

- Mahidol University Faculty of Veterinary Science, Sai Yoke Campus, served as site for critical reagent program in sheep to develop antiserum for use in Influenza A assays (CTA) (AFRIMS-Enterics and AFRIMS-DVM)
- Toyama Chemical Company, Toyama Japan, AFRIMS-Virology and AFRIMS-DVM engaged to perform work on novel anti-viral compounds for dengue fever virus using a collaborative research and development agreement (CRADA)
- Novartis, WRAIR-ET, and AFRIMS-DVM engaged on work with antimalarial compounds in mice (CRADA)
- The National Science and Technology Development Agency, Thailand, engaged on use of the circumsporozoite protein vaccine model for dengue fever virus (AFRIMS-Immunology and AFRIMS-DVM)

## **FUTURE PLANS AND STRATEGIES**

- Continue to use and improve animal models of malaria in the mouse and monkey for testing new antimalarial drugs.
  - Expand staff to complete malaria mission requirements
  - Adapt further the mouse malaria model to test novel hemoglobin based oxygen carriers being developed by the US Navy.
  - Continue GLP capabilities for preclinical studies. A follow on study is expected for advancing a Shigella vaccine.
  - Continue to develop partnerships for testing vaccines and therapeutics against Dengue Fever in the Rhesus Macaque model.
  - Develop partnerships and collaborations for Influenza A surveillance.
  - Expand zoonotic surveillance mission under the Bio Engagement Program
  - Provide expertise and assistance to support development of a regional primate center in Thailand
  - Continue to support the USAMC-AFRIMS research mission by providing veterinary expertise and animal resources for product testing.
  - Continue to maintain and exceptional animal care and use program and prepare for the 2010 AAALAC site visit.

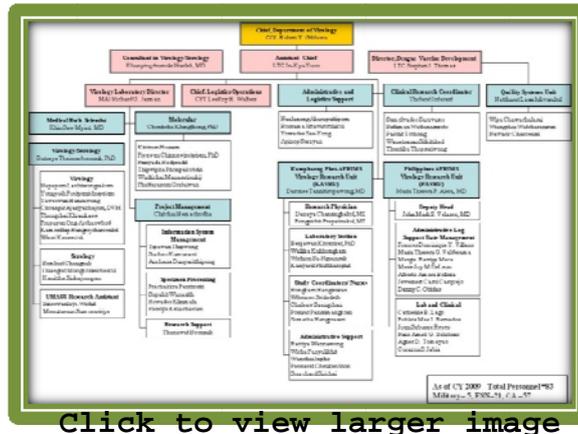
## **DEPARTMENT OF VIROLOGY**

### **DEPARTMENT MISSION**

To develop and evaluate products, and collects epidemic data to protect the Soldier from infectious diseases



## PERSONNEL



## IN-HOUSE TRAINING PROGRAMS AND OUTSIDE TRAINING OF PERSONNEL

### In-House

- AFRIMS Annual Occupational Health, Safety and Biosurety Training
- Good Clinical Practice (GCP) (Refresher), QAU
- Good Clinical Laboratory Practice, QAU
- Monthly Logistics Workshop, Logistics
- Hand Receipt Training, Logistics
- SOP Writing Workshop, QAU
- Equal Employment Opportunity and Prevention of Sexual Harassment, on-line
- Basic Course of Human Subject Research, Collaborative Institutional Training Initiative (CITI), on-line

### Officer

- DoD Government Purchase Card Refresher Training, on-line
- Annual Counter Intelligence, Operations Security, and Anti-Terrorism 2009, Security

### Officer

- Army Wide Network Security Focus (WNSF) Training, on-line
- DoD Information Assurance Awareness, on-line
- Ethics Training, on-line
- Suicide prevention, COL Kent Kester
- GIS training, Uniformed Services University of the Health Sciences
- AT Level 1 Awareness Training (AT/FP), on-line
- Material Transfer Agreement, Mr. Winchester, MRMC
- Publications, CRADA, MTA, MOU, MOA, LOI and Patent Application Approval Process, QAU

- English Language Program
- Hot Weather Training, Self-training
- Religious Accommodation, Self-training
- Sexual Assault Prevention and Response Program Unit Training, Self-training

**Outside**

- E2 Travel Training, U.S. Embassy, Bangkok
- Voucher Examination / FSN Pay and Allowances / Introduction to Grants and Cooperative Agreements (PY-220) / Monitoring Grants and Cooperative Agreements (PY-222) / Principles of Appropriations Law (PA-215) / Accounting I / Travel Regulations for Uniformed Personnel, JFTR Vol. 1, U.S. Department of State RM/GFS, Bangkok
  - Dangerous Goods Awareness with Concentration on preparing, handling & transporting Infectious Substances by Air, World Courier, Bangkok
  - Good Practice for Routine Clinical Laboratory, the Association of Medical Technologists of Thailand, Chiangmai
  - Standard Course in Clinical Trials, Faculty of Medicine, Chulalongkorn University, Bangkok
  - 9th International Advanced Course on Vaccinology in Asia-Pacific Regions, International Vaccine Institute, Seoul, South Korea
  - 9th FERCAP International Conference “Achieving Quality and GCP Compliance at the Clinical Research Site, WHO-TDR Clinical Coordination and Training Center, Pathumthani
  - Community Research forum “Achieving Quality and GCP Compliance at the Clinical Research Site”, Thai Food and Drug Administration (FDA) and Thai FDA foundation, Chiangmai
  - The Twelfth Annual Conference on Vaccine Research, National Foundation of Infectious Diseases, Maryland
  - Good Clinical Practices, Thai Ministry of Public Health, Kamphaeng Phet
  - South East Asia Bio-Engagement Workshop, Washington DC
  - Arbovirus surveillance procedures for international visitors, the Division of Vector-borne Infectious Diseases-Arbovirus Diseases Branch (DVBID-ADB), Denver
  - Microneutralization assay procedure and visit Flow Cytometry Laboratory, WRAIR, Washington DC
  - GIS training, Landsystems representative, Cebu
  - Asian Clinical Tropical Medicine, Mahidol University/Medical College of Georgia, Bangkok
  - Vaccinology course, Ministry of Public Health, Bangkok
  - Annual Study Coordinator and CRA training, Thailand Center of Excellence for Life Sciences and Pharmaceutical Research and Manufacturers Association, Bangkok
  - XI International Symposium on Respiratory Viral Infections, The Macrae Group LLC, Bangkok
  - Certificate in Emerging Infectious Diseases Epidemiology (CEID) Course, University of Iowa, USA
  - The information of the first influenza dynamics and evolutionary analysis (IDEA), NIH, MD
  - Virology Association Conference, Virology Association, Bangkok
  - 58th American Society of Tropical Medicine and Hygiene (ASTMH), Washington DC
  - Joint International Tropical Medicine Meeting (JITMM), Bangkok

**AWARDS**

None

## ACCOMPLISHMENTS

### 1. The Dengue Hemorrhagic Fever Project III: Continued Prospective Observational Studies of Children with Suspected Dengue

Three hundred and fifty six subjects were enrolled during four year period. There were 188 PCR positive and 168 PCR negative cases. Cases by DENV type included: DENV- 1 = 81 (43%); DENV- 2 = 17(9.04%); DENV- 3 = 38 (20.21%); and DENV- 4 = 52 (27.65%). A total of 189 cases were diagnosed as dengue based on clinical and laboratory data. Clinical characterization of dengue cases included; 128 cases (67.7%) of dengue fever, DHF grade I = 13 cases (6.9%); DHF grade II = 36 cases (19%); DHF grade III = 11 cases (5.8%); and DHF grade IV = 1 case (0.5%).

There was one protocol deviation during the reporting period. Volunteer CHD08-016-024 was withdrawn from the study by the PI because the subject did not meet enrollment criteria. There were no serious or unexpected adverse events

#### Future Plans:

In December 2008, after five years, the protocol expired. One new protocol was written to complete long term follow up in 97 study subjects enrolled during year 2006 to 2008. Analyses using acute and short and long term follow up samples include exploring immune and clinical markers of disease severity (NS1 protein/antibody levels, immune activation markers), the role of host versus virus in determining disease severity, characterization of the dengue specific T cell response with regard to the magnitude of T cell expansion during infection, and the functional characteristics of these cells. Measurements of makers of endothelial cell activation are planned.

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

Results from the primary vaccination series (doses #1 and 2) demonstrate the WRAIR/GSK vaccine candidate to be safe and immunogenic. A significant proportion of the cohort develops neutralizing antibodies to all four dengue virus types following two doses of vaccine. Long term follow up had finished and reveals no evidence vaccine recipients have an increased incidence of dengue compared to controls in the period remote from vaccination. There were no overt safety signals in the subset of volunteers receiving a third dose of dengue vaccine remote from the primary vaccination series. Final safety and immunogenicity analyses are underway.

#### Future Plans:

Complete laboratory testing, safety analyses, amend clinical study reports, file for an 18-month protocol extension, and draft scientific manuscripts.

### 3. 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

The enrollment and study follow ups were completed in February 2009. Neutralizing antibody assays are complete. Laboratory testing to characterize cell mediated immune responses are pending. Generating an amendment to the final clinical study report is ongoing. Investigators are planning to publish study results.

In summary, a 3<sup>rd</sup> dose of vaccine administered to a small number of Thai children primed to dengue and JE was well tolerated without any safety signals. Dengue neutralizing antibody responses waned over time. There was a modest boosting with a 3<sup>rd</sup> dose of vaccine but this was short lived with unknown immunologic significance. Year 4 increases in immune responses likely indicate a natural infection boosting effect.

**Future Plans:**

Laboratory testing to fulfill the study objectives and endpoints will be completed. Data analysis will continue. The final study report will be generated and submitted to regulatory agencies. Scientific manuscripts will be drafted.

**4. 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**

A total of 120 volunteers were enrolled and randomized to receive one of two dengue vaccine formulations or placebo in April 2007. One hundred sixteen subjects received dose 2 and complete study visit. There are 4 subjects lost to follow up (3 subjects after 1<sup>st</sup> dose and 1 subject after 2<sup>nd</sup> dose). The final study visit occurred 14 February 2008. There are 3 SAEs reported (1 abdominal hernia, 1 ectopic pregnancy and 1 appendicitis) none of which were dimmed related to study vaccine. There are 2 pregnancy cases reported. There were no volunteers which met clinical or laboratory criteria for suspected dengue and there were no alert lab values. An interim safety analysis performed on cleaned data collected for 31 days after the first study vaccine dose (days 0-30 post dose 1) in the entire cohort (2 vaccine and 1 placebo group). The summary results showed the vaccine was well tolerated in a highly primed adult population. Immunogenicity data continues to be generated.

**Future Plans:**

Cellular and humoral assays are being completed. Data analysis is ongoing. A final study report is planned.

**5. A Phase II, Prospective, Randomized, Double Blind, Placebo Controlled Field Efficacy Trial of a Candidate Hepatitis E Vaccine in Nepal WRAIR# 749, HSRRB Log# A-9117.1**

The clinical phase of the trial has been successfully completed according to the protocol amendment 9. The DSMB unblinded 111 cases of suspected hepatitis following SOP provided by GSK on 23 June 2004. The study results were reported in the HEV Symposium of the American Society of Tropical Medicine and Hygiene (ASTMH) annual meeting in December 2005 as well as in the *New England Journal of Medicine* in March 2007.

The clinical portion of the study was completed in January 2004. A final clinical study report was submitted to the Food and Drug Administration on 21 August 2006.

The vaccine was found to be 96% (86-99) effective (95% CI) among those who received three vaccine doses.

**Future Plans:**

The WRAIR protocol # 749 reached five year term in October 2006. Memorandum requesting extension of this protocol for 18 months was submitted to Office of Research Management (ORM) on 12 January 2007. The study remains open in order to complete the data analysis and manuscript generation pertaining to this study.

The source document in all subject folders at WARUN and the Regulatory Documents at AFRIMS have been scanned and secured. The electronic files have also been secured off-site. Hard copy source documents and regulatory documents at WARUN has been forwarded to Archivist, Division of Regulated Activities and Compliance U.S. Army Medical Research & Material Command, 1452 Campus Drive, Fort Detrick, MD 21702 and received on 29 September 2009.

### 6. Japanese Encephalitis Surveillance in Nepal

In this reporting period, 1874 blinded clinical encephalitis samples were provided by National Public Health Laboratory (NPHL) in Nepal to AFRIMS for JE IgM-capture ELISA. Overall, we detected JE IgM in 11.69 % (219 samples) of the 1874 available samples.

Result of blinded samples tested by AFRIMS JE IgM-capture ELISA assay.

Shipment Date	Specimen	Results		Total
		Positive JE / Borderline	Negative	
10-Dec-08	SERUM	130	615	745
	CSF	29	193	222
	Total	159 (16.44 %)	808 (83.56 %)	967
10-Sep-09	SERUM	44	557	601
	CSF	16	290	306
	Total	60 (6.61 %)	847 (93.38 %)	907
N/A	<b>TOTAL</b>	<b>219 (11.69%)</b>	<b>1,655 (88.31%)</b>	<b>1,874</b>

#### Future Plans:

A request was submitted to extend the study for an additional 18 months to complete the tests required 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 US 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 already 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 will allow immediate processing of influenza samples, and ensure on-time reporting. Construction of a BSL-3 laboratory is well underway at AFRIMS. The 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.

Progress on influenza surveillance in U.S. citizens at U.S. Embassies/Consulates in Asia: subject enrollment started since the protocol was approved by HURC on Feb 2006. There are now a total of 19 medical units/clinics from 14 countries in Asia participating in this study - Thailand (Bangkok, Chiangmai), Burma (Rangoon), Bangladesh (Dhaka), India (New Dehl, Mumbi, Chennai), Pakistan (Islamabad), Mongolia (Mongolia, Ulaanbaatar), Laos (Vientiane), Malaysia (Kuala Lumpur), Sri Lanka (Colombo), Vietnam (Hanoi and Ho Chi Minh City),

Cambodia (Phnom Penh), Nepal (Kathmandu), China (Beijing), and Philippines (Manila). Total number of subjects enrolled since the study started until Dec 09 is 452; majority was from the US Embassy Medical Unit in Bangkok and New Delhi. Rapid diagnostic tests performed on site showed the following: 52 (11.5%) FLU A, 6 (1.3%) FLU B and 7 (1.5%) FLU A+B. Real-Time PCR for Influenza A/B performed at AFRIMS showed the following: 13 (2.9%) FLU A/H1, 55 (12.29%) FLU A/H3, 81 (17.9) FLU A/SW H1 and 18 (4.0%) FLU B. Virus isolation results were available on 211 specimens: 51 (24.1%) Influenza A, 12 (5.7%) Influenza B, 18 (8.5%) pH1N1, 1 (0.7%) Parainfluenza 2, 2 (1.5%) Parainfluenza 3, 1 (0.5%) herpes simplex virus, and no respiratory virus was isolated in 127 (59.9%).

		Real-Time PCR for Influenza A/B (AFRIMS)							
Country Site	Collection Site	Flu A	Flu A/H1	Flu A/H3	Flu A/SW H1	Flu A/Un-subtype	Flu B	Neg	Grand Total
Bangladesh	US EMB Dhaka			5				2	7
Burma	US EMB Rangoon		2				1	4	7
Cambodia	US EMB Phnom Penh			1					1
China	US EMB Beijing			9	14		5	47	75
India	US EMB Mumbai							2	2
	US EMB New Delhi		1	15	30	2	2	83	133
Laos	US EMB Vientiane							3	3
Malaysia	US EMB Malaysia		1	1					2
Pakistan	US EMB Islamabad				1			4	5
Philippines	US EMB Manila		7	7	16		4	43	77
Thailand	US EMB ChiangMai				1			1	2
	US EMB TH	3	2	14	18		6	90	133
Vietnam	US EMB Hanoi	1		2	1				4
	US EMB Ho Chi Minh			1					1
Grand Total		4	13	55	81	2	18	279	452

Progress on influenza surveillance in Nepal: During the period from initiation of the study until 30 November 2009, 2946 subject specimens were received from active sentinel surveillance sites and outbreaks in Nepal of which 2029 subject specimens have been received in this reporting period of 1 December 2008 to 30 November 2009. Rapid diagnostic test showed 1060 (35.9%) FLU A and 68 (2.3%) FLU B positive of the total subject specimens tested till 30 November 2009. Real-time PCR performed at WARUN on 1506 specimens till 30 November 2009 showed 108 (7.2%) as FLU A/SWH1, 7 (0.5%) FLU A/H1, 686 (45.6%) FLU A/H3, 20 (1.3%) FLU A/ Un-subtyped, 19 (1.2%) FLU A/ Sub-type not done and 5 (0.3%) FLUB

Progress on influenza surveillance in the Philippines: 340 influenza-like illness (ILI) cases were investigated from the 5 Barangay Health Center (BHC) sentinel sites, Cebu City. Male to female ratio of the ILI cases was 1.2 and with age range of 6 months to 56 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 58% and 24%, respectively. Breakdown of the influenza PCR results are as follows: 101 (30%) Influenza A/SW H1, 44 (13%) Influenza A/H3, 8 (2%) Influenza B, 6 (2%) Influenza A/H1, 4 (1%) Influenza A (un-subtyped), and 177 (52%) were negative. For those positive for Influenza A/SW H1, majority were in the 6 months to 4 years old (35%) and 5 to 9 years old

(31%) age groups. In Metro Manila, 548 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 1.1 and with age range of 6 months to 73 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 34% and 22%, respectively. Influenza PCR results are as follows: 226 (41%) Influenza A/SW H1, 56 (10%) Influenza A/H3, 7 (1%) Influenza B, 4 (1%) Influenza A (un-subtyped), and 253 (46%) were negative. There was a single specimen each of Flu A and Flu A/H1 + SW H1. For those positive for Influenza A/ SW H1, majority were in the 5 to 9 (19%), 10 to 14 (19%), and 15 to 19 (19%) years old age groups. In addition, the VSMMC-AFRIMS Collaborative laboratory has been identified as a sub-national referral laboratory for testing influenza A/SW H1 and has been qualified by the AFRIMS Virology Quality Systems Unit (VQSU) and the Research Institute of Tropical Medicine (RITM) to perform influenza PCR testing for seasonal flu as well as influenza A/SW H1. The laboratory is also providing assistance to the Philippines Ministry of Health in testing of service specimens for A/SW H1 especially during the peak of the influenza A/SW H1 outbreak in the Philippines which occurred from June to July 2009. AFRIMS was also able to detect the first cases of influenza A/SW H1 among the Philippine military which resulted in creation and rapid implementation of guidelines and SOPs by the military, specifically to address and contain a potential outbreak among military personnel housed within the barracks and military camps.

Progress on influenza surveillance in Thailand: Total number of subjects enrolled from Kamphaeng Phet and Sangkhlaburi since the study started in April 2007 until Dec 2009 was 904. Oseltamivir (O) resistance was assessed at the US CDC on four H1N1 viruses isolated from specimens collected in Nov 2007 in Kamphaeng Phet. One of the four H1 strains was found to be O-resistant.

<i>Count of Study</i>		<i>Real-Time PCR for Influenza A/B (AFRIMS)</i>						
<b>Collection Site</b>	<b>Study</b>	<b>Flu A/H1</b>	<b>Flu A/H3</b>	<b>Flu A/SW H1</b>	<b>Flu A/Un-subtype</b>	<b>Flu B</b>	<b>Neg</b>	<b>Grand Total</b>
KPP Province Hospital	Non-protocol			4			9	13
	Protocol	16	44	71	1	69	270	471
KRCH, Sangkla	Non-protocol		8			2	16	26
	Outbreak		2			88	61	151
	Protocol	41	20	5		11	166	243

Progress on influenza surveillance in Bhutan: AFRIMS has established a relationship with Bhutan Public Health Laboratory and Bhutan Ministry of Health to enhance the country's capabilities to perform influenza and other emerging pathogen surveillance by significantly increasing laboratory and information technology infrastructure and through the provision of training. Influenza-like illness specimens are collected from Thimphu, Punakha and Paro with expansion to Phuentsholing, Gelephu, and Mongar in later phases of the study. Thimphu is the capital city of Bhutan with an estimated population of 80,000, the highest in the country. Paro and Punakha are two districts frequented by tourists and are located close to Thimphu. Three hospitals (Jigme Dorji Wangchuck National Referral Hospital, Thimphu; Punakha Hospital; Paro Hospital) are initially involved in the study.

One thousand eight hundred thirty nine (1839) ILI service specimens were collected from Bhutan from Oct-Dec 2009. Influenza real-time PCR subtyping done at AFRIMS showed the following results: 109 (5.9%) positive for influenza A (H1), 186 (10.1%) positive for influenza A (H3), 167 (9.1%) positive for influenza A (SW H1), and 128 (7%) positive for influenza B. No respiratory virus was detected in 1238 (67.3%) of the specimens tested.

**Future Plans:**

1. To expand surveillance sites in Nepal, Bhutan and the Philippines.
2. To expand surveillance to other countries in the region.
3. Set up BSL-3 facility at AFRIMS.
4. Set up Real-Time RT-PCR in Bhutan.

**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. One hundred forty-four subjects (93%) were diagnosed as acute secondary dengue infection, six (4%) cases were diagnosed as acute primary infection (4%), acute dengue infection in 1 (1%), and 3 (2%) were suggestive of secondary dengue infection. The clinical diagnoses of laboratory-confirmed dengue infections were as follows: DHF gr 2: 69 (45%); DHF gr 1: 43 (28%), DSS (DHF gr 3/gr 4): 33 (21%), DF: 5 (3%), and 4 (3%) systemic viral infection. All 4 serotypes circulated with DEN 3 predominating. Serotype distribution were as follows: DEN 1: 10 (12%); DEN 2: 9 (11%); DEN 3: 65 (76%); DEN4: 1 (1%).

**Future Plans:**

Data analysis is ongoing and manuscripts are being prepared for publication.

**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.

#### **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**

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

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.

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

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.

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

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.

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.

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.

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

#### **Future Plans:**

1) EDE hopes to strengthen the Philippine Integrated Disease Surveillance and Response (PIDSRS) 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) Based on the successful implementation of CDES in Guadalupe Health Center, it will be implemented in additional two (2) health centers in Cebu City, Philippines. CDES manual will be released soon. The current system in place will significantly increase the timely acquisition and transmission of data from the clinics to the City Health Department and to the National Epidemiology Center. Furthermore, when CDES is standardized then, the project can be replicated in other resource-limited settings.

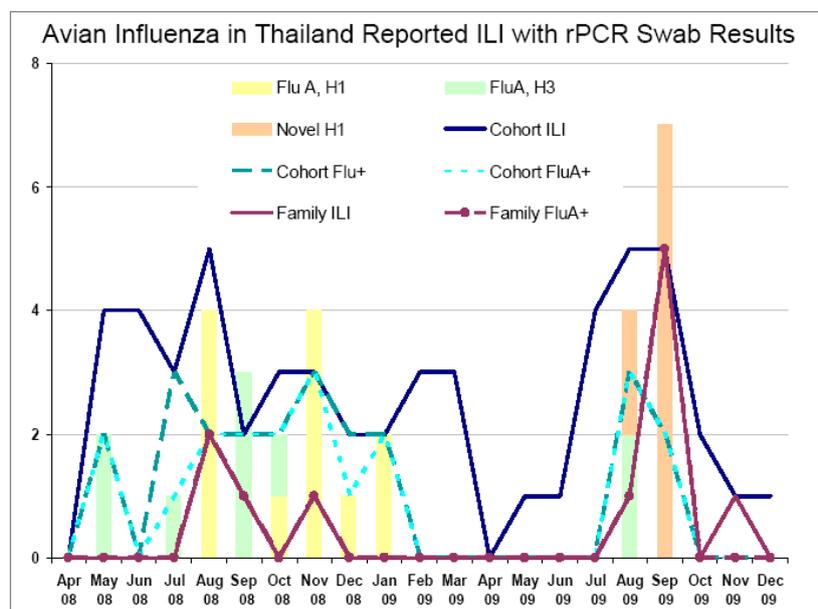
3) The Dengue SMS system is under consideration for expansion; to collect data on other syndromes such as gastrointestinal illness, influenza, etc. and make it as a real time syndromic surveillance system.

## 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 2009, 40 cohort subjects were withdrawn from the study. Twelve subjects were withdrawn due to death, seven due to inability to comply with the study protocol, 20 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 replacement subjects were enrolled from the same villages as the withdrawn subjects to maintain the cohort at 800. Annual follow-up blood draws and questionnaires were initiated on the cohort subjects in April 2009 and completed in September 2009.

**Human Influenza-Like Illness Investigation and Family Study of Influenza Transmission:** During the year 2009, 27 ILI investigations were conducted (Figure 1). Seven ILI investigations were found to be positive for influenza virus by RT-PCR testing; the remaining 20 were negative for influenza virus. All 7 ILI cases were positive for influenza A (seasonal H1=2, H3=5, novel H1=3). Seven family transmission studies were conducted; 24 case contact subjects were enrolled. Of the 24 case contact subjects, 6 had ILI symptoms and all were positive for novel H1 influenza A virus 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

Enrollment sera from 800 cohort subjects were evaluated for antibodies against human seasonal influenza H1 and H3 viruses by hemagglutination inhibition (HI) assay and against avian influenza viruses by microneutralization (MN) assay. An HI titer of  $\geq 80$  and a MN titer of  $\geq 10$  were considered as evidence of respective infection. As shown in the table below, about 4% of the 800 cohort subjects had previous evidence of human influenza H1 infection and 31-68% had previous human influenza H3 infection. Interestingly, 4-6% of subjects also showed serological evidence of previous infection with highly pathogenic avian influenza (HPAI) H5N1 viruses isolated from Thailand in 2005 and 2006. Risk factor analyses for positive serology against the HPAI H5N1 virus using proportional odds modeling are being conducted. Variables examined will include poultry or swine exposure, human H1 and H3 influenza virus exposure, indoor water source, human influenza vaccination, and use of tobacco products. Thus far, no evidence of previous infections with low pathogenic avian influenza (LPAI) viruses (avian H1, H4, H5, H6, H7, H8, H9, H10, H11, and H12) has yet been identified (data not shown).

Virus	Positive (%)	Negative	Not Done
Human H1N1 Brisbane	33 (4.1)	760	1
Human H1N1 New Caledonia	32 (4.0)	765	3
Human H3N2 Panama	250 (31.3)	549	1
Human H3N2 Brisbane	539 (67.6)	258	3
Avian H5N1 A/Thailand/384/2006	28 (3.5)	772	0
Avian H5N1 A/Thailand/676/2005	45 (5.6)	755	0

### Virus culture and characterization

A total of 23 RT-PCR influenza A positive respiratory samples collected during 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), RT-PCR, and HI. By IFA, 10 isolates were found to be of H1 subtype and 5 to be of H3 subtype. By RT-PCR, two isolates were found to be of seasonal H1 subtype, two to be of H3 subtype, and the remaining two to be of novel H1 subtype. All positive isolates had the same subtype as shown by RT-PCR analysis of the specimens prior to virus culture. As determined by HI assay, antigenicity of all H1, H3, and novel H1 isolates was related to that of A/Brisbane/50/2007-like virus (H1N1), of A/Brisbane/10/2007-like virus (H3N2), and of A/California/7/2009-like virus (H1N1), respectively. Further characterization by nucleotide sequence of the hemagglutinin and neuraminidase genes is underway.

### Future Plans:

Weekly and annual follow-ups, ILI investigation, and Family Study of Influenza Transmission of the 800 cohort subjects will be continued.

## 12. Prospective Study of Influenza A Transmission From and To Household Animals in Thailand

As of 30 December 2009, blood, respiratory and cloacal swab samples were collected by Kamphaeng Phet (KPP) Provincial Livestock Office (PLO) personnel from animals (chickens, dogs, and trapped rodents) belong to 5 human cohort subjects who had influenza A infection. Twenty three blood samples were collected from chickens (12), dogs (4), and trapped rodents

(7), 11 respiratory samples (nasal swab or tracheal lavage) from dogs and rodents, and 12 cloacal swab samples from chicken. Virus culture from respiratory and cloacal swab samples was conducted by Thai National Institute of Animal Health laboratory using egg inoculation technique. It was found that all 23 samples were culture negative as determined by hemagglutination assay. Serological analysis from animal specimens is now underway.

#### **Future Plans:**

The 800 human cohort subjects will continue to be monitored for evidence of influenza A infection with positive influenza A cases triggering household animal contact investigations.

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