



- Mandatory Command SOPs
- Mandatory Departmental SOPs
- Hands-on Training in the Microneutralization and Hemagglutination Inhibition Assays for the Detection of Neutralizing Antibodies
- Equal Employment Opportunity and Prevention of Sexual Harassment
- Basic Course of Human Subject Research, Collaborative Institutional Training Initiative (CITI)

Initiative (CITI)

- DoD Government Purchase Card Refresher Training
- Annual Counter Intelligence, Operations Security, and Anti-terrorism
- DoD Information Assurance Awareness
- Intro Biostatistics
- Ethics Training
- Level B Training SERE 100 Code of Conduct
- Motorcycle Safety Training
- Management of Durable Property
- Multi-Center Trial Model - Use of a Core Protocol with Site-specific Addenda
- National Disability Employment Awareness Training
- Occupational Health and Safety Communication for Research Animals
- OPSEC & SAEDA Training
- AT Level 1 Awareness Training
- English Language Program
- The Humane Care and Use of Nonhuman Primates
- US Army Medical Research Acquisition Activity Sponsored Contracting/Grant

Officer's Representative

- What is Research? / Informed Consent and Vulnerable Populations / CITI
- Weapons of Mass Destruction Preparedness
- Institutional Animal Care & Use Committee Training

Outside Training

- Second National Vaccine Conference
- The 57th Anniversary Celebration and Annual Scientific Meeting of the Philippine

Association of Military Surgeons Inc

- The 9th Annual Conference International Society for Disease Surveillance
- Applied Infectious Diseases Epidemiology
- Certificate Course in Emerging Infectious Disease Epidemiology
- EPI I: Principles
- MS-Word 2007 Intermediate
- Relational Database Management System (RDBMS)
- Series#1 Applied Regression Methods in Medical Research
- The Science of Small Clinical Trials
- Transport of Biomedical Training Course
- Vaccinology Course
- Virology Association Conferences
- WHO Collaborating Centre for Case Management of Dengue/DHF/DSS
- Workshop on Laboratory Investigation of Influenza Viruses
- Advancing Ethical Research Conference by Public Responsibility in Medicine and

Research (PRM&R)



- Zoonotic Diseases Workshop
- Joint International Tropical Medicine Meeting
- Surveillance Establishment and Preparedness for West Nile Fever Outbreak in Thailand
- USPACOM Disease Surveillance Workshop

AWARDS

None

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 window due to the length of the approval process and 2 subjects were lost to follow up due to relocation). The project was implemented 7 June 2010. From 7 June to 15 November 2010, 53 subjects were enrolled. Of the 53 subjects, 46 have completed all study activities and 7 remain active.

Clinical data has been collected but not analyzed. Samples have been collected but not tested. At the time of this report there are no results to summarize.

Future Plans: Complete clinical activities for the 7 remaining subjects, complete laboratory testing as outlined in the protocol, and analyze and report the data generated

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

Study will involve children who have been admitted with suspected dengue virus infections to the Queen Sirikit National Institute for Child Health (QSNICH), Bangkok, Thailand. The proposed research will extend our past studies of dengue immune-pathogenesis to more severely ill children, with the additional objectives of testing the utility of new non-invasive continuous monitoring devices and identifying positive and negative predictors of disease complications.

Enrollment of subjects was started in August 2010. 55 subjects were enrolled. Follow-up visits (4-5 days after discharge) were completed for subjects that enrolled in CY10. Specimens were collected and archived at AFRIMS.

Laboratory testing is pending. Data will be transferred to appropriate collaborators for data analysis. Data analysis of available data is planned.

Future Plan: Continue to follow up study subjects that were enrolled in CY10. Start to enroll subjects in 2011 dengue season (May-December 2011).

3. A Phase I/II Trial of a Tetravalent Live Attenuated Dengue Vaccine in Flavivirus Antibody Naive Infants

The investigational F17Post-transfection DEN vaccine was safe and well-tolerated when given to a small number of children who were previously vaccinated with two doses of F17 Pre-transfection vaccine at either 1st/10th or full dose. The safety evaluation of the DEN



vaccine (F17Pre) during the approximately 42 months after the primary vaccination, and one year after the booster vaccination with dengue F17Post vaccine, revealed no clinically significant findings. There was no instance of dengue disease of notable severity occurring in children who received the WRAIR/GSK vaccine, although the study cohort was exposed to DEN as evidenced by the 33% incidence of serologically-confirmed DEN infection in the control group during the median 53 month period of surveillance.

Measured neutralizing antibody responses following a primary 2-dose vaccination course decreased over time. A median of 42 months after primary vaccination, 27.6% (95% CI: 12.7%, 47.2%) of the subjects receiving DEN vaccine full dose had a tetravalent DEN neut. antibody profile. A booster dose of F17Post-transfection DEN vaccine elicited a tetravalent response in approximately 79% of the subjects 30 days after administration but one year later, only 45% of the subjects retained a tetravalent profile.

There were no tetravalent antibody profiles in the 1/10 dengue dose group (also boosted with full dose F17Post vaccine) or in the control group. Two of the four subjects who received the 1/10 dose DEN vaccine for primary vaccination followed by the F17Post-transfection DEN vaccine full dose had a tetravalent profile 30 days following booster vaccination, but this did not persist one year later.

The limited response to the administration of a booster dose suggests that most subjects were sufficiently immune 42 months after the completion of a primary vaccination course to restrict replication of the administered (attenuated) vaccine viruses. Whether this level of immunity would restrict replication of wild-type DEN is unknown.

As noted immediately following a 2-dose, JEV vaccination course administered to all subjects during the primary vaccination phase of the Dengue-001 Study, the presence of JEV antibodies at a median of 10.5 months after vaccination was less in the DEN vaccine groups than in the control group. This observation is of uncertain significance, as the group sizes were small. Additional studies will be required to evaluate whether prior administration of live DEN vaccine interferes with subsequent immunization by inactivated JE vaccine.

The study is now closed. Study results from the primary and long-term follow-up phases of the study will be published in the scientific literature. The final clinical study report will be submitted.

4. A Phase I/II, Open, Five-year, Clinical Follow-up Study of Thai Children Who Participated in Dengue-003 ("A Phase I/II Trial of a Tetravalent Live Attenuated Dengue Vaccine in Flavivirus Antibody Naive Children") with Evaluation of a Booster Dose Given One Year after Primary Dengue Vaccination Series

A third dose of the investigational WRAIR/GSK DEN vaccine (F17) appeared to be safe and was acceptably reactogenicity when given to this small group of healthy Thai children who were primed by previous DEN vaccination and JEV vaccination. The third dose boosted neut. antibody titers that had waned over approximately 12 months since the primary series was completed, thus suggesting that immunologic memory had been established despite waning antibodies for perhaps several DEN types. Nevertheless, the boosting effect was transient. There was evidence of subsequent natural boosting between Years 2 and 3, in 3 of 7 subjects.

Despite the apparent natural boosting, most likely reflecting environmental exposure to DEN (or JEV), no subject was hospitalized for suspected or confirmed dengue. Thus in this limited sample, there was no evidence that waning antibody titers increased the risk of severe dengue upon natural challenge in this at-risk population. Although a third dose of the dengue vaccine candidate appears to be well-tolerated, its clinical benefit remains uncertain.



The study is now closed. A clinical study report will be completed and submitted. Manuscripts will be submitted to scientific journals for publication.

5. Phase II, Randomized, Double-blind, Single Center, Controlled Study of the WRAIR Live Attenuated Tetravalent Dengue Vaccine Compared to a Placebo Control, Administered on a 0-6-month Schedule, to Healthy Adults

In flavivirus-primed and -unprimed Thai adults, the TDEN vaccine candidates were well tolerated and elicited high rates of seroconversion and antibody responses following 2 doses of vaccine administered at 0 and 6 months. There was a trend towards a superior safety and immunogenicity profile in one of the TDEN candidates compared to the other. These results support expanded studies of the TDEN candidates in primed and unprimed adults.

Future Plans: All clinical activities are complete. Analyses of all safety and immunogenicity data are complete. Cellular and humoral assays are being completed. The final clinical study report will be submitted to the appropriate regulatory agencies.

6. Japanese Encephalitis Surveillance in Nepal

In this reporting period, there were no samples sent to AFRIMS for testing.

Future Plans: To extend the study to continuation of testing samples and to identify other etiologies of encephalitis in JE negative specimens, data analysis, final study report and manuscripts.

7. Influenza Surveillance in Southeast Asia

AFRIMS works in close collaboration with the U.S. and Thai CDCs, the Thai Ministry of Public Health, 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.

Area	Collection Sites	Flu A/H1	Flu A/SW H1	Flu A/H3	Flu A/Unsubtype	Flu B	Neg	Total
U.S. Embassy	Rangoon						2	
	Beijing					1	6	
	Cairo		7				7	
	Nepal						3	
	New Delhi		2			2	30	
	Jakarta		4			7	9	
	Manila		2	4		2		
	Vientiane						3	
	Thailand		13	4			46	
	Total			28	8		12	106
			18.18%	5.19%		7.79%	68.83%	
Bhutan			104		2	61	216	383
			27.15%		0.52%	15.93%	56.40%	
Nepal			12		1	132	245	390
			3.08%			33.85%	62.82%	
Thailand	Phramongkutkloao Hospital		346	81	7	220	863	1,517
	Kamphaeng Phet Hospital		4	1		1	1	7
	Total		350	82	7	221	864	1,524
			22.97%	5.38%	0.46%	14.50%	56.69%	
Grand Total			494	90	10	426	1,431	2,451

Influenza surveillance in the Philippines: 96 influenza-like illness (ILI) cases were investigated from 5 barangay health center (BHC) sentinel sites, Cebu City. Male to female ratio of the ILI cases was 0.67 and with age range of 6 months to 33 years old. Majority of the ILI cases were from the 6 months to 4 years old age group and 5 to 9 years old age group at 73% and 19%, respectively. Breakdown of the influenza PCR results are as follows: 8 (8%) influenza A/SW H1, 14 (15%) influenza A/H3, 15 (16%) influenza B, 1 (1%) influenza A (un-subtyped), and 57 (60%) were negative. In Metro Manila, 91 ILI cases were gathered from the Armed Forces of the Philippines Medical Center (AFPMC), Quezon City. Male to female ratio of the ILI cases was 1 and with age range of 6 months to 33 years old. Majority of the ILI cases were also from the 6 months to 4 years old age group and 5 to 9 years old age group at 51% and 26%, respectively. Influenza PCR results are as follows: 6 (7%) influenza A/SW H1, 20 (22%) influenza A/H3, 14 (15%) influenza B, 5 (5%) influenza A (un-subtyped), and 46 (51%) were negative. AFRIMS personnel and representatives from AFPMC Department of Research and Training visited Camp Panacan Station Hospital (CPSH) in Davao City and Camp Navarro General Hospital (CNGH) in Zamboanga City for the purpose of expanding influenza collection sites in Mindanao. The expansion of the collection sites was coordinated through the Office of the Surgeon General



(OTSG) and the tri-service medical commands. A total of 39 medical and paramedical personnel from CPSH (21), CNGH (16), and the AFPMC Trauma Center (2) located in Jolo, Sulu were trained in influenza specimen collection and rapid testing. Collection of specimens will start once the ultra low freezers are received by the station hospitals. AFPMC has also given permission to AFRIMS to renovate a space at the ground floor of the Victoriano Luna General Hospital with a floor area of approximately 2,600 square feet for a mixed use collaborative influenza molecular laboratory. Renovation through a private contractor is being coordinated through the U.S. Naval Facilities Engineering Command (NAVFAC). Inauguration of the laboratory will be done by the Philippines Secretary of Defense, AFP Chief of Staff and U.S. Ambassador to the Philippines on 30 March 2011.

8. Sentinel Surveillance for Emerging Diseases Causing Hospitalized Dengue-like Illness in Cebu, Philippines (SEDC)

From January to December 2008, 176 patients were enrolled into the study. There were 14 patients lost to follow-up and 2 patient withdrawals. No Serious Adverse Events (SAEs) were reported. One hundred fifty-four (89%) were laboratory confirmed dengue infections. Demographics of the laboratory confirmed dengue cases are as follows: age range of 2 to 32 years old with majority (96%) with age less than 15 years old; male to female ratio was 1.2:1.0.

Serum samples were sent to AFRIMS, Bangkok for dengue/JE EIA and dengue RT-PCR/ Nested PCR testing. One hundred fifty four (154) out of the 176 patients (87.5%) were confirmed positive for dengue infection. All serotypes were documented to circulate with DEN-3 (76%) predominating. One hundred forty eight (148) of the 154 (96%) confirmed dengue cases were due to acute secondary dengue infection. Clinical diagnosis of these confirmed dengue cases were classified as follows: 5/176 (2.8%) were dengue fever (DF); 47/176 (26.7%) were dengue hemorrhagic fever (DHF) grade I; 75/176 (42.6%) were DHF grade II; and 28/176 (15.9%) as dengue shock syndrome (DSS).

Future Plans: Data analysis and manuscript preparation will be performed.

9. Use of Geographic Information System (GIS) to Establish a Community-based Biosurveillance Infrastructure in Cebu City, Philippines

After cleaning of data and linking of available household data with physical location of polygons representing households, as of 31 December 2009, a total of 9,040 households in Guadalupe and Banawa have been linked to their respective polygons. This represents a total of 41,250 individuals corresponding to a 92% population coverage (as compared to the 2007 population census). Average household size was calculated at 4 members per household. Male to female ratio was reported as 0.93 with majority of the population reported as either single (58%) or legally married (32%). Majority of the population belonged to the 20-29 and 30-39 years old age groups at 20% and 14%, respectively. Projected health facility utilization data of adults and children for a hypothetical fever was elicited from 9,024 households while actual health facility utilization during the past year for a febrile illness was determined from 6,188 households. Out of 8,998 households, 5,840 (65%) indicated that a member of their household had a fever during the past year. The total number of individual household members who reported having had a fever during the past year was 10,583 (26%). Projected health facility utilization for adults when presented with the situation of a hypothetical fever, majority (43%) answered that they would prefer to self-medicate or consult in hospitals unlisted in the choices given, 18% would go to a private clinic, 21% would go either to the Guadalupe Health Center or the Banawa Health Center and 10% would consult at the Chong Hua Hospital Outpatient Department.



Projected health facility utilization if any of the children in the household presented with hypothetical fever, majority (54%) of the adult household members answered that they would prefer to administer antipyretic medicines to their children or consult in hospitals unlisted in the choices given, 19% would go to a private clinic, 17% would either go to the Guadalupe Health Center or Banawa Health Center and 6% would consult the Chong Hua Hospital Outpatient Department. Out of 9,858 households who answered the question whether they plan to transfer or move out of their present residence in the near future, only 211 (2%) answered in the affirmative while 2,128 (22%) were unsure or answered “don’t know.” Majority of households had at least one member with a mobile phone (82%) and 37% of the households have a telephone landline. Ownership of pets or animals was reported at 40% of the households. From 1 July 2009 to 31 May 2010, no additional households were enrolled. Clinically diagnosed dengue cases from the health center and city health office were plotted and maps were generated to reflect disease distribution.

Future Plans: There are plans to integrate the GIS data with the data gathered from other future projects to produce disease incidence maps and assist the local government with data plotting

10. Collaborative Electronic Disease Surveillance Project in the Philippines

10.1 EDE has been proposed to DOH-NEC Manila to be integrated in the Philippines Integrated Disease Surveillance and Response (PIDSRS) program on 16 March 2009. Work with NEC IT personnel is ongoing.

10.2 EDE was modified by JHU/APL to accept text files as input and broaden types of databases with which it will work such as Microsoft Office Access and Fox Pro.

10.3 EDE was demonstrated in Cebu City Epidemiology and Surveillance Unit (CESU) on 18 March 2009.

10.4 The current version of EDE was installed in Cebu City Epidemiology and Surveillance Unit within the Cebu City Health Department on 14 August 2009.

10.5 CDES was demonstrated to the Cebu City Health Department on 18 March 2009.

10.6 CDES program was piloted at Guadalupe Health Center in the first quarter of 2009 until the present. Encoding of patient visits/encounters to CDES program is currently on going to include evaluation and provision of feedback by users.

10.7 CDES introduced electronic data capture to the personnel in the Guadalupe Health Center, a primary care unit in the health system. This new practice will shorten the lag time between patient visit and receipt of visit information at the City Health Department.

10.8 CDES was originally created in Open Office after an initial evaluation in Cebu City; it was suggested to be modified to MS Access based for easy exporting of data in March 2009.

10.9 CDES was created to mimic the logbooks of the Field Health Service Information System (FHSIS) of the Philippines Department of Health, and health programs being used in the health centers.

10.10 SMS texting of fever cases was promoted by the Cebu City Health Office to the 84 city health clinics. So far, over 60 of the clinics have been reporting fever cases electronically. This information is being input into a central database at the City Health Office and being analyzed using EDE.



Future Plans:

1. EDE hopes to strengthen the Philippines Integrated Disease Surveillance and Response (PIDSAR) and the Cebu City Epidemiology Surveillance Unit and to improve its capacity to detect disease trends in the community and to provide early warning for new emerging and re-emerging infectious/severe disease outbreaks.

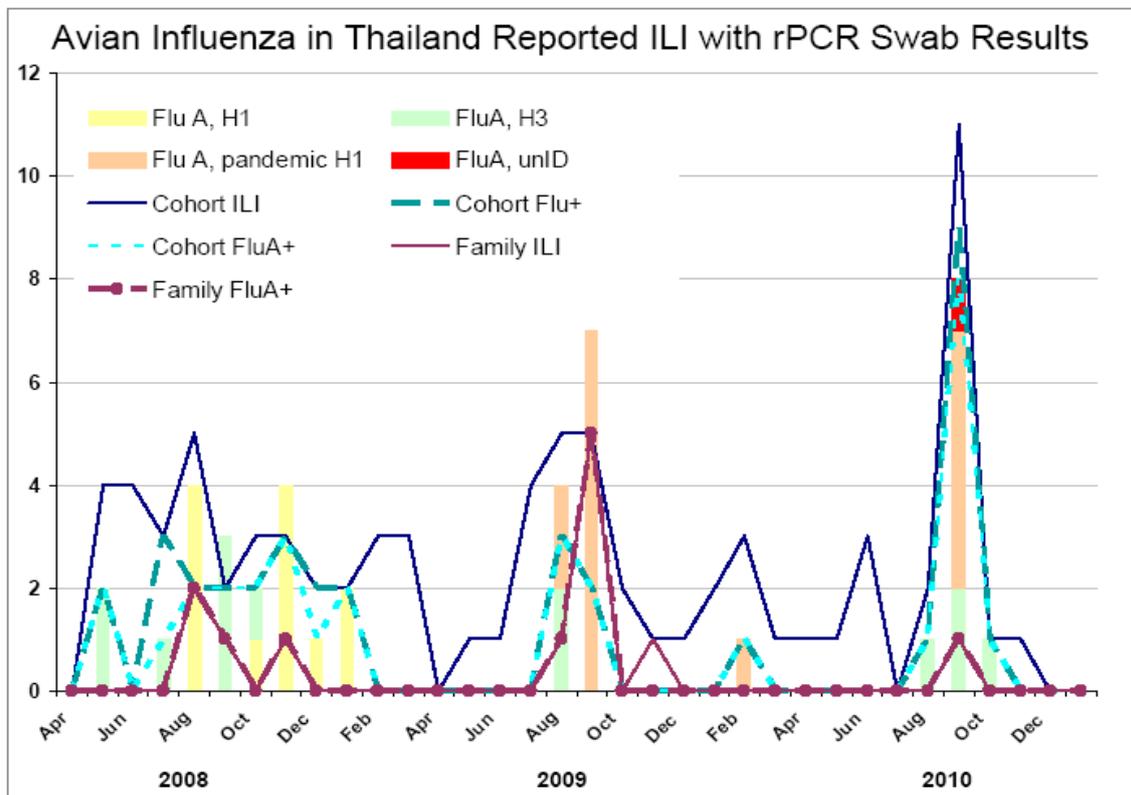
2. The SMS fever surveillance system will continue to be promoted. Data quality and timeliness will be assessed. Practical use of the EDE program for this information will be evaluated.

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

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. As of 31 December 2010, 58 cohort subjects were withdrawn from the study. Twenty-six subjects were withdrawn due to death, eight due to inability to comply with the study protocol, 23 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. 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 were initiated in May 2010 and completed in October 2010.

Human Influenza-Like Illness Investigation and Family Study of Influenza Transmission: During the year 2010, 26 ILI investigations were conducted (Figure 1). Twelve ILI investigations were found to be positive for influenza virus by RT-PCR testing; the remaining 14 were negative for influenza virus. Eleven of the ILI cases were positive for influenza A (seasonal H1 = 0, H3 = 4, novel H1 = 6, unidentified subtype = 1) and one was positive for influenza B. Eleven family transmission studies were conducted; 31 case contact subjects were enrolled. Of the 31 case contact subjects, one had ILI symptoms and was positive for influenza A virus subtype H3 the same as the respective index cohort subjects.

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



Serological Analysis

The annual follow up bleeds that were completed in September 2009 were sent to the Thai NIH for H1 and H3 HI testing and H5 MN testing during the first quarter. Results of the HI testing from the 804 samples are shown in the table below. An HI titer of ≥ 80 and a MN titer of ≥ 10 were considered as evidence of infection. As demonstrated in the table, many cohort subjects showed positive HI titers against pH1N1. These results suggest that a significant proportion of pandemic H1N1 infections may be subclinical in adults.

Virus	Positive (%)	Negative	Pending
Human H1N1 Brisbane	41 (5.37)	763	0
Human H3N2 Brisbane	142 (21.45)	662	0
A/Thailand/102/2009 Swine lineage (H1N1)	88 (12.29)	716	0
Avian H5N1 A/Thailand/384/2006	10 (1.25)	789	5

Virus Culture and Characterization

A total of 23 RT-PCR influenza A positive respiratory samples collected from May 2008 to August 2009 from subjects whose symptoms met the ILI case definition were subsequently cultured. Of these, two respiratory samples were culture negative; the remaining 21 samples were culture positive. Further characterization of these isolates was conducted by indirect immunofluorescence assay (IFA), hemagglutination inhibition assay (HI), RT-PCR, and sequencing. Samples collected in 2008 were subtyped by IFA and those collected in 2009 were subtyped by RT-PCR. The results are shown in the table below.

As determined by HI assay, antigenicity of all H1 isolates from sample collected in 2008 and 2009 is related to A/Brisbane/59/2007-like virus (H1N1). However, antigenicity of H3 isolates from sample collected in 2008 and 2009 is different. The 2008 H3 isolates is related to A/Brisbane/10/2007-like virus (H3N2) whereas the 2009 H3 isolates is related A/Perth/16/2009-like virus (H3N2). Interestingly, based on the nucleotide sequence of the hemagglutinin gene, all 2008 H1 isolates are more closely related to A/South Dakota/6/2007-like virus (H1N1) while the 2009 H1 isolates are more closely related to A/Brisbane/59/2007-like virus (H1N1). Based on the nucleotide sequence of the neuraminidase gene, 9 of 10 of the 2008 H1 isolates and both of the 2009 H1 isolates show the H274Y mutation (oseltamivir resistance) whereas 1 of 5 of the 2008 H3 isolates and none of the 2009 H3 isolate show the H274Y mutation.

In 2009, 2 pdmH1 isolates were cultured and both are related to A/California/07/2009-like virus (H1N1) and A/California/04/2009-like virus (H1N1) as determined by HI assay and the nucleotide sequence of the hemagglutinin gene, respectively. Based on the nucleotide sequence of the neuraminidase gene, none of the isolates show the H274Y mutation.

Year	Number of Isolates	Subtyping (IFA or RT-PCR)	Strain Analysis (HI)	Gene Sequencing	
				Hemeagglutinin Gene	Neuraminidase Gene
2008	9	H1	A/Brisbane/59/2007-like virus (H1N1)	A/South Dakota/6/2007-like virus (H1N1)	H274Y mutation (Oseltamivir Resistance)
2008	1	H1	A/Brisbane/59/2007-like virus (H1N1)	A/South Dakota/6/2007-like virus (H1N1)	Oseltamivir Sensitive
2009	2	H1	A/Brisbane/59/2007-like virus (H1N1)	A/Brisbane/59/2007-like virus (H1N1)	H274Y mutation (Oseltamivir Resistance)
2008	1	H3	A/Brisbane/10/2007-like virus (H3N2)	A/Brisbane/10/2007-like virus (H3N2)	H274Y mutation (Oseltamivir Resistance)
2008	4	H3	A/Brisbane/10/2007-like virus (H3N2)	A/Brisbane/10/2007-like virus (H3N2)	Oseltamivir Sensitive
2009	1	H3	A/Perth/16/2009-like virus (H3N2)	A/Perth/16/2009-like virus (H3N2)	Oseltamivir Sensitive
2009	1	H3	A/Perth/16/2009-like virus (H3N2)	Unamplified DNA	Unamplified DNA
2009	2	pdmH1	A/California/07/2009-like virus (H1N1)	A/California/04/2009-like virus (H1N1)	Oseltamivir Sensitive

Future Plans: Weekly and annual follow-ups, ILI investigation, and Family Study of Influenza Transmission of the cohort subjects will be continued.

12. Prospective Study of Influenza A Transmission from and to Household Animals in Thailand

As of 31 December 2010, animal specimens were collected from pets, livestock, and rodents trapped from ill human cohort subjects' households. Throat or cloacal swabs and blood samples were collected from 5 cats, 32 chickens, 7 cows, 12 dogs, 2 geese, and 9 rodents. The samples were sent to the Thai National Institute of Animal Health laboratory for testing. Virus culture by egg inoculation is in progress.

Future Plans: The human cohort subjects will continue to be monitored for evidence of influenza A infection with positive influenza A cases triggering household animal contact investigations.



13. Dengue Virus Circulation, Evolution, Virus-vector, and Virus-host Interaction in Kamphaeng Phet Province, Thailand

This project is to establish baseline data of wild-type DENV genetic diversity and microevolution in a geographically defined area over time and characterize the impact of mosquito-virus interactions on DENV genetic diversity and microevolution.

13.1 Hospital-Based Study: Ninety-three (N = 93) in-patients at the Kamphaeng Phet Provincial Hospital with PCR proven dengue virus infections were screened, briefed, and enrolled as index cases. Of the 93 subjects, 47 (50.5%) were male and 46(49.5%) were female. The subject ages were 2 years 7 months old to 56 years old. Of the 93 index case subjects enrolled, 15 were DENV-1, 56 were DENV-2, and 22 were DENV-3 virus types. Serology classified the assays as 2 acute primary dengue infection, 3 recent secondary dengue infections, 73 acute secondary dengue infections, and 6 were without serologic diagnosis due to availability of only a single specimen (unpaired); 9 serological result is pending.

13.2 Cluster Investigations: Total of 93 cluster investigations were initiated during the reporting period, corresponding to the number of enrolled index cases. The study team GPS mapped 4,439 houses, 793 eligible contacts were briefed, 438 subjects consented and were enrolled. Of the 438 subjects who were enrolled, 197(45%) were male and 241 (55%) were female. The subject ages were 9 months old to 94 years old. Of the 438 contacts enrolled during cluster investigations, 1 was PCR proven DENV-1, 11 were DENV-2, 19 were DENV-3, and 407 were negative. Eleven eligible contacts reported fever before the convalescent blood collection (between the acute and scheduled convalescent blood draw), a second acute blood sample was collected, of these 1 was DENV-1 PCR+, 3 were DENV-2, 3 were DENV-3, and 4 were negative. Serologic characterizations included 14 acute primary dengue infections, 3 recent secondary dengue infections, 68 acute secondary dengue infections, 1 JE virus infection and 317 without evidence of a recent flavivirus infection; there was no serologic diagnosis available for 25 subjects due to single specimen, and 10 are pending for HAI assay completion.

13.3 Vector Studies: Investigators collected 3,548 *Aedes*. mosquitoes during the 93 cluster investigations. PCR of three thousand-six hundred samples on the female mosquitoes (the sex which transmits dengue) has been completed, of these 5 mosquitoes were DENV-1 PCR+, 36 were positive for DENV-2, 22 were positive for DENV-3 and 3,427 tested negative.

Future Plans: Continue year 3 of the grant (dengue season 2011) and make plans for renewal.

14. Kamphaeng Phet Prospective Dengue Surveillance Study

Study objectives are designed to collect dengue epidemiology (clinical, serologic, virologic) data in a pediatric cohort in KPP. Exploratory objectives are to evaluate influenza epidemiology in the same cohort. An immunology sub-study immunologically profiles the cohort before, during, and after dengue virus infection. All objectives collect information important to understanding dengue virus circulation dynamics and if KPP can be the site of a future dengue vaccine efficacy trial.

14.1 Between 21 May to 3 October 2010; a total of 1,886 subjects (200 subjects for each of age group 1-3 and 1,286 subjects for age group 4) were enrolled. Of the 1,886 active subjects, 978 (51.9%) are male and 908 (41.1%) female. Varicella vaccine provided to 1,305 subjects.

14.2 Surveillance data: 166 subjects reported fever $\geq 38^{\circ}\text{C}$ during the reporting period; 1 DENV-1, 6 DENV-2 and 3 DENV-3 were detected by PCR from 10 subjects. Of 94 positive influenza cases; 35 cases were Flu A (H1N1). Fifteen cases were Flu A (H3), 2 cases were Flu A (unidentified subtype), 35 cases were Flu B and 7 cases were Flu A+ Flu B. Sixty-two (72) illness cases were negative dengue and influenza PCR results.



Future Plans: Complete visit 2 activities as scheduled and continue febrile illness surveillance.

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

USAMRMC has great interest in Sanofi Pasteur's yellow fever-dengue vaccine (CYD) as a potential vaccine for US 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.

At study site: subject enrollment (visit 1) both study sites (Kamphaeng Phet and Cebu) has been completed. Enrollees are being followed weekly by study staff for evidence of dengue-like illnesses.

At AFRIMS: a standard laboratory panel to determine other causes of fever in both acute and convalescent sera for serology testing: leptospirosis, influenza A, typhoid fever, hepatitis A, rickettsias and chikungunya virus is ongoing.

Future Plans: Termination visit (visit 2) will be completed by May 2011. Continued laboratory testing of samples received from each study site will be performed at AFRIMS.

16. 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.

Protocol was submitted to IRBs. IRB approval is pending. Study has not yet been initiated.

Future Plan: Study will be implemented in 3rd quarter 2011.

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