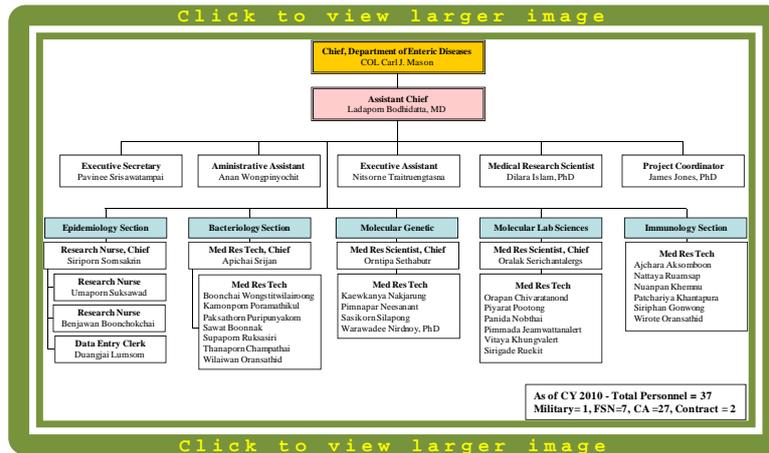


DEPARTMENT OF ENTERIC DISEASES

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 (Study coordinator)
 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 Puripunyakom – 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
 Sirigade Ruekit – 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 Infectious Diseases Fellows from Siriraj Hospital, Rajavithi Hospital, Chulalongkorn Hospital, Queen Sirikit National Institute of Child Health, Phramongkutklo Hospital, Maharaj Nakorn Chiang Mai, Ramathibodi Hospital. 11 March 2010.
- Laboratory Internships from Department of Biology, Faculty of Science, Srinakharinwirot University. 1 April-14 May 2010.
- Laboratory Training for collaborators from Kathmandu, Nepal on Norovirus PCR, *E. coli* PCR and ELISA diagnostic techniques. 30 August-2 September 2010.
- Laboratory Training for collaborators from Hanoi, Vietnam on hemagglutination inhibition assay for Avian Influenza (H5N1). 27-30 July 2010, 3-6 August 2010, 31 August-3 September 2010.

Outside Training Received or Provided by Department

- Laboratory Training for WARUN Technicians. 22-26 February 2010, 7-9 June 2010, 16-20 August 2010, 13-17 December 2010.
- International Research in Infectious Diseases Conference. Washington DC, U.S.A. 18-20 May 2010.
- American Society of Microbiology, 110th General Meeting. San Diego, U.S.A. 21-28 May 2010.
- Keystone Symposia, Vaccine Mechanisms. Seattle, U.S.A. 27 October-1 November 2010.



- 59th American Society of Tropical Medicine and Hygiene Conference. Atlanta, U.S.A. 1-5 November 2010.

AWARDS

Department of Enteric Diseases staff received no awards for work at WRAIR in CY2010.

ACCOMPLISHMENTS

1. Surveillance of Antimicrobial Resistance of Enteric Pathogens in Indigenous Populations in Multiple Sites within Thailand: A 2-year prospective surveillance of diarrhea etiology was completed. Over 4,000 stool samples were collected from diarrhea cases and non-diarrhea controls in Bangkok and 4 regional sites (Chiangrai, Phitsanulok, Nakon Ratchasima, Surat Thani). Completion of rotavirus and norovirus genotyping is pending. Rotavirus and *Campylobacter* were identified as leading causes of acute diarrhea in this project.

2. Development and Standardization of Real-time PCR Assays for Detection and Characterization of Enteric Pathogens: Probes and primer sets have been developed and evaluated for enteric pathogens: *Shigella*, ETEC, *Campylobacter*, Cryptosporidia, Cyclospora, Norovirus, Sapovirus, Rotavirus and Astrovirus. Standardized and validated methods were applied to a routine detection of pathogens in clinical specimens. During CY2010, 3,500 frozen clinical stool specimens received from multiple study sites in Thailand and Nepal were processed and investigated for Noro-virus, Sapovirus, Rotavirus and Astrovirus infection. Rotavirus and Norovirus were the major enteric pathogens detected by real time PCR. Sapovirus and Astrovirus were also considered important. For transition to JBAIDS platform, Cryptosporidium wet reagents optimization were completed and Cryptosporidium freeze-dried reagents were formulated.

3. Capsule Genotyping System for *Campylobacter jejuni* and Sequencing of Capsule Locus of *C. jejuni* type strain HS O:42: The multiplex PCR of capsular genotyping demonstrated that 350/469 (75%) of *C. jejuni* isolates had known capsular genotypes while 119 *C. jejuni* isolates in the study remained untypable. Six common major capsular genotypes detected by multiplex PCR were O:2(16.6%); O:17(10.4%), O:3(8.9%); O:4 complex (7.9%), O:5(6.1%), and O:4(6.1%) which accounted for 56% of all isolates in this study. The distribution of these major serotypes were similar in *C. jejuni* isolates from Thailand, Cambodia, and Nepal. The capsular genotypes O:6 was detected more frequently in Nepal, whereas capsular genotypes O:9/37 and O:15 were detected only in Thailand. *C. jejuni* isolates from Cambodia has shown similar distribution to Thailand which O:2, O:4 complex and O:5 were detected as three major capsular genotypes. The 119 untypable *C. jejuni* isolates were submitted for Penner serotypes by passive agglutination assay at the Stanten Serum Institute, Denmark. One hundred six isolates can be typed by passive agglutination assay and two common Penner serotypes are predominate as O:5 (37/119) and O:37(11/119). Serotype O:5 is one of the most predominant serotype in Thailand, and thus should be included into the capsular genotypic panel.

4. Evaluation of Live, Attenuated Oral *Shigella dysenteriae* 1 Vaccine Candidates in Rhesus Monkeys (*Macaca mulatta*) in an Intra-gastric Challenge Model: WRSd3 and WRSd5 vaccine candidates are characterized by using:

- Slide agglutination test with commercially available *S. dysenteriae* 1-specific sera to confirm identity (IAW SOP# ETR-BT-011). *S. dysenteriae* 1 (vaccine candidates or wild type) will agglutinate with *S. dysenteriae* 1-specific sera.



- Invasion assay in HEp-2 cells IAW SOP# ETR-IM-018. The invasion assay will evaluate the ability of the vaccine strains to invade epithelial cells. Vaccine strain(s) will be added to HEp-2 cells in duplicate wells and incubated at $37\pm 1^{\circ}\text{C}$, $5\pm 1\%$ CO_2 , $95\pm 5\%$ humidity in a CO_2 incubator for 90-120 min then wash. Gentamicin will be added to the cultures and incubated at $37\pm 1^{\circ}\text{C}$, $5\pm 1\%$ CO_2 , $95\pm 5\%$ humidity in a CO_2 incubator for 90-120 min. After removal of gentamicin, the HEp-2 cells will be lysed using Triton X-100 at RT for 10-15 min and the intracellular bacteria will be enumerated by growing on tryptic soy agar plate. The number of viable bacteria on the plate(s) will be counted and expressed as the CFU. Invasiveness of the test strain is expressed as a percentage of the internalized bacteria by the input bacteria, used to infect HEp-2 cells.

- PCR assay utilizing multiple primers, to confirm the presence or absence of genes. Conventional PCR assays were performed for evaluation of gene deletion in live, *Shigella* vaccine strains. DNA template were prepared from TA *Shigella* strains using heat lysis method (IAW SOP# ETR-ML-001). The lysate was subjected to PCR amplification followed by gel electrophoresis to detect the presence of amplified products (IAW SOP # ETR-ML-007).

- Stability Assay: Strains will be sub-cultured on CRA plates, consecutively three times to show stability (SOP# ETR-IM-020). MCB strains will be streaked on 4 CRA plates after dilution and will be incubated for 16-20 h at $37\pm 1^{\circ}\text{C}$ and then 5 single well-isolated colonies from each plate will be sub-cultured on 20 CRA plates. After growth for 16-20 h at $37\pm 1^{\circ}\text{C}$, 1 single well-isolated colony from each plate will be sub-cultured on 20 CRA plates. Each colony morphology (red colony: virulent and white colony: avirulent) will be recorded to measure stability. The strain will be accepted as stable if $>80\%$ of single well-isolated colonies are virulent.

5. Surveillance of Antimicrobial Resistance of Enteric Pathogens in Indigenous Populations in Nepal: Surveillance completed in all 3 sites. A total of 3,600 stool samples were collected from subjects with and without diarrhea. The leading pathogens significantly identified in children with acute diarrhea were rotavirus, enteroaggregative *E.coli* (EAEC) and norovirus. In adults with acute diarrhea, *Vibrio*, ETEC, and *Campylobacter* were detected as major pathogens. Completion of rotavirus and norovirus genotyping is pending.

6. Establishment of a *Shigella sonnei* Challenge Model for Evaluation of Future Vaccine Candidates: This study was completed. The 75% attack rate of clinical disease was achieved in the third group of volunteers challenged with 1600 cfu of *S. sonnei* strain 53G. The information on the 1600 cfu challenge dose from this study was applied in the evaluation of WRSS1 vaccine efficacy and probably will be used to test the other next generation vaccine candidates against *S. sonnei* in Thailand. Manuscripts are being submitted for publication.

7. Surveillance of Respiratory Pathogens in Patients Attending Royal Thai Army Hospitals: Of the samples collected at the eight sites from a total of 2,657 volunteers, 2,186 samples collected have been influenza negative by on site rapid testing; 471 were positive (345 influenza A & 113 influenza B & 13 both influenza A & B). More comprehensive PCR testing at AFRIMS on 2,355 samples found 1,793 negative and 562 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 safety, immunogenicity, and efficacy trial of WRSS1, an oral live attenuated *Shigella sonnei* vaccine, in Thai adults was conducted at the Vaccine Trial Centre of the Mahidol University Faculty of Tropical Medicine. The protocol entitled "Safety, Immunogenicity and Efficacy Studies of WRSS1, a Live Attenuated *Shigella*



sonnei Vaccine Candidate, in Healthy Thai Adults” and other regulatory documents were reviewed by relevant IRBs (Faculty of Tropical Medicine IRB and WRAIR IRB, DHSP), USAMMDA, NIAID (a financial sponsor) and U.S. FDA.

The first part, a double-blind, placebo control study for WRSS1 vaccine safety and immunogenicity evaluation, was conducted on 31 May 2010. One volunteer had diarrhea with mucous stools in the night of study day 1 (30 May 2010). Thus, he was withdrawn from the study on study day 0 before vaccination.

A total of 19 volunteers orally received bicarbonate buffer solution prior to receiving either a single dose of 10^4 CFU of WRSS1 vaccine or placebo. The actual post-administration inoculum count was 1.49×10^4 CFU/ml. No immediately side effect was observed. Nine of 13 (69%) vaccinees and 0 of 6 placebo recipients had shedding of WRSS1 vaccine strain. One of 13 (7.69%) vaccinees and 2 of 6 (33.33%) placebo recipients had abdominal pain. Two of 13 (15.38%) vaccinees and 2 of 6 (33.33%) placebo recipients had abnormal stools but did not yet meet diarrhea definitions. One of 13 (7.69%) vaccinees had nausea. All 19 subjects completed their admission in the VTC facility and were treated with 3 days of ciprofloxacin as scheduled on study day 8. All subjects were symptom free, completed antibiotic treatment, culture negative and no abnormalities detected before discharge on study day 11. All subjects returned for out-patient follow up visit on study day 14, 28 and 60. No unexpected and serious adverse event was reported during admission and follow up.

As per protocol, the randomization code was broken and clinical, laboratory and safety data was submitted and reviewed by DSMB and medical monitor. The DSMB convened a meeting on 23 June 2010 and approved the continuation of study to the second part (efficacy testing).

The second part, WRSS1 vaccine efficacy evaluation, was conducted on 28 July 2010 (approximately 60 days after vaccination). A total of 20 volunteers, 10 previous vaccinees and 10 naïve controls, were orally challenged with 1600 CFU of a wild type *Shigella sonnei* strain 53G after bicarbonate buffer solution. The actual post-challenge inoculum count was 1710 cfu. During the course of admission, 6 of 10 vaccinees and 7 of 10 naïve controls had shedding of *S. sonnei* 53G in at least one of their stool samples.

All 20 subjects completed their admission in the VTC facility and were treated with 3 days course of ciprofloxacin as scheduled on study day 5. All were symptom free, completed antibiotic treatment, culture negative and no abnormalities detected before discharge on study day 8. All returned for out-patient follow up visit on study day 14, 28 and 60. One subject missed a window period (+/-2 days) of a follow-up visit on study day 28 for 3 days due to work commitment abroad. No unexpected and serious adverse event was reported during admission and follow up.

Clinical and laboratory data were transcribed to CRFs and faxed to Data Management Unit (BIOPHICS, Mahidol University, Bangkok, Thailand). Data has been entered in the database and checked for accuracy. The database will be locked once it is completed and data analysis will be performed.

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 β -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 β -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 one *Shigella* strain, or that encoding CTX-M-IV for ten *Shigella* strains. Three *Shigella* strains were found to carry two ESBL genes encoding TEM and CTX-M-I- β -lactamase. Sequencing analysis of all 17 ESBL genes has confirmed the types of β -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

- Further characterize the genotype of Norovirus, Rotaviruses and Astrovirus by PCR and/or nucleotide sequencing.
- Assay validation of *Cryptosporidium* freeze-dried reagents is expected to be completed in March 2011.
- Norovirus assay transition to JBAIDS platform will be continued.
- Continue to collaborate with NMRC to evaluate capsule genotypes of *C. jejuni* isolates by using new primer sets, especially in serotypes O:5 and O:9/37. Primer sets for these serotypes will be included in the multiplex PCR panel and re-evaluated with *C. jejuni* isolates at AFRIMS.
- *C. jejuni* type strain O:31 will be sent from NMRC to AFRIMS. Sequencing of capsule locus will be amplified, sequenced, and analyze by long-PCR reaction and short gun cloning.
- Study of cross reactivity of human sera of diarrhea patients with *Campylobacter* infection to common capsular antigen extracts in this geographical area will be initiated in CY 2011.
- Continue further analysis of *Shigella* strains harboring ESBLs to confirm activity of these beta-lactamases and roles of plasmid on spreading of β -lactam antibiotic resistance.
- Surveillance of antimicrobial resistance of enteric pathogens in Bhutan will be implemented in CY11 in collaboration with Ministry of Health, Bhutan.
- Study of epidemiology of travelers diarrhea and post-infectious sequence is planned for implementation in CY11 at Bumrungrad Hospital.
- Continue evaluation of higher dose (10^5) and multiple doses of WRSS1 vaccine is planned.
- Continue studies on pathogen discovery with UCSF and Washington University.