer and trouble-shooting was provided to 23 participants. The training has enhanced the quality of study conduct, timeliness of reporting, and improved operational issues in detail.

U.S. Embassies

ILI specimens were collected from six U.S. Embassy Medical Units in the region. A total of 61 specimens were submitted from Medical Units in Dhaka (1), Manila (2), New Delhi (4), Beijing (21, of which 14 that arrived at the end of the quarter are pending results), Jakarta (8), Bangkok (25) (see Table 1 for further details).

Response to Second Respiratory Outbreak in RTA Barracks in Uttaradit Province, Thailand

During November 2012, AFRIMS Deartment of Virology was called on to provide laboratory support to the RTA in response to a severe respiratory outbreak in the RTA infantry barracks in Uttaradit, Thailand. This was the second such outbreak this year in the same location. We received 66 respiratory specimens collected from the most severe cases, including almost two dozen hospitalized cases. We immediately tested these specimens for influenza virus as well as for adenovirus, since during the earlier outbreak several adenovirus positives cases were found. However, influenza and or adenovirus positive cases were very few. We implemented multiplex PCR analyses of 33 of the most severe specimens in an effort to determine etiology. We found that in a relatively high percentage of cases (58% of all tested), *Haemophilus influenzae* B (HIB) was present. Also of significance was the presence of *Moraxella catarrhalis* and rhinovirus. Enteroviruses (we have performed no typing at this point) was found in 4 cases. These results were provided to the RTA medical department for further consideration.

Laboratory

Virus Isolation: Selected specimens were cultured in various cell lines to include MDCK, H292 and LLCMK2 cells and recently incorporated the HEP2 cells in order to increase the isolation and identification of other respiratory pathogens such as parainfluenza (1-3), adenovirus and RSV. Specimens sent for isolation included influenza PCR-positive specimens, influenza PCR-negative specimens, and discordant specimens. In the final quarter of CY2012, we attempted to isolate proportionally equal numbers of specimens from all sites. We recently implemented changes in our isolation process, which have resulted in significant improvements in the number of isolates. These changes will likely become more apparent in future reports and we are confident they will translate into a more efficient process and a larger isolate repository. Of the specimens collected and analyzed by PCR, 618 influenza-positive and influenza-negative specimens entered Virology isolation. During this same period, we completed the isolation process for 698 specimens (some of these specimens entered isolation prior to the beginning of the first quarter of 2013). We were able to identity, isolate and store 7 influenza A pH1N1, 27 influenza A/H3, 145 Flu B (Brisbane and Wisconsin), 7 adenovirus, and 2 parainfluenza 1 (no parainfluenza 2 or 3) (see Table 2). There was no unsubtyped influenza A isolates. There were 510 negative samples, of which 42 specimens were positive for cytopathogenic effect (CPE). The Virology Core Laboratory continued to assist in determining non-influenza etiologies of ILI cases from Cambodia by isolating and identifying (by IFA) specimens that are negative for influenza PCR.

Influenza Pyrosequencing: From 1 October to 31 December 2012, the total number of 29 influenza samples was tested for pyrosequencing including 13 influenza A/H3, 16 pandemic 2009 influenza A/H1. All of them showed resistance to M2 blockers (amantadine and rimantadine) and susceptibility to NA inhibitors (oseltamivir) that are common in these influenza subtypes.

Influenza Virus Sequencing:

Figure 3 Neighbor Joining Tree for Influenza A/H3: The Neighbor Joining tree (Fisheye view) of 175 influenza A virus H3N2 complete HA gene sequence included 153 sequences from our study (33 Nepal, 18 Bhutan, 23 Philippines, 65 Thailand, 7 Indonesia, 3 India, 2 Afghanistan, 1 China, and 1 Egypt), and 10 vaccine sequences from GenBank. Eleven new sequences were collected during June-October 2012, including sequences from Philippines (8), and Thailand (3) isolates. These new sequences indicated by colors representing countries that recently collected: green-Thailand and yellow-Philippines. Black letters represented viral

sequences collected in the past and vaccine sequences were in red. All new viral sequences isolated from Thailand and Philippines fell into clade 2011-2012, a new branch of the tree. The sequences obtained from the old viruses isolated since 2008-2009, 2009-2010, and 2010-2011 were distinctively separated onto different parts of the tree. They were mixed sequences from Thailand, Nepal, Philippines, Egypt and Bhutan. The sequence of the vaccine strain A/Perth/16/2009 was in the same 2009-2010 clade. However, the HA sequence of the current vaccine A/Victoria/361/2011 was not available in GenBank database. Therefore, in this study, we used the vaccine candidate A/Brisbane/11/2010 to compare with the new 11 sequences. The alignment of the new sequences with the vaccine candidate showed 97.8-98.4% nucleotide identity.

Figure 4 Neighbor Joining Tree for Influenza B: The Neighbor joining phylogenetic tree (Fisheye view) of 246 influenza B virus complete HA gene sequences contained 234 sequences from our study (100 Thailand, 59 Nepal, 38 Bhutan, 29 Philippines, 3 China, 3 Indonesia and 2 India), and 8 vaccine strains from GenBank in red. There were 39 new sequences, indicated by colors representing countries that were recently collected: green-Thailand, blue-Bhutan, yellow-Philippines and pink-Nepal. Black letters represented viral sequences collected in the past and vaccine sequence was in red. Twenty sequences collected from Thailand (15), Philippines (2), Bhutan (2), and Nepal (1) were found in Victoria lineage whereas 19 sequences collected from Thailand (8), Nepal (7), Bhutan (3) and Philippines (1) were in the Yamagata lineage. All new sequences continued to be related to their respective vaccine strain with sequences in the Victoria lineage showing 98.5-99.3% nucleotide identity to B/Brisbane/60/2008 and 98.3-98.7% nucleotide identity to B/Bangladesh/5945/2009, which was the new Victoria vaccine candidate. The alignment of the new sequences in the Yamagata lineage showed 97.2-97.6% nucleotide identity to B/Florida/4/2006 and 97.6-99.4% nucleotide identity to B/Wisconsin/01/2010 Northern Hemisphere 2012-2013. The one potential N-linked glycosylation site at N211 was found in all strains in the Yamagata lineage, except B/Florida/-4/2006.

Figure 5 Neighbor Joining Tree for Influenza A pH1N1: The Neighbor Joining phylogenetic tree (Fisheye view) of 199 influenza A virus pandemic H1N1 complete HA gene sequences contained 185 sequences from our study (89 Thailand, 44 Nepal, 42 Bhutan, 5 Philippines, 3 Egypt and 2 Indonesia) and vaccine sequence from GenBank. Twenty-four new sequences indicated by colors representing countries that recently collected: green-Thailand, blue-Bhutan and pink-Nepal. Black letters represented viral sequences collected in the past and vaccine sequence was in red. Even though all new sequences fell into 2 clades (clade 2 (2012) and clade3 (2010-2012), the nucleotide identity showed 98.1-98.5% nucleotide identity compared to the California vaccine strain. Furthermore, clades 2 and 3 carried distinct amino acid changes, when compared to the California vaccine strain, clade 2: P100S, D114N, D146N, H155R, S202T, S220T, T226K, R240Q, V266L, I338V, E391K and S468N. clade 3: L8M, S101G, S160G and A214T. The 2012 (clade 2 and 3) were currently on separated branches of the tree. Overall diversity continued to accrue in these 2 clades which also seemed to be becoming less and less related to the currently used vaccine and therefore should be monitored closely.

Table 1 Real-time PCR Results for Influenza Testing

Area	Site	A/H1	A/pH1	A/H3	Flu A/ Un-subtype	Flu B	A/pH1 + Flu B	Neg	Pending	Total
U.S. Embassy	Dhaka	0	0	0	0	0	0	1	0	1
	Manila	0	0	0	0	0	0	0	2	2
	New Delhi	0	0	0	0	0	0	4	0	4
	Beijing	0	0	0	0	0	0	7	0	7(14 untested)
	Jakarta	0	0	0	0	0	0	8	0	8
	Thailand	0	2	2	0	3	0	18	0	25
U.S. Embassy Total		**0**	**2**	**2**	**0**	**3**	**0**	**38**	**2**	**47**
Bhutan	Mongar	0	0	0	0	2	0	2	0	4
	Thimphu	0	1	1	0	6	0	13	0	21
	Paro	0	0	0	0	0	0	7	0	7
	Punakha	0	0	0	0	4	0	5	0	9
	Phuntsholing	0	0	0	1	0	0	19	5	25
	Gelephu	0	1	0	0	1	0	10	0	12
	Tashigang	0	1	0	0	6	0	12	0	19
	Damphu	0	0	0	0	0	0	4	0	4
	Phuntsholing	0	1	0	0	1	0	8	0	10
Bhutan Total		**0**	**4**	**1**	**1**	**20**	**0**	**80**	**5**	**111**
Nepal	NPHL, NIC	0	13	0	0	38	2	81	0	134
	Bharatpur	0	14	0	0	27	0	40	0	81
	Kathmandu	0	0	0	0	0	0	4	0	4
	Siddhi	0	1	0	0	2	0	1	1	5
	Sukra Raj	0	8	0	0	11	0	7	0	26
	Tribhuvan	0	1	0	0	3	0	6	0	10
	WARUN	0	0	0	0	0	0	2	0	2
	Pokhara	0	19	0	0	12	0	32	0	63
Nepal Total		**0**	**56**	**0**	**0**	**93**	**2**	**173**	**1**	**325**
Philippines	AFPMC	0	0	0	0	30	0	81	0	111
	AFGH	0	0	1	0	3	0	2	0	6
	Lorega	0	0	1	0	0	0	2	0	3
	Tisa	0	0	4	0	0	0	7	0	11
	Sambag I	0	0	7	0	0	0	10	0	17
	Sawang Calero	0	0	1	0	0	0	3	0	4
	Kamputhaw	0	0	9	0	0	0	32	0	41
	Lahug	0	0	2	0	0	0	4	0	6
	Mambaling	0	0	0	0	0	0	12	0	12
	Talamban	0	0	5	0	0	0	13	2	20
	Apas	0	0	1	0	0	0	5	1	7
	Pardo	0	0	1	0	0	0	0	0	1
Philippines Total		**0**	**0**	**32**	**0**	**33**	**0**	**171**	**3**	**239**
Thailand	PMKH	0	10	26	0	51	0	132	20	239
	KAVRU	0	18	8	0	56	1	96	0	179
	KRCH	0	0	0	0	0	0	6	0	6
	MOPH	0	0	0	0	0	0	30	0	30
	QSNICH	0	0	0	0	0	0	1	0	1
	RTA	0	0	4	1	0	0	57	0	62
Thailand Total		**0**	**28**	**38**	**1**	**107**	**1**	**322**	**20**	**517**
Grand Total		**0**	**90**	**73**	**2**	**256**	**3**	**784**	**31**	**1,239**

Table 2 Virus Isolations

Total isolations completed in January 2013	**698**
Influenza A pdmH1	7
Influenza A/H3	27
Influenza B	145
Influenza A/unsubtype*	1
Adenovirus	7
Parainfluenza 1	2
RSV	0
Negative (CPE positive)	42
Negative (CPE negative)	468
Total isolations completed in October 2012	**582**
Influenza A pdmH1	0
Influenza A/H3	29
Influenza B	82
Influenza A/unsubtype*	1
Adenovirus	16
Parainfluenza (PI1and PI3)	10
RSV	1
Negative (CPE positive)	28
Negative (CPE negative)	415
*very low viremia	

Figure 1 Influenza Subtypes in October-December 2012

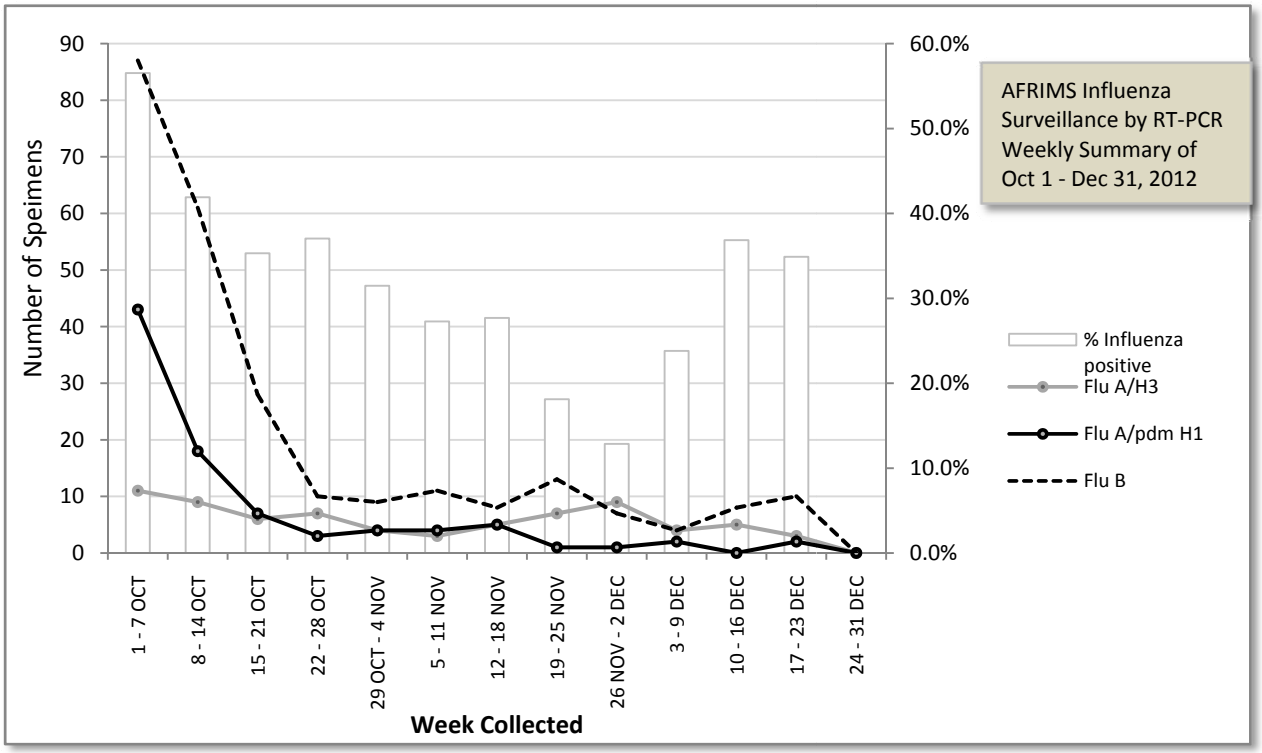


Figure 2 Proportion of Positive Influenza Specimens by Month

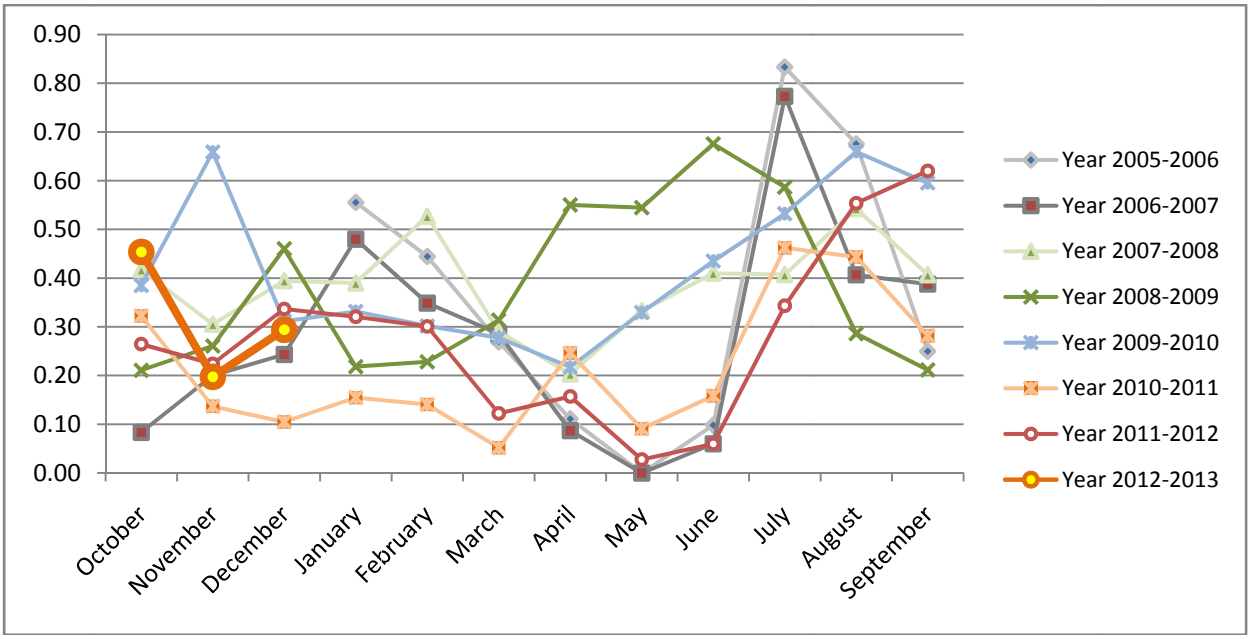


Figure 3 Neighbor Joining Tree for Influenza A/H3 (enlarge for clarity)

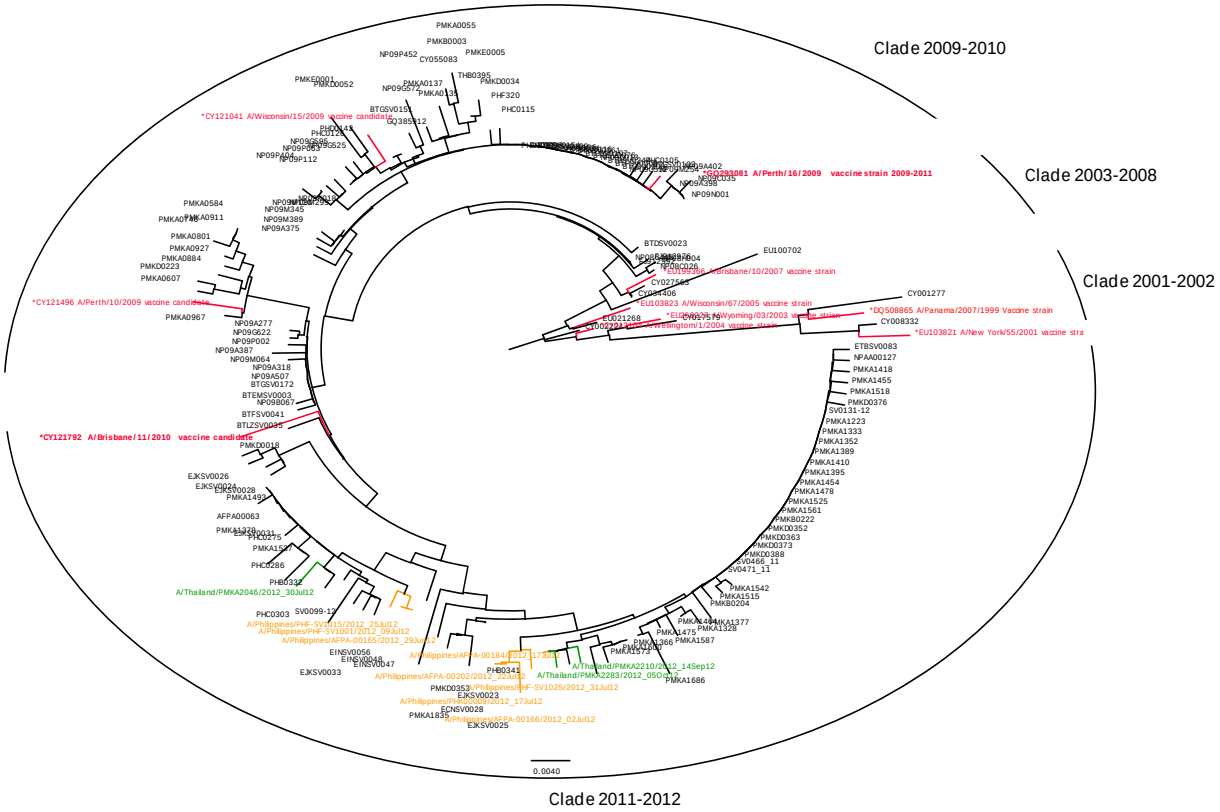


Figure 4 Neighbor Joining Tree for Influenza B

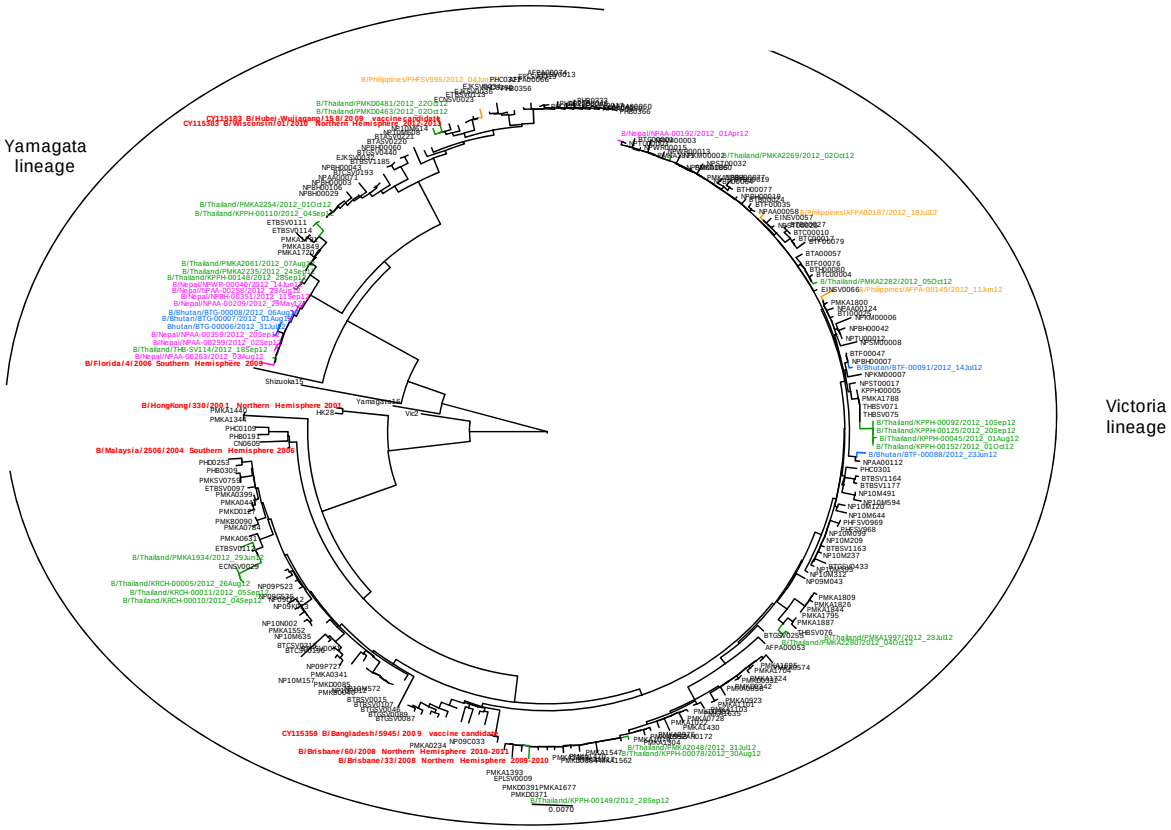
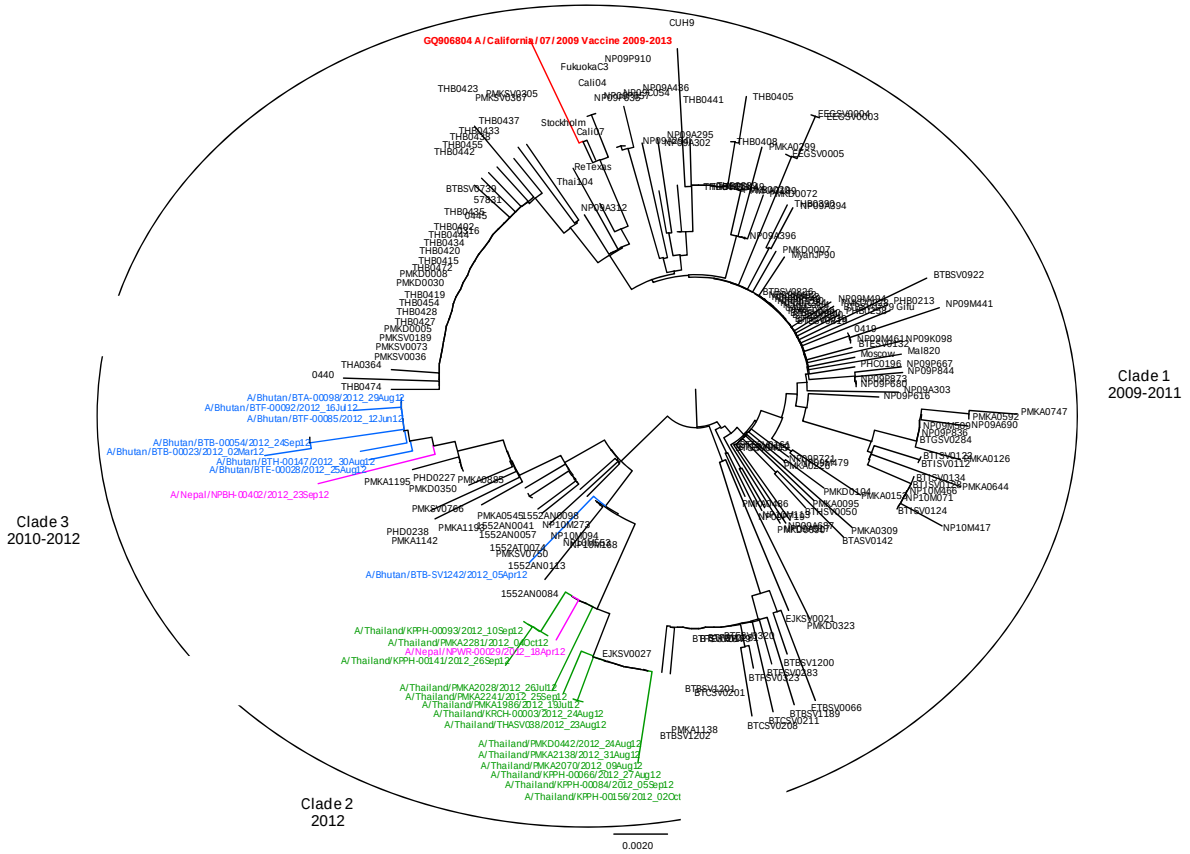


Figure 5 Neighbor Joining Tree for Influenza A pH1N1:



Future Plans: The enrollment from protocol listed sites is expected to continue. Results from the data analysis will be presented in scientific publications or scientific meetings. Any changes to expand the study sites will be amended and will be re-submitted to IRBs for review and approval.

5. Collaborative Electronic Disease Surveillance Project in the Philippines

Fever text messages are received by an SMS/GPRS wireless modem with SIM card associated with a mobile number. The program performs automatic error detection on authentication, data range and data type and data format. It is an interactive system which sends an auto-reply to providers. The server for the database and the web are located in CESSU. The physical and environmental security of the system was secured by isolating it in a locked cabinet and accessible only by authorized persons.

PAVRU developed a website which can be viewed in a static IP address (http://122.3.121.50/) that is password protected. The dash board displays fever time series in real time and line listings of cases. SMS metrics also viewed in the dash board are total valid/-invalid/zero text, timeliness, map of cases. See Figure 1. Reports are generated depending on the time range set and submitted to the CHO, epidemiologist, and assistant epidemiologist and medical health area officers as feedback. To improve the data quality, continuous targeted trainings for providers are conducted. CESSU staff can download SMS data from the website and analyze it by using EDE, see Figure 2. The age and gender distribution display are the next metrics planned to include in the report.

An interactive user guide for new users can also be viewed and downloaded from the website and a mechanism to enable downloading of the SMS raw data for further analysis by authorized personnel is also incorporated in the system. A "push" SMS is also being sent every day to remind providers to send fever cases and report if there are zero cases. Area supervisors and nurse supervisors are required to conduct monthly monitoring.

Data collected since July 2010 up to September 2012 corresponded to 5,488 fever cases within the BHC system. The SMS surveillance system is currently based on data collected from 84 BHCs out of 85 participating BHCs. The surveillance is representative of the city's population of about 822,628 people. Overall, data was transmitted within the same day in 73% of cases.

SMS data was analyzed using EDE. The time series is monitored daily and weekly. The SMS fever data time series (2011) was compared to dengue hospitalized cases of 2011. The fever SMS pattern was similar to the pattern of dengue cases suggesting that dengue was likely the cause of fever during the time period. This also provided information on seasonal trend of fever and dengue (see Figure 2). CESSU is in the process of instituting a documentation system of observed fever spikes, investigations done, and public health actions taken.

Figure 1 SMS Fever Surveillance Dash Board

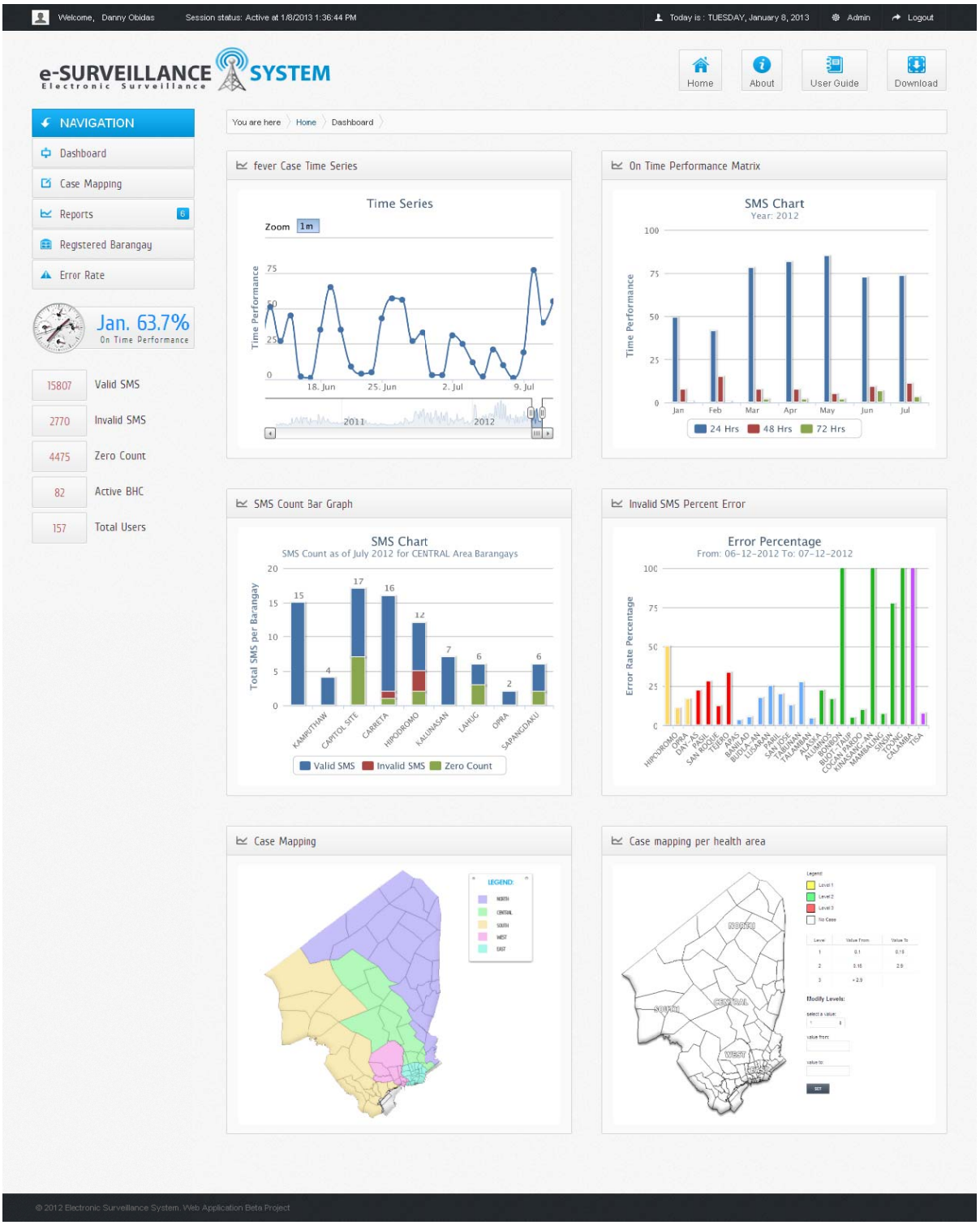
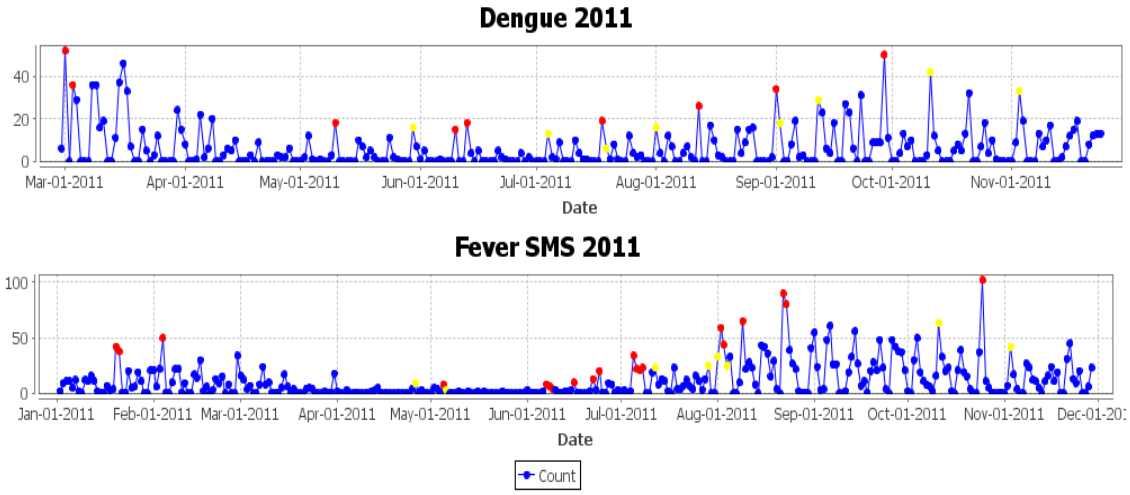


Figure 2 SMS Fever Trend in 2011 Compared with Dengue Cases in the Same Year



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.

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

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. 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.

Eighty-one ILI investigations were conducted among 76 cohort subjects (5 subjects experienced 2 unique ILI events) and 83 household contacts were enrolled in the family transmission study with 12 case contact (14%) developing ILI.

6.1 Acute Human Influenza A Infections: Molecular analyses were performed on nasal and pharyngeal swabs collected during ILI episodes (Figure 1). For the cohort subjects, 31 (38%) of the 81 reported ILIs were molecularly positive for influenza A virus by either one or both respiratory swabs; of the 12 ILI cases among family contacts, 11(92%) were RT-PCR positive for influenza A. Among the 42 participants positive for influenza A, 12 were the classical human H1N1 influenza A virus, 15 for the influenza A (H1N1) pdm09 virus, and 13 subjects were positive for human H3N2 influenza A virus; one participant's influenza subtype was indeterminate. In addition, one ill participant had a dual infection with an H3N2 influenza A virus and an influenza B virus.

Incident influenza A infections were also assessed by a 4-fold or greater increase in HI assay antibody titers between acute sera and convalescent sera collected 60 days later. One symptomatic participant negative by RT-PCR had a 5-fold titer increase against human H3N2 influenza. As paired sera was collected from all ILI case contacts, regardless of experiencing ILI symptoms, 11 family members experienced a 4-fold or greater increase in antibody titers from the acute to convalescent blood draws, yet never reported experiencing symptoms consistent with ILI. This included one subject seropositive for classical H1N1, 5 subjects for pH1N1, and 5 subjects seropositive for H3N2. When examining serological reactivity for those with positive RT-PCR results, none of the 12 subjects RT-PCR positive for classical H1N1had a corresponding increase in HI titer; 8 (53%) of 15 subjects RT-PCR positive for pH1N1 had a 4-fold or greater increase in antibody titer, while 3 (21%) of the 14 subjects positive for H3N2 had a 4-fold or greater titer increase.

Lastly, numerous asymptomatic acute influenza A virus infections were identified through 4-fold or greater increases in antibody titers amongst the cohort by analyses of annual follow-up sera. For classical human H1N1 influenza, an additional 60 subjects experienced acute influenza infections, based on HI titer increases, of which 57 (95%) did not report experiencing an ILI during the respective follow-up period. For the three cases that did report an ILI, one subject's ILI swabs and sera were negative for influenza, one subject was only RT-PCR positive for human H3N2, and one subject was both RT-PCR positive and seropositive for pH1N1.

Thirty-two subjects' titers increased $\geq$4-fold against human H3N2 influenza over the annual follow-up period. Thirty (94%) had no corresponding report of ILI during the respective follow-up time. One subject experienced an ILI molecularly and serologically confirmed as a pH1N1 infection, and the other subject's ILI samples were negative for influenza.

Against the pandemic H1N1 influenza virus, annual antibody titers increased $\geq$4-fold among an additional 48 subjects. None of the subjects save for one (98%) reported experiencing an ILI during the respective follow-up period. The ILI swabs and sera of the subject who reported an ILI were negative for influenza.

Over the 2 years of follow-up, the primary attack rate for human influenza infections among the study cohort was 21.5%; the secondary attack rate among family contacts was 27.5%.

6.2 Avian Influenza Virus Infections: No AIV infections were detected by molecular analyses of the respiratory swabs nor by serological analyses of the paired ILI sera. Contrasting from the sera collected at the time of enrollment, there was little serological activity against the HPAI H5N1 virus among the sera collected at annual follow-up visits. One subject had an elevated titer (1:20) at the 24-month follow-up visit; the subject's sera collected previously at the time of study enrollment and at 12-months were not seropositive for H5N1 HPAI.

Serological activity against low-pathogenic avian influenza (LPAI) viruses was sparse, with the exception of A/Hong Kong/1073/1999(H9N2). For the 12-month follow-up, 21 subjects had elevated antibody titers against this avian-like H9N2 influenza virus: 10 (1:10), 8 (1:20), and 3 (1:80). For the 24-month follow-up, 40 subjects had elevated titers against this

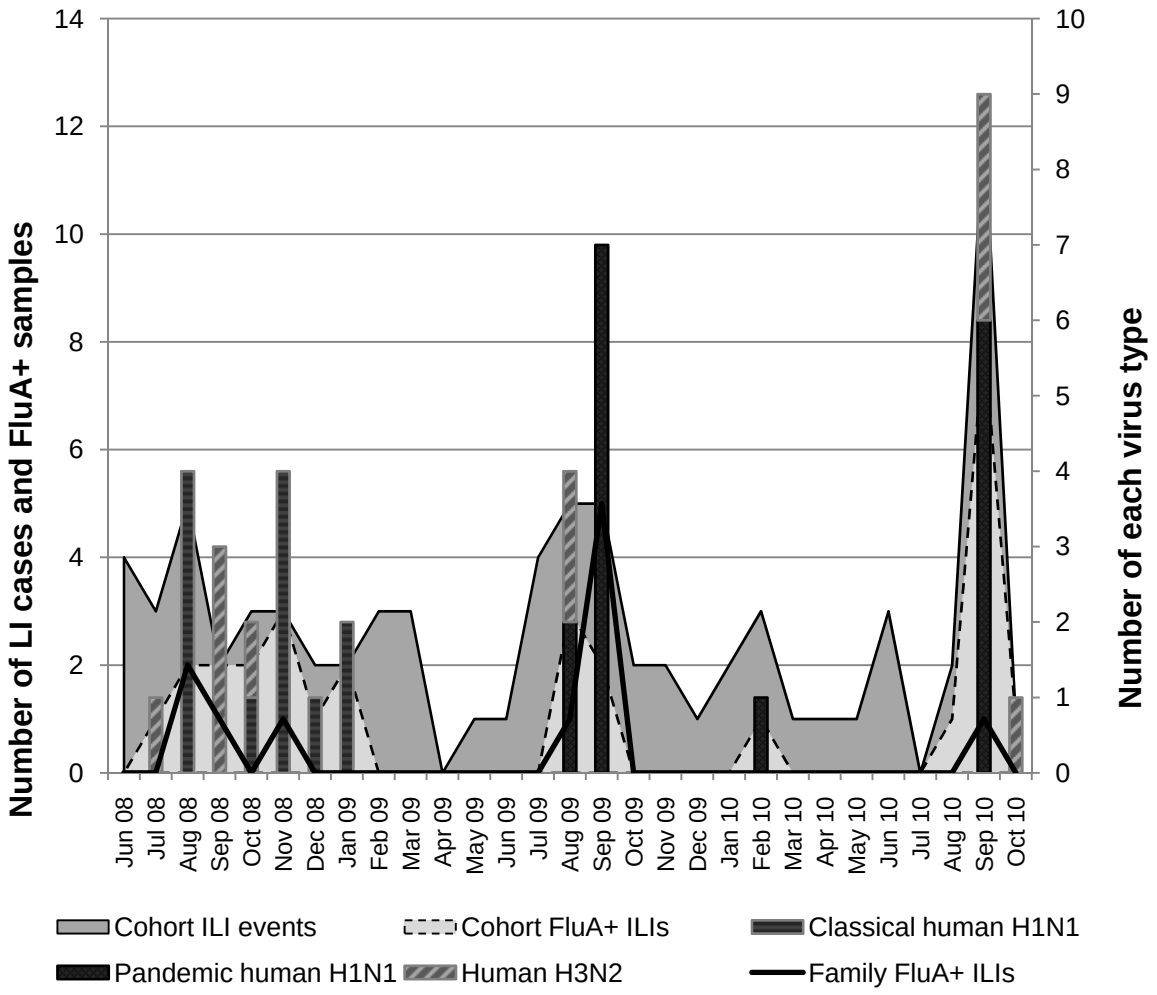
virus: 21 (1:10), 13 (1:20), 5 (1:40), and 1 (1:160). Five participants had elevated titers at all 3 encounters; 16 subjects had elevated titers at both the 12- and 24-month follow-up visits. In bivariate analyses, prior receipt of a human influenza vaccine was significantly associated with elevated antibodies against H9N2 for both 12- and 24-month follow-ups ([OR=11.4, 95% CI, 2.5-41.2] and [OR=5.0; 95% CI, 1.9-12.3], respectively). No potential risk factors could be identified for H9N2 seropositivity at 12 or 24 months. Other LPAIs with low serologic activity included the H6N1 virus (1 positive at 1:20 at 12-months), H7N7 virus (1 positive at 1:40 at 24-months), and H12N5 virus (2 positive subjects with titers at 1:40 and 1:320 at 24-months).

There was considerable serological activity against the 3 swine influenza viruses (SIVs). However, as less than 50 respondents reported any swine exposure, this seroreactivity was likely a reflection of cross-reactivity due to human influenza virus infection.

6.3 Virus Culture and Characterization: Further characterization of the 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 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 oseltamivir-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.

Future Plans: Data analysis of the results and preparation for publication will be continued.

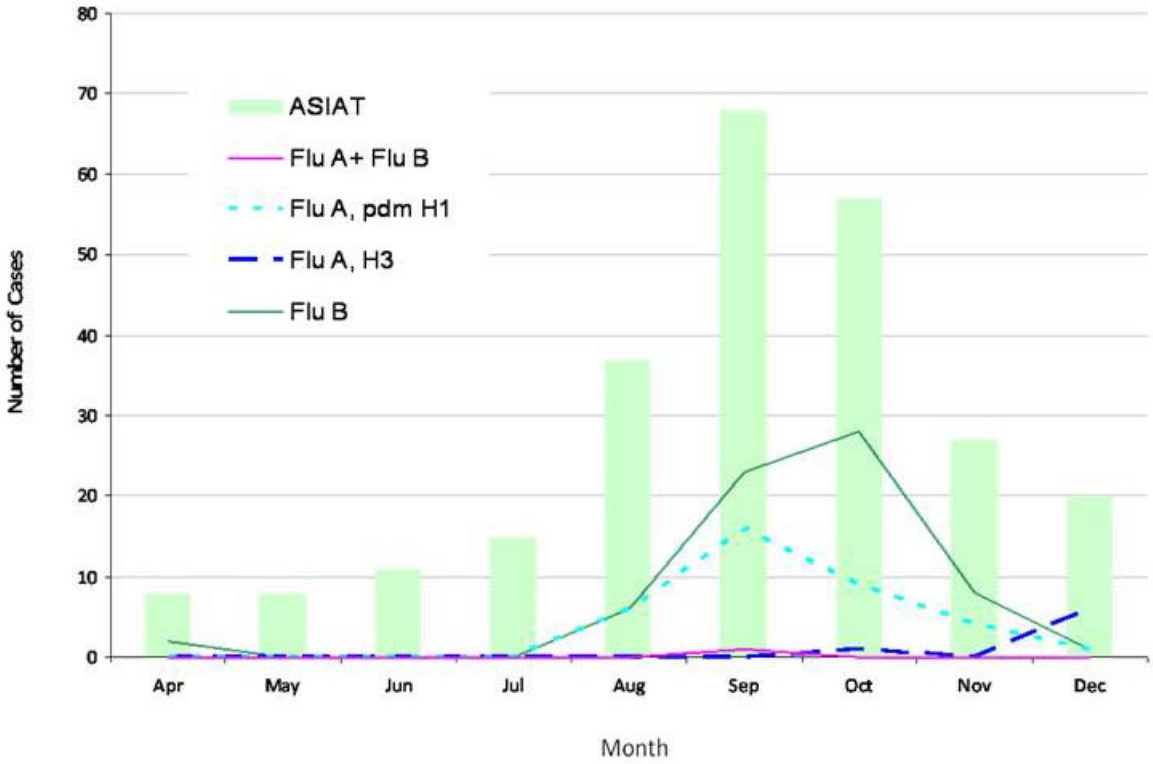
Figure 1 Reported Influenza-Like Illnesses with Real-Time RT-PCR Results of Respiratory Swabs Collected from Study Cohort Members and Their Family Contacts at Time of Illness; June 2008 - November 2010; Kamphaeng Phet, Thailand.



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

Since the start of the sentinel human influenza surveillance study (ASIAT) in April 2012 until 31 December 2012, 247 ILI subjects were enrolled. Of these subjects, 43 were positive for influenza A (pandemic H1 = 36, H3 = 7), 68 for influenza B, and 1 for both influenza A/-pdmH1 and influenza B (Figure 1). Of the 44 influenza A subjects, 4 live outside the study site (Muang District, Kamphaeng Phet Province), 14 do not have any pet or livestock, and 2 did not permit the study staff to conduct household animal investigation. Of the remaining 24 subjects, there were 20 households that allowed study staff to collect animal samples from their pets/livestock (some subjects reside in the same houses). A total of 74 animals (4 chickens, 10 fighting cocks, 40 dogs and 6 rodents) were sampled. Respiratory or cloacal samples were collected for virus detection and isolation, and serum specimens for detection of antibody to influenza viruses. Preliminary result of virus culture from respiratory/cloacal samples sent to the NIAH laboratory using egg inoculation technique was negative. Virus isolation by cell culture and RT-PCR testing for influenza viruses are in progress.

Figure 1 Influenza Surveillance at Kamphaeng Phet Provincial Hospital during 2012



Future Plans: A sentinel human influenza surveillance of patients presenting to the Kamphaeng Phet Provincial Hospital will be continued to monitor for evidence of influenza A infection with positive influenza A cases triggering household animal contact investigations. Testing of respratory/cloacal and serum samples at the NIAH laboratory will also be continued.

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

8.1 Hospital-Based Study: As of 31 December 2012, three hundred twenty-six (N = 326) in-patients at the Kamphaeng Phet Provincial Hospital with PCR proven dengue virus infections were screened, briefed, and enrolled as initiate cases. Of the 326 subjects, 167 (51.2%) were male and 159 (48.8%) were female. The subject ages were 2 years 2 months old to 72 years 4 months old. Of the 326 initiate cases enrolled, 77 were DENV-1, 196 were DENV-2, 37 were DENV-3 virus and 16 were DENV-4 virus types. Serology classified the assays as 5 acute primary dengue infection, 4 recent secondary dengue infections, 287 acute secondary dengue/flavivirus infections, 10 were without serologic diagnosis due to availability of only a single specimen (unpaired), and 20 serological result is pending.

8.2 Cluster Investigations: Total of 326 cluster investigations were conducted until 31 December 2012, corresponding to the enrolled initiate numbers. The study GPS team mapped 17,462 houses, 2,918 eligible contacts were briefed, 1,251 associates consented and were enrolled. Of the 1,251 associates who were enrolled, 534(42.7%) were male and 717 (57.3%) were female. The subject ages were 6 months old to 96 years old. Of the 1,251 contacts enrolled during cluster investigations, 33 were PCR proven DENV-1, 67 were DENV-2, 28 were DENV-3, 6 were DENV-4 serotypes and 1,117 were negative. Thirty-nine contact enrollees 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+, 15 were DENV-2, 3 were DENV-3, and 17 were negative. Serologic characterizations included 1 Acute

Dengue Infection, 29 Acute Primary Dengue Infection, 229 Acute Secondary Dengue/Flavivirus Infection, 20 Recent Secondary Dengue Infection, 5 JEV Infection, 884 with No Evidence of Recent Flavivirus Infection, 40 with No Serologic Diagnosis (single specimen) and 43 are pending for EIA and HAI assay completion.

8.3 Vector Studies: Investigators collected 9,430 *Aedes* mosquitoes during the 326 cluster investigations. PCR of 7,950 samples on the female mosquitoes (the sex which transmits dengue) has been completed. Of these, 11 mosquitoes were positive for DENV-1 PCR, 72 were positive for DENV-2, 27 were positive for DENV-3, 3 were positive for DENV-4 and 7,836 were negative.

Future Plans: Complete database check and analysis.

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

Kamphaeng Phet Site: The AFRIMS field station in Kamphaeng Phet, Thailand, supported the conduct of the CYD14 Phase III dengue vaccine trial in collaboration with the Sponsor (Sanofi Pastuer), Thai Ministry of Public Health, and Mahidol University. Subject enrollment was completed in October 2011. Five hundred eighty five subjects were enrolled. The third (final) dose of vaccination for all subjects was completed in October 2012. Active surveillance for febrile episodes continues. At the time of this report there are no results to summarize.

Cebu Site: The AFRIMS field station in Cebu, Philippines, supported the conduct of the CYD14 Phase III dengue vaccine trial in collaboration with the Sponsor (Sanofi Pasteur), Philippines Department of Health, Cebu City Health Department, BioPath Diagnostics, Inc., and Chong Hua Hospital. Subject enrollment was completed in December 2011. Nine hundred thirty four subjects were enrolled. The third (final) dose of vaccination for all subjects was completed in December 2012. Active surveillance for febrile episodes continues. At the time of this report there are no results to summarize.

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

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

In the event of fatal dengue cases at Vicente Sotto Memorial Medical Center, Cebu, Philippines, and provided that consent is obtained from next-of-kin, autopsy specimens are collected for further histopathological, virological, and immunological testing. The study was approved by the Ethics Committee of VSMMC. Autopsy specimens were obtained from two fatal dengue cases in 2012. Initial pathology and histopathology are performed at VSMMC. Further laboratory evaluations are performed at AFRIMS, Bangkok.

Future Plans: Enrollment of fatal dengue cases will continue.

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

Enrollment Phase: There were 1,000 subjects enrolled from March to May 2012: 107 (53%) were male and 94 (47%) were female. Breakdown of age groups were, as follows: 202 (20%) from >6 months to 5 years (20%); 200 (20%) from >5 years to 15 years; 202 (20%) from >15 years to 30 years; 203 (20%) from >30 years to 50 years; 201 (20%) from >50 years. 8 subjects who withdrew from the study during this phase were replaced to maintain the cohort size of 1,000.

Surveillance Phase: There were 221 subjects who had acute fever illness episodes

(June to December 2012).

Total acute fever illness episodes by age group for each corresponding age groups, as follows:

1st Episode:

Age group	Male	Female	Total
6 months to 5 years?	50	38	88
6 years to 15 years?	27	25	52
16 years to 30 years?	8	13	21
31 to 50 years?	10	9	19
51 years and above?	12	4	16
Total	**107**	**89**	**196**

2nd Episode:

Age group	Male	Female	Total
6 months to 5 years?	7	7	14
6 years to 15 years?	3	5	5
16 years to 30 years?	1	0	1
31 to 50 years?	3	0	3
51 years and above?	0	0	0
Total	**14**	**9**	**23**

3rd Episode:

Age group	Male	Female	Total
6 months to 5 years?	2	0	2
6 years to 15 years?	0	0	0
16 years to 30 years?	0	0	0
31 to 50 years?	0	0	0
51 years and above?	0	0	0
Total	**2**	**0**	**2**

Twenty out of 221 were FLU RT-PCR (+). Of the 20 positives: Flu A/H3: 12/20 (60%); Flu B: 6/20 (30%); pdmH1: 1/20 (5%); and Flu A unsubtypable: 1/20 (5%).

Twelve out of 221 were DEN RT-PCR (+). Breakdown as follows: DEN1: 9/12 (75%); DEN2: 2/12 (17%); and DEN3: 1/12 (8%)

Future Plans: Study duration will be for at least 2 years. Follow-up of cohort subjects on a weekly basis to monitor their health status will continue. Annual follow-up of cohort subjects will be initiated in February 2013.

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

This is an ancillary immunological study to the CYD14 dengue vaccine trial being conducted in Cebu, Philippines. The purpose of the ancillary study is to obtain reagents and data from vaccinated subjects in order to conduct humoral and cellular immunity studies with the ultimate goal of elucidating an immunological correlate of protection. A total of 612 subjects who are participating in the CYD14 trial were enrolled into the ancillary study in 2012.

Acute Febrile Episodes: From the start of the study, there were 133 acute febrile

illness episodes reported. 59 cases had 1 acute febrile episode; 32 with 2 acute febrile episodes; 18 with 3 acute febrile episodes; 9 with 4 acute febrile episodes; 7 with 5 acute febrile episodes; 5 with 6 acute febrile episodes; 2 with 7 acute febrile episodes; and 1 with 8 acute febrile episodes.

Future Plans: All subjects will be followed for acute febrile episodes for the duration of the parent CYD14 dengue vaccine trial, and possibly longer.

13. Sentinel Human Influenza Surveillance in Royal Thai Army Recruits

During this quarter, 118 acute respiratory specimens were collected from RTA Barrack camp 3 and 4. All were negative for influenza by PCR. The illness was clustered in the latter half of the quarter, starting November 21. In addition, in camp 3 (one of our two study sites) which consists of 191 new recruits, 29 showed up at the medical unit with fever and rash in December 2012 without history of MMR vaccination. These acute cases were referred to PMK Hospital (tertiary care military hospital) for laboratory diagnostic and treatment. Results revealed 26 with Rubella and 5 with Measles by ELISA; 3 were with both. After this group, 38 more appeared with similar symptoms in the same week.

Results from multiplex-PCR performed to identify cause of respiratory illnesses captured in previous quarters are now available. The information has been publicized in a poster presentation during ICMM Conference in June 2012. The three most common causes (number of specimens) identified are *Haemophilus parahaemolyticus*(44), *Haemophilus influenza* Type B (25) and Rhinovirus (22).

NOTE: Baseline and follow-up blood for serology were also collected.

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