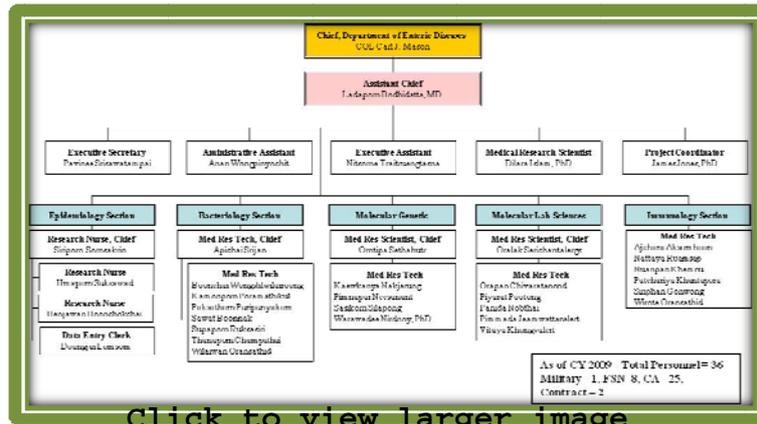


## DEPARTMENT OF ENTERIC DISEASES

### DEPARTMENT MISSION

Develop and evaluate interventions to diagnose, treat, and prevent diarrheal disease.

### PERSONNEL



#### Management & Administration

COL Carl J. Mason – Department Chief  
 Dr. Ladaporn Bodhidatta – Assistant Department Chief  
 Pavinee Srisawatampai – Executive Secretary  
 Anan Wongpinyochit – Administrative Assistant  
 Nitsorne Traitruengtasna – Executive Assistant

#### Epidemiology Section

Siriporn Sornsakrin – Supervisor  
 Umaporn Suksawad – Research Nurse  
 Benjawan Boonchokchai – Research Nurse  
 Duangjai Lumson – Data Entry Clerk

#### Bacteriology Section

Apichai Srijan – Supervisor  
 Sawat Boonnak – Medical Research Technician  
 Boonchai Wongstitwilairoong – Medical Research Technician  
 Paksathorn Puripanyakom – Medical Research Technician  
 Wilawan Oransathid – Medical Research Technician  
 Supaporn Ruksasiri – Medical Research Technician  
 Kamonporn Poramathikul – Medical Research Technician  
 Thanaporn Champathai – Medical Research Technician

#### Molecular Genetic

Orntipa Sethabutr – Supervisor  
 Warawadee Nirdnoy, PhD – Medical Research Technician  
 Pimnapar Neesanant – Medical Research Technician  
 Sasikorn Silapong – Medical Research Technician  
 Kaewkanya Nakjarung – Medical Research Technician

### **Molecular Lab Sciences**

Oralak Serichantalergs – Supervisor  
Orapan Chivaratanond – Medical Research Technician  
Vitaya Khungvalert – Medical Research Technician  
Piyarat Pootong – Medical Research Technician  
Panida Nobthai – Medical Research Technician  
Pimmada Jeamwattanalert – Medical Research Technician

### **Immunology Section**

Dilara Islam, PhD – Research Scientist  
Ajchara Eksomboon – Medical Research Technician  
Nattaya Ruamsap – Medical Research Technician  
Patchariya Khantapura – Medical Research Technician  
Nuanpan Khemnu – Medical Research Technician  
Siriphan Gonwong – Medical Research Technician  
Wirote Oransathid – Medical Research Technician

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

### **In-House Training Provided by Department**

- Laboratory Training for Ministry of Public Health's field epidemiologists (FETP), 12 February 2009
- Laboratory Internships from Department of Biology, Faculty of Science, Srinakharinwirot University, 23 March - 1 May 2009
- Laboratory Internships from Department of Microbiology, Faculty of Science, Chulalongkorn University, 1 April - 29 May 2009
- Diarrheal Disease Internship from John Hopkins University, 15 August - 15 December 2009
- Laboratory Training for collaborators from Hanoi, Vietnam on Testing of Vietnamese Shigella Isolates for Antibiotic Susceptibility Study, 13-26 September 2009
- Laboratory Training for collaborators from, Nairobi, Kenya on Testing of Kenyan Shigella Isolates for Antibiotic Susceptibility Study, 26 September - 10 October 2009

### **Outside Training Received or Provided by Department**

- Laboratory Training for WARUN technicians, 19-24 January 2009, 22-25 July 2009 and 30 March - 3 April 2009
- U.S.-Japan CMSP: 13th International Conference on EID in the Pacific Rim, Kolkatta, India, 6-9 April 2009
- BioNumerics and GelCompar II Training, Brussels, Belgium, 18-24 April 2009
- Training on Sublingual Immunization Technique and Whole Blood ELISPOT Assay at International Vaccine Institute, Seoul, Korea, 2-5 May 2009
- International Research in Infectious Diseases Conference, Washington DC, U.S.A. 12-15 May 2009
- HJF Financial Transition Training, Washington DC, U.S.A., 12-21 May 2009
- ASM 109th General Meeting, Philadelphia, U.S.A., 16-21 May 2009
- FCA - Disease Surveillance Workshop, Vientiane, Laos, 9-10 June 2009

- Comprehensive COTR Workshop, Washington DC, U.S.A., 6-10 July 2009
- Vaccines for Enteric Diseases Conference 2009, Malaga, Spain, 9-12 September 2009
- 58th ASTMH Conference, Washington DC, U.S.A., 18-22 November 2009
- Laboratory training for technicians at Regional Medical Sciences Center, Chiang Rai Province, Thailand, 14-18 December 2009

## AWARDS

Department of Enterics Disease staff received no awards for work at WRAIR in CY2009.

## ACCOMPLISHMENTS

**1. Surveillance of Antimicrobial Resistance of Enteric Pathogens in Indigenous Populations in Multiple Sites within Thailand.** Protocol approval process and field laboratory setup and training were completed. Surveillance was conducted in all 5 sites. Over 1800 stool samples were collected from Bangkok and regional sites. We completed the 2 year enrollment and laboratory assay of specimens from a Bangkok site (Phramongkutklo Military Hospital) in June 2009. Campylobacter and rotavirus were identified as leading causes of acute diarrhea in Bangkok and regional sites, respectively.

**2. Development and Standardization of Realtime PCR Assays for Detection and Characterization of Enteric Pathogens.** Probes and primer sets have been developed and evaluated for Shigella, ETEC, Campylobacter, Cryptosporidia, Cyclospora, Norovirus, Sapovirus, Rotavirus and Astrovirus. Standardized and validated methods were applied to routine detection of pathogens in clinical specimens. During CY2009, over 2,000 frozen clinical stool specimens received from multiple study sites were processed and investigated for Norovirus, Sapovirus, Rotavirus, and Astrovirus infection. Rotavirus and Norovirus were the major pathogens detected by real time PCR. Validated and evaluated assays for ETEC and Shigella spp. freeze-dried (FD) reagents were transferred to JBAIDS platform.

**3. Capsule Genotyping System for *Campylobacter jejuni* and Sequencing of Capsule Locus of *C. jejuni* Type Strain HS O:42.**

3.1 Ten primer sets of capsule genotypes have been tested with 103 strains of *C. jejuni* isolates. Primer sets successfully discriminated most of Thai strains at the level of Penner complexes were primers for HS O:1, O:2, O:3, O:4, O:23/36. The HS O:10, O:53 primer sets correctly identified strains of HS10 and HS 53. Additionally, primer sets for HS O:23/36 and HS O:2 also discriminated additional *C. jejuni* isolates from the same PFGE cluster that shared closed similarities. Relationship of the probes and capsules types needs further evaluation.

3.2 For capsule locus sequencing of type strain HS O:42, primer set for the conserve site of capsule locus (*kpsC-R* and *hddA-L*) or *kpsC-R* and *dmhA-L* could amplified PCR product around 8-9 Kb. TOPO shotgun subcloning kit generated 75 clones. These 75 clones have been submitted for sequencing by Macrogen, Korea. The sequences showed good quality. All 75 sequences were edited, aligned and assemble. Blast search showed this sequence (conserved site) of capsule was similar to HS O:41 type strain from previous publication. Only 4 gaps were generated and 4 PCR primers of these gaps were designed by Dr. Poly at NMRC. PCR of these 4 primer sets could amplify products of 700bp, 600 bp, 500 bp, and 400 bp. These PCR products were sequenced and all sequences assembly can fill to the gap. Sequencing of the conserved part of capsule of HS O: 42 was completed.

**4. Evaluation of Live, Attenuated Oral *Shigella dysenteriae* 1 Vaccine Candidates in Rhesus Monkeys (*Macaca mulatta*) in an Intra-gastric Challenge Model.** The result showed that all 5 vaccine candidates are effective.

**Clinical Outcomes of Immunized Monkeys and Control Monkeys are shown in Table (below):**

<b>Monkey Groups:</b>	<b>Clinical observation: (After immunization)</b>	<b>Clinical observation: (After challenge)</b>
WRSd1	Soft stool (5/6) & vomitus present in the morning (3/6) only after 1st immunization dose	Loose stool with mucous and blood (1/6) Normal (5/6)
WRSd2	Normal	Loose stool with mucous and blood (1/6) Normal (5/6)
WRSd4	Normal	Normal stool with mucous (1/6) Normal (5/6)
WRSd3	Vomitus present in the evening (2/6) after the 1st immunization dose	Loose stool with mucous and blood (1/6) Normal (5/6)
WRSd5	Vomitus present in the evening (2/6) after the 1st immunization dose	All normal
PBS (Controls)	All normal	Loose stool with mucous and blood (9/18) Loose stool with mucous (2/18) Loose stool (2/18) Vomitus present in the morning (1/18) Normal stool with mucous (1/18) Normal (3/18)

The final analysis of the data and results are on-going.

**5. Surveillance of Antimicrobial Resistance of Enteric Pathogens in Indigenous Populations in Nepal.** Surveillance has been completed in all 3 sites. A total of 3600 stool samples have been collected from subjects with and without diarrhea. The leading pathogens significantly identified in children with acute diarrhea were rotavirus (>20%), Enterotoxigenic *E.coli* (ETEC) and *Shigella*. In adults with acute diarrhea, ETEC, *Campylobacter* and *Shigella* were detected as major pathogens.

**6. Establishment of a *Shigella sonnei* Challenge Model for Evaluation of Future Vaccine Candidates.** The first cohort of 12 healthy Thai adults volunteers were challenged with 93 cfu of *S.sonnei* 53G on 10 September 2007. The disease of fever, diarrhea, and/or dysentery was observed in 3 out of 12 volunteers (25%). The adverse events were generally as a result of the challenge. One serious adverse event with an elevation of total bilirubin, which returned to normal during follow up, was reported in one volunteer. Study medical monitors were in agreement that the data from the first cohort did not meet the target attack rate and no safety concern and the second cohort was scheduled. The second cohort of 12 healthy Thai adults volunteers were challenged with 440 cfu of *S.sonnei* 53G on 15 Jan 08. The disease was observed in 6 out of 12 (50%). The adverse events were generally as a result of the challenge. No serious adverse event was observed during the admission and follow up. Since the target attack rate was still not achieved in the second cohort and there was no safety concern, the third cohort of 12 healthy Thai adults volunteers were challenged with 1680 cfu of *S.sonnei* 53G on 02 April 2008. The disease was observed in 9 out of 12 volunteers (75%) and exceeded the target attack rate of 70%. Dysenteric stool was observed in 9 out of 12 volunteers (75%). Excretion of *S. sonnei* in at least one stool was observed in 11 out of 12 volunteers (92%).

Adverse events were generally as a result of the challenge. One serious adverse event was reported in one volunteer who had an increased total bilirubin, classified as grade IV according to CBER's guideline, with no other associated signs and symptoms at Day 14. The total bilirubin level returned to normal without any treatment. Data management conducted by the Center of Excellence for Biomedical and Public Health Informatics, Mahidol University was completed. Data was analyzed. Two manuscripts have been prepared.

**7. Surveillance of Respiratory Pathogens in Patients Attending Royal Thai Army Hospitals.** Of the samples collected at the six sites from a total of 1,797 volunteers, 1,524 samples collected have been influenza negative by on site rapid testing; 273 were positive (221 influenza A & 51 influenza B & 1 both influenza A & B). More comprehensive PCR testing at AFRIMS on an initial 38 samples found 34 negative and 4 positive (4 influenza A/H3). Further testing on these samples is pending.

**8. Safety, Immunogenicity and Efficacy Studies of WRSS1, a Live Attenuated *Shigella sonnei* Vaccine Candidate, in Healthy Thai Adults.** A human use protocol was prepared, submitted for review and approved by AFRIMS Scientific Review Committee. The protocol was submitted for ethical and regulatory review by Faculty of Tropical Medicine IRB, the WRAIR IRB, DHSP, USAMMDA, NIAID (a financial sponsor) and US FDA.

**9. Antimicrobial Drug Combinations to Treat Antimicrobial Agent Resistant Shigellosis in Developing Countries.** Similar to other studies, the prevalence of ESBL-producing *Shigella* was low in this study and no plasmid-mediated class C  $\beta$ -lactamase producing *Shigella* strains were detected. Our findings from this study suggested that mecillinam, an amidinopenicillin, when used alone, appears to act as a poor substrate for certain ESBL-producing *Shigella* detected in this study with all MICs shown in susceptible range. Little potentiation was observed with the addition of clavulanic acid. Real-time and conventional PCR was useful for the molecular typing of extended-spectrum  $\beta$ -lactamases (ESBLs) of *Shigella* spp. Fourteen *Shigella* strains harboring ESBLs as confirmed phenotypically were identified to have one ESBL gene encoding CTX-M-I for *Shigella* strain, or that encoding CTX-M-IV for *Shigella* strains. Three *Shigella* strains were found to carry two ESBL genes encoding TEM and CTX-M-I- $\beta$ -lactamase.

Sequencing analysis of all 17 ESBL genes has confirmed the types of  $\beta$ -lactamase as identified by real-time and conventional PCR. Preliminary data for intracellular study indicates that intracellular bactericidal effect of control antibiotic ciprofloxacin is the best among the tested 15 antimicrobial agents. Based on the obtained data, it seems bactericidal effect of test antimicrobial agents is dependent on host cells indicates combination of antibiotics may work better to kill intracellular *Shigella*, this needs to be explored further.

## FUTURE PLANS AND STRATEGIES

- Transfer evaluated assays for Cryptosporidium and Noroviruses FD reagents to JBAIDS platform in CY2010 and further characterize the genotype of Norovirus/Rotaviruses by PCR and nucleotide sequencing.
- Continue to collaborate with NMRC to evaluate capsule primer sets with other Thai *C. jejuni* strains and continue to sequence the variable part of capsule locus of other type and unknown strains
- GLP study to evaluate and compare 2 best oral live attenuated *S. dysenteriae* vaccine candidates in primates.
- Data analysis and manuscript preparation for the study Surveillance of Antimicrobial Resistance of Enteric Pathogens in Indigenous Populations in Nepal

- Complete manuscripts preparation and submit for publication for the study on Establishment of a *Shigella sonnei* Challenge Model for Evaluation of Future Vaccine Candidates
- Continue Surveillance of Respiratory Pathogens in Patients Attending Royal Thai Army Hospitals
- Safety, Immunogenicity and Efficacy Studies of WRSS1, a Live Attenuated *Shigella sonnei* Vaccine Candidate, in Healthy Thai Adults clinical trial will be implemented and completed in year 2010.

## GLOBAL EMERGING INFECTIOUS DISEASE SURVEILLANCE (GEIS)

### GEIS MISSION

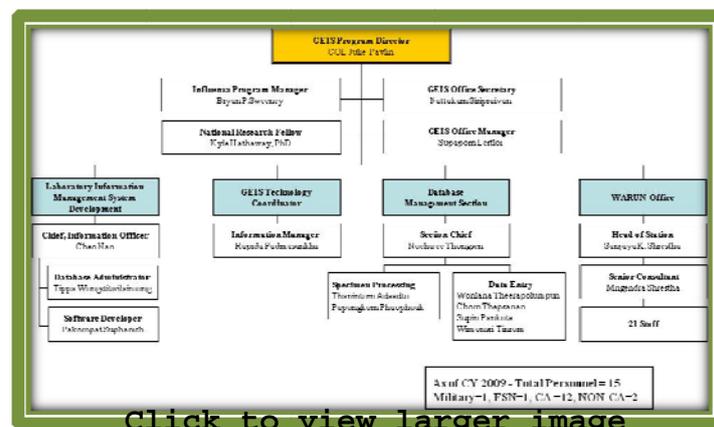
GEIS develops, monitors, and improves surveillance systems/networks throughout South and Southeast Asia to monitor a number of infectious diseases, including influenza, malaria, drug-resistant enteric organisms and febrile illnesses. Technology transfers and training conducted by AFRIMS promotes host nation ownership of these programs, ensuring sustainability. The local infrastructure improvement allows rapid diagnosis of diseases at the local level, improved patient care, and real-time surveillance of infectious diseases previously unavailable. This information is then available to the world health community and to the U.S. Department of Defense, providing improved global health security and force protection.

The pillars of the DoD-GEIS program at AFRIMS support the overall AFRIMS mission. They include:

Surveillance focusing on

- Respiratory illnesses, especially influenza
- Enteric pathogens and antibiotic resistance
- Malaria and antimalarial resistance
- Etiologies of acute febrile illnesses
- Infectious disease outbreak response
- Training and capacity building

### PERSONNEL



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