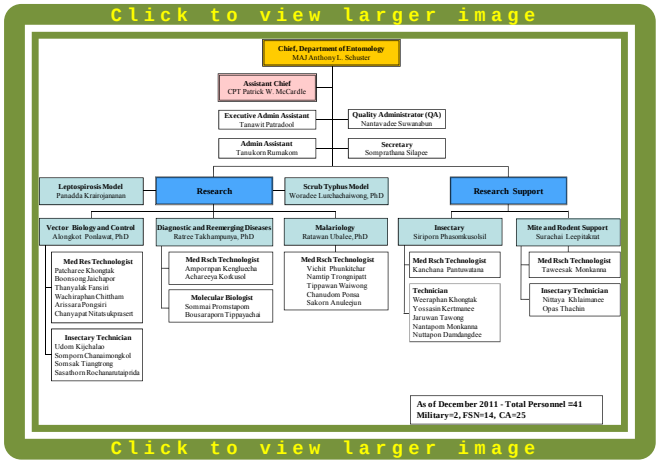
DEPARTMENT OF ENTOMOLOGY

DEPARTMENT MISSION

To **identify** vector- and rodent-borne disease threats of military and global health importance and to **develop and evaluate interventions and products** in order to mitigate those threats.

PERSONNEL



IN-HOUSE TRAINING PROGRAMS AND OUTSIDE TRAINING OF PERSONNEL

In-House Training Programs and Outside Training of Personnel

- Accident, Illness and Complaint Reporting, Records and Investigations
- Animal Bites and Other Injuries/Reports
- Animal BSL-3 Hazard Training
- B-Virus Post-exposure Intervention and Prophylaxis
- Bio-safety in Laboratory
- Blood-borne Pathogens Exposure Control Plan
- BSL-3 Laboratory Training Program
- Computer Security Awareness
- Composite Risk Management
- Chemical Safety
- Counter Intelligence, Operations Security, and Anti-terrorism
- Equal Employment Opportunity and Prevention of Sexual Harassment (EEO & POSH)
- Ethics Training
- Fire Prevention, Protection, Report and Investigation
- Fire Fighting Training
- Human Subject Protection Program
- The Humane Care and Use of Lab Animals
- Hazard Communication Program
- IACUC Training
- Laboratory Waste Management

- Occupational Health Program & Communication
- Post-exposure Prophylaxis and Interventions
- Respiratory Protection Program
- Routine Prophylaxis and Screening and Post-exposure Prophylaxis and Intervention
- Staff Training on Command SOPs, Protocols, Departmental SOPs
- Safety Equipment Usage
- Emerging Infectious Disease Research, University of Florida, May 2011
- APMEN GIS Training Course, Shanghai, PR China, November 2011

Outside Training Provided by Department

- Dengue Prevention Consortium, Ministry of Public Health, Bangkok, Thailand
- Malaria Vector Identification Workshop, Ministry of Public Health, SuratThani, Thailand
- Mosquito Surveillance and Identification Workshop, Siem Reap , Cambodia
- TALAS Annual Training

RESEARCH AREAS

Vector Biology and Control: Develops and evaluates mosquito surveillance traps. Evaluates control or preventive measures including insecticide barrier treatments, insecticide-impregnated bed nets and tents, and personal-use and spatial repellents. Studies are aimed at understanding basic vector biology and behavior through vector competence and oviposition assays. Taxonomic keys are continually developed and updated for the mosquitoes of Thailand. Apply Geographic Information Systems (GIS)-based tools to evaluate the risk of vector-borne disease outbreaks. This capability incorporates environmental factors and field collection data (i.e., vector/pathogen distributions) in order to delineate vector-borne disease (i.e., dengue, chikungunya, Japanese encephalitis, malaria) risk areas.

Diagnostic and Reemerging Diseases: Develops and evaluates conventional and rapid field assays for the detection of emerging/re-emerging pathogens. Conducts cutting edge bio-informatics and molecular research on arthropod-borne disease vectors (i.e., molecular identification of mites) and pathogens (*Leptospira*, *O. tsutsugamushi*, *Bartonella* spp., *Plasmodium* spp., Chikungunya virus, and dengue viruses) and on relationships among the pathogen, vector/ reservoir and host.

Malariology: Conducts research on malaria biology and transmission. On-going research includes studies on malaria parasite development in mosquitoes, erythrocyte and liver cells. Develops *in vitro* systems for the screening of antimalarial compounds against exoerythrocytic, gametocytic and sporogonic stages of malaria parasites. Studies are also focused on gametocytogenesis in *Plasmodium falciparum* and the production of different stages of human and animal malaria parasites in order to support drug and vaccine development and transmission blocking strategies.

Leptospirosis Model: Conducts research on leptospirosis pathogen biology and host-vector relationships. Evaluate environmental factors that affect transmission and maintenance of pathogen across diverse geographical differences. Correlate vector, reservoir, and host genotypes across divergent geographical areas with a focus on phylogenetic relationships.

Scrub Typhus Model: Conducts research on scrub typhus epidemiology within vectors, resevoirs and hosts. Establish and maintain colonies of Leptotrobidium mites to conduct vector competency and vaccine challenge studies. Develop repeatable standardized chigger-mouse

model to further investigate transmission studies. Develop chigger-nonhuman primate model to more closely mimic human reactions to chigger feeding and pathogen transmission in order to support drug and vaccine development and transmission blocking strategies.

Insectary: Produce world class superior quality standard mosquitoes for malaria, dengue, and Japanese Encephalitis research. Rears and researches biology of 5 endemic Thai malaria vectors for transmission studies in support of internal Veterinary Medicine, Entomology and Immunology transmission blocking, vaccine, and prophylaxtic compound challenges.

Mite and Rodent: Maintain endemic scrub typhus chigger colonies for biological studies and ongoing transmission studies. Provide infected and non-infected chigger mites to collaborative laboraties investigating pahthophysiological effects of mite feeding.

ACCOMPLISHMENTS

1. **Title: Field Evaluation of Surveillance Devices for Dengue and Malaria Vectors**
   Funding Source: MIDRP U

Project Summary: This study evaluated the BG-Sentinel Trap (BGS-Trap) to improve collection of dengue and malaria vectors in Thailand. The efficacy of the BGS-Trap baited with different attractants was compared with the CDC backpack aspirator and CDC light trap, the gold standard methods for capturing *Ae. aegypti* and malaria vectors, respectively. Trap evaluations against *Ae. aegypti* were conducted in Ban Non Hua Chang located in Muang district, Lopburi province. Results demonstrated that the BGS with BG-Lure and CO_2 and the BGS baited with CO_2 collected significantly more *Ae. aegypti* females than the CDC backpack aspirator ($P < 0.05$). The BGS trap (with BG-Lure and CO_2) augmented with either a blue or yellow infrared light stick or without a light stick collected significantly more *Ae. aegypti* females than the CDC back-pack aspirator ($P < 0.05$). We conclude that the BGS trap is a potentially effective surveillance tool for dengue vectors in Thailand when baited with CO_2 and the BG-Lure or baited with CO_2 only. For the trap evaluation against anopheline mosquitoes, experiments were conducted in Ban KhunHuay, Mae Sot district, Tak province. The CDC light trap baited with both the BG-Lure and CO_2 is most effective at collecting Anopheline mosquitoes, while the lowest number was captured with a BGS Trap complemented with a BG-Lure ($P < 0.05$). CDC light traps with light sources collected significantly more Anopheline females than the BGS Trap ($P < 0.05$). We conclude that the BGS trap is not appropriate for use as surveillance tool for Anopheline mosquitoes but the CDC light trap (with BG-Lure and CO_2) with an incandescent bulb is effective at collecting malaria vectors in Thailand.

CY12 Plan: Continue evaluations of BG-Sentinel traps complemented with different attractants which would presumably render the traps to be significantly more effective.

2. **Title: Dengue Vaccine and Viral Evolution in Rural Thailand**
   Funding Source: NIH RO1 (Multi-center collaboration)

Project Summary: We collected adult mosquitoes within a 200 m radius of patients infected with dengue during cluster investigation to determine if genetic diversity of dengue viruses changes. In 2011, 110 clusters were investigated (more than 10,000 houses in 34 villages have been mapped). Immature mosquitoes were collected from the field and transported to the AFRIMS Entomology Lab, where they were allowed to emerge as adults. Genetic diversity among adult mosquitoes was determined and vector competence similarly was assessed at the AFRIMS lab. The answer to this specific aim will be determined by comparing results from dengue virus genetic analyses and vector competence assays to the genetic identity of mosquitoes.

A minimum of two DENV serotypes will be studied, which serotypes will depend on those most frequently recovered in cluster investigations. These activities are to support a larger effort to determine the evolution of the dengue virus in response to a vaccine.

CY12 Plan: Supportive efforts will continue thru the next 4 years.

3. Title: Chikungunya Virus (CHIKV) Surveillance in Vector Mosquitoes in Thailand

Funding Source: GEIS

Project Summary: Rayong and SuratThani provinces were selected as the study sites for this study based on the CHIKV cases reported to Thai MOPH. Mosquitoes were collected from two sites in which disease incidence was different (low and high CHIKV cases) in each study area. *Ae. albopictus* and *Ae. aegypti* colonies were established from all four study sites and F2 populations of field mosquitoes were used in vector competence assays using an oral infection technique. Passages of CHIKV isolates were propagated by the Virology Department. The vector competence of these two mosquito species populations will be determined by comparing the efficiency of virus spread and transmission of the old strain, the Asian strain of CHIKV endemic to Thailand for decades, with the newly imported variant virus which has been associated with the major outbreaks since 2005. The vector competence experiments are currently being performed in the BSL-3 laboratory.

Ecological niche modeling of two vectors of chikungunya in Thailand has been performed. Mosquito data was collected from AFRIMS' projects and from mosquitomap.org website for generation of the model. Climate data of all environmental variables and elevation data were used for this model. After MaxEnt software was run, we found that the data for *Ae. aegypti* and *Ae. albopictus* in Thailand needed to be reformatted and re-sampled. Since we had used data not designed for modeling, the sampling appeared to be clustered which had a very negative effect on the model. We had tried sampling on a 30 kilometer grid. However, this left one small area in Thailand with extremely high probabilities of occurrence. Therefore we need to use another program to clean up the samples.

We currently have clinic-level CHIKV data from 4 study sites which have different incidence. We have a mosaic of Landsat as Geocover Land Cover which has 30 meter resolution and land cover data from Land Development Department as well. The data with these resolutions will allow us to examine and calculate the area inside of 4 study sites for different land cover classes to test the associations between land cover types especially percent of rubber tree plantations and incidence of chikungunya in these areas.

We completed the first vector competence experiment and discontinued study as no GEIS funding was provided for continuation of this project.

CY12 Plan: N/A

4. Title: Genetic Basis of Natural Compatibility between Dengue Viruses and Mosquito Vectors

Funding Source: ANR, France

Project Summary: The present project constitutes a complementary approach to an existing collaborative project involving several research groups in the U.S.A. and in Thailand. In the present project, we want to gain a better understanding of vector-mediated constraints to arboviral emergence and evolution by investigating the genetic basis of natural vector-virus interactions between *Ae. aegypti* and dengue viruses.

CY12 Plan: Continue the vector competence study.

5. Title: Development and Evaluation of Novel Push-Pull Strategies for the Control of Disease Relevant Mosquito Species

Funding Source: MIDRP U

Project Summary:

1) Outdoor mosquito enclosures: Three large mosquito enclosures (LME) (16.8 m long x 12 m wide x 5.5 m high) were constructed in a dengue-endemic area at Nongpling sub-district, Muang district, Kamphaeng Phet province (KPP) (Fig. 1). A water drainage system was installed and connected to the enclosures. Each LME is covered with polyester netting thereby allowing the free entry of both wind and precipitation and creating climatic conditions within the enclosures that mimic ambient conditions. The roof of each LME is covered with thick white polyethylene plastic to afford some degree of protection from intense seasonal rains. Entrance into the large screened cage is through a double layer door system to prevent escape of released mosquitoes from within the cage.

2) Outdoor mosquito enclosure calibration: Calibrations were conducted in order to demonstrate that mosquitoes show relatively equal preference for the same trap model located at 4 corners of each LME. Procedures were performed using commercially-available surveillance traps that have shown efficacy in collecting the mosquitoes species used in our study. BG-Sentinel traps with BG-Lure and CO_2 were used and operated according to the manufacturer's instructions to collect *Ae. aegypti*, while CDC light traps baited with dry ice were used for *An. minimus* collection. Each LME was divided into four equal sections (each 8.4 x 6 m). Four traps of the same type were randomly placed at the center of each section. Two hundred host seeking females (4-7 day-old) and two hundred males were released into the cage at its midpoint. *Aedes aegypti* were released for 3 hrs from 7-10 am, while *An. minimus* were released for 13 hrs from 6 pm - 7 am. Traps were switched on before mosquitoes were released at the midpoint of the LME. Four replicates were performed with each trap being rotated clockwise after each replicate. Collected mosquitoes were sorted by sex and counted. Each LME was voided of mosquitoes after each replicate using traps and CDC backpack aspirators.

Figure 1 Large mosquito enclosures (LME) (16.8 x 12.0 x 5.5 m) constructed in Nongpling subdistrict, Muang District, Kamphaeng Phet Province, Thailand

3) Indoor and outdoor preferences of mosquitoes within LMEs: Both vector species used in this study are different in their host seeking behavior. Female *Ae. aegypti* seek their hosts during the day time. They also prefer resting inside of structures where humans dwell whereas *An. minimus* normally live outside seek human or animal hosts during the night. An experiment was performed to determine whether semi-field conditions within LMEs affect the normal behavior of these two mosquito species. Grass and potted foliage plants were grown in each LME to represent the outdoor environment inside the LME. A PVC-coated polyester tent (3.0 x 3.0 x 2.10 m with its roof 2.65 at the apex) with mesh-net door and 3 windows were positioned 3 meters from the center of the each LME (Fig. 2) to mimic a human dwelling. BG-sentinel traps with BG-lure and CO_2 and CDC light traps with CO_2 were used in this experiment. *Ae. aegypti* were collected in the morning (0700-1000 hrs, while *An. minimus* collection was performed at night time (0600-0700 hrs). One trap was place inside the tent and the other one was placed outside the tent (6 m from the tent). A plastic cup containing 400 mosquitoes (200 males, 200 females) was placed at the midpoint of the LME. Each mosquito cup was remotely opened from the door after traps had already become operational.

Figure 2 A PVC-coated polyester tent (3 x 3 x 2.1 m) with mesh-net door and 3 windows (60 x 60 cm) placed inside each LME.

4) Attractive bait "Pull" evaluations: Three candidates of "Pull" devices including ProvectorTM BT (MEVLABS, Inc. U.S.A.), Dry ice and the BG-Lure were evaluated against *Ae. aegypti*. For *An. minimus*, NettyTMwith EntobacD (MEVLABS, Inc. U.S.A.), Dry ice, and the BG-Lure were tested. ProvectorTM BT consists of a durable plastic flower with a replacement bait pad containing the new formulation of *Bacillus thuringiensis israelensis* (Bti) called Entobac. The NettyTM device is the lethal insecticide bait that consists of a piece of paper with a color pattern on one side and EntobacD pesticide (a combination of Bti and deltamethrin) on the opposite side that can rapidly kill mosquitoes. Pull device evaluations using cubic sticky trap were carried out under the non human-bait condition.

5) Evaluation of the "Pull" devices versus the "Push-Pull" devices: The BG-sentinel trap baited with BG-lure and CO_2 and a CDC light trap baited with CO_2 and lure were used as the "Pull" devices for *Ae. aegypti* and *An. minimus* collection, respectively. Metofluthrin impregnated net (MIN) (SumiOne®) was evaluated in this experiment as the "Push" device. We evaluated the efficacy of the "Pull" devices and the "Push-Pull" devices, and compared with the control (without devices) against *Ae. aegypti* and *An. minimus*. One hundred host-seeking females (4-7 d-old) were remotely released from the midpoint of each LME. *Ae. aegypti* mosquitoes were released for 2 hrs (0700-0900 hrs while *An. minimus* experiments were released for 13 hrs (1730-0630 hrs). Doors and windows of the tents were remained opened throughout these periods. After the operation time period, mosquitoes inside the tent in each LME were captured and counted. Devices were rotated counterclockwise after each collection time which comprised one replicate of the study. Six replicates of each mosquito species were conducted.

6) Evaluation of the "Push" devices versus the "Push-Pull" devices: We evaluated the efficacy of the "Push" devices and the "Push-Pull" devices, and compared with the control (without devices) against *Ae. aegypti* and *An. minimus*.

CY12 Plan: Complete all experiments in large mosquito enclosures.

6. Title: Development of a Rapid Diagnostic Assay for the Detection of Chikungunya Virus (CHIKV) in Human Serum

Funding Source: MIDRP L

Project Summary: Rapid and accurate diagnosis of Chikungunya virus (CHIKV) infection remains a critical goal for deployed populations particularly since CHIKV symptoms are similar to many other febrile-like illnesses. Our goal was to develop a rapid (less than 2 hours) point-of-care CHIKV assay which employed an easy and rapid RNA extraction method and the use of loop-mediated isothermal amplification (LAMP) to increase the sensitivity and specificity values that are comparable to existing diagnostic methods and significantly reduces the amount of time required to obtain a clinical diagnosis relative to established methods. Several RNA extraction methods were compared; viral RNA minikit (Qiagent, U.S.A.), Dynabeads® Streptavidin kit (Invitrogen, U.S.A.), Dynabeads® SILANE viral NA kit (Invitrogen, U.S.A.). The LAMP technique was used to amplify and detect CHIKV RNA using new designed LAMP primers. Sensitivity and specificity were assessed using archived human serum and the cross-reactivity with other viruses was also tested. A simple viral RNA extraction procedure using Dynabeads® SILANE viral NA kit (Invitrogen, U.S.A.) produced the detectable signals as strong as the RNA extracted using the Qiagen kit and the time required for this extraction was as low as 25-30 mins. The new LAMP primers were designed from NSP1 and NSP3 regions. The LAMP primers were optimized and validated with clinical specimens received from Virology Department, AFRIMS. Our results showed that the NSP1 LAMP primers set produced higher sensitivity than NSP3 primers and the reference E1 LAMP primer (Parida, MM. 2007). NSP1 primers could detect the CHIKV virus as low as 1.0 PFU/ml while E1 primer can only detect 10.0 PFU/ml.

CY12 Plan: N/A

7. Title: Rodent-Borne Surveillance in Thailand Focusing on Leptospirosis, Scrub Typhus, Rickettsial Disease, and Bartonellosis.

Funding Source: GEIS

Project Summary: To better understand the epidemiology of leptospirosis, scrub typhus, and bartonellosis we are conducting systematic sampling of rodents from the Northeast (high incidence of leptospirosis) and Central regions (low incidence of leptospirosis) during

the dry and wet seasons. Conduct monthly rodent collections in the Northeast and the Central regions in Thailand and process and detect pathogens in animal tissues and ectoparasite specimens by real-time PCR technique. Analyze and compare results of rodent and ectoparasite collections during the wet and dry seasons. Our expectation was that there would be a high incidence of *Leptospira*-positive rodents in the Northeast region relative to the Central region as numbers of human cases in the former region are historically and currently much greater relative to the Central region. Our results from Sing Buri proved this hypothesis to be false based on the surveillance conducted in the wet/dry seasons (2010 and 2011) and in the wet season 2011. However, we are assuming that the quality of medical surveillance is the same across all of the provinces and that the human case numbers from the Ministry of Public Health Thailand (MoPH) are real and accurate. Indeed such an assumption may be false. In Thailand, leptospirosis is a reportable disease that is most commonly observed during the rainy season. However, leptospirosis is also often misdiagnosed in Thailand as symptoms typically resemble those of other diseases such as dengue, scrub typhus, and malaria. An overview of the national surveillance system for leptospirosis in Thailand conducted by the Centers for Disease Control and Prevention found that there was no clear case definition for the disease, laboratory confirmation was infrequently used, and there was an inability to link clinical, epidemiologic, and laboratory data. One study found that the accuracy of clinical diagnosis varied from 0% to 50% among 700 suspected cases across 9 provinces in Thailand. Only 143 of the 700 cases (20%) were confirmed by laboratory testing. Most of the confirmed cases were found in the north and northeast regions of Thailand. It is highly probable that the medical surveillance system currently in place in Sing Buri is giving little or no consideration to leptospirosis as a cause of disease or perhaps leptospirosis is mistaken for other febrile diseases. We plan to determine the pathogenicity of the *Leptospira* discovered in Sing Buri and present the Ministry of Public Health with a report of our findings. Indeed, our results highlight that the medical surveillance system currently in place may be mistaking cases of leptospirosis as something other than leptospirosis. This could have serious implications with regard to the Thai people receiving appropriate medical care (i.e. antibiotics) in a timely manner.

CY12 Plan: Sampling will continue in the Northeast and Central regions (wet and dry seasons). Moreover, we are planning to collect rodents in flooded areas by focusing on leptospirosis.

8. Title: Development of a Molecular Taxonomic Key for the Identification of Scrub Typhus Vectors from the PACOM Region

Funding Source: MIDRP U

Project Summary: Develop a molecular taxonomic key to identify chigger specimens collected from Thailand. Each morphologically-identified species can be associated with DNA barcode (COI gene, 12-16S rDNA). Chiggers were collected from wild-caught rodents trapped from different parts of Thailand. Some chiggers were subjected to DNA extraction individually. Since we planned to collect DNA barcode information as well as information on morphological characteristics, some chiggers were reared and maintained in our laboratory. These chiggers were the representative chigger isolates/species from each part of the country. Their F1 progeny were divided into 2 groups. One group was subjected to molecular ID by sequencing and another was slide-mounted. The mounted slides will be sent to Walter Reed Biosystematics Unit (WRBU) for morphological ID or for documentation of key characteristics. Additionally, sequence analysis of these chiggers will reveal the extent of geographical diversity of chigger species from throughout Thailand. The PCR amplification and DNA sequencing of 16S-12S rRNA were performed in parallel with the COI gene. The sequence analysis was done for 20 field chiggers along with

references from GenBank and our colonies. The evolutionary relationship among field chiggers and the reference sequences was analyzed from the full-length COI gene (amino acid and nucleotide sequences), 16S rRNA, and 12S rRNA sequences. Based on the tree topology generated from full-length COI gene (nucleotide and amino acid), 12S rRNA, and 16S rRNA, the field chiggers were divided into 7 groups. Sequence similarity showed that group 6 is most similar to *L. deliense*, group 7 is closed to *L. imphalum* and the remaining field chiggers are separated into 5 groups with high bootstrap support for trees constructed from full-length COI nucleotide and 16S rRNA sequences. Comparison of multiple DNA sequences and morphological ID would enable us to adjust and develop the more accurate/rapid molecular assay for chigger species identification in the near future. Our plan to expand this capability to other countries would be accelerated by the success of this study performed on chiggers in Thailand.

CY12 Plan: Expand our effort to other regions (i.e., Korea, Japan).

9. Title: Detection of *Plasmodium vivax* Using Rolling Circle Amplification Assay in the Liver Stage Culture

Funding Source: ILIR

Project Summary: This project applied the rolling circle amplification (RCA) technique toward the establishment of a novel tool to detect the development of *P. vivax* liver-stage parasites. Since little is known regarding biomarkers for the *P. vivax* liver-stage or hypnozoite form, the immunofluorescene staining technique using antibodies against some parasitic proteins is not currently a feasible option. The *in situ* RCA assay is a viable method for future research involving *P. vivax* liver-stage biology. We established the *in vitro* and *in situ* RCA assays for malaria parasite detection in patient blood and in liver cells, respectively. C-probes were designed to specifically detect *P. vivax* (Pv c-probe) or *Plasmodium* spp. (universal c-probe) in *in situ* RCA assay. The *in vitro* RCA assay was successfully optimized using parasitic DNA as a positive control. The fluorescent signal obtained from the RCA assay was significantly higher relative to the signal resulting from the real-time PCR method (9 versus 0.35). However, the RCA assay required more time and additional steps than the real-time PCR assay. Therefore, the RCA technique might not be suitable for rapid detection of the malaria parasite. The *in situ* RCA assay for the detection of the malaria parasite in infected cells was performed with a blood smear (thick film) collected from a *P. vivax*-infected patient and with *P. berghei*-infected HepG2 cells collected at 48 hrs post-infection. The *in situ* condition worked exceptionally well with *P. berghei*-infected HepG2 cells using the universal probe. *P. vivax* in an infected blood smear generated a strong signal (green, Alexa Fluor®488) from *in situ* RCA assay using specific Pv c-probe. The *in situ* RCA assay was evaluated in *P. vivax*-infected HepG2-A16 cells and it showed positive signal obtained from invasion experiment at 3 days post-infection.

CY12 Plan: N/A

10. Continued Development and Validation of a Diagnostic Assay for Detection of the Etiologic Agent of Leptospirosis from Clinical Samples Using the JBAIDS

Funding Source: MIDRP L

Project Summary: Continued development and validation of a diagnostic assay for detection of the etiologic agent of leptospirosis from clinical samples. Second Target PCR Assay and Internal Positive Control were developed. We successfully completed the design of a pathogenic *Leptospira* spp. second target assay (16s rRNA gene). The assay was down selected from three candidates of primer/probe sets. The optimization and testing of the 16s rRNA second target assay in the wet reagent format was successful. The limit of detection was established at 100 fg of DNA concentration. The LOD was estimated at ≤ 100 fg or ≤ 20

genome equivalent (ge) based on linear regression analyses results. The assay was proven to have high sensitivity and specificity in testing using a broad panel of diverse *Leptospira* spp. and relevant cross-reactivity test organisms. The Joint Project office JBAIDS Molecular Assay Transition Package was submitted to the JPO/JBAIDS project manager on 10 MAY 2011 - DTO CB.56 Molecular Assay Transition Package; Report Number: Ento_AFRIMS_Lepto/JBAIDS_ MIDRP-PAL_FY10_2011May10. A generic JBAIDS IPC is in development by Idaho Technology Inc. The ITI IPC will undergo beta test under the Lepto/JBAIDS FY12 MIDRP project.

CY12 Plan: Manuscript completed - McAvin *et al*. A field-expedient method for sensitive and specific detection of leptospirosis causative agents in rodents.

11. Mosquito-Borne Arbovirus Surveillance in Vector Populations in Support of Pathogen Discovery, Disease Risk Assessments, Modeling, and Predictive Analysis in the Pacific Region

Funding Source: GEIS

Project summary: in CY 11, we evaluated over 2,000 pools of mosquitoes from Korea for flavivirus detection as part of arbovirus surveillance throughout Korea. Results were provided to Korea.

CY12 Plan: N/A

12. Title: Performance of *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) Transmission Blocking Assays in Support of Transmission Blocking Vaccine Studies

Funding Source: NIH

Project Summary: To evaluate blocking efficacy of sera samples taken from humans/ animals administered with a candidate vaccine. The immune sera samples are mixed with malaria infected blood samples and fed to the laboratory-rear *An. dirus* mosquitoes. The engourged mosquitoes are maintained. Approximately 7-10 days, 5-10% of the mosquitoes are checked for the presence or absence of oocysts. The efficacy of the immune sera inhibiting parasite development is evaluated by determining the numbers of the oocysts in the mosquito midgut compare to those of the mosquitoes fed with non-immune sera.

CY12 Plan: Continue to support efforts to evaluate blocking efficacy of candidate vaccines.

13. Title: Screening of Antimalarial Compounds in Mice and Monkeys for Blood and Liver Stage Activities

Funding Source: MIDRP

Project Summary: To support Department of Veterinary Medicine, we provide *Plasmodium cynomolgi* (monkey malaria) and *P. berghei* (mouse malaria) infected mosquitoes and live sporozoites harvested from salivary glands for the screening of antiamalrial compounds. *An. dirus* mosquitoes fed on *P. cynomolgi* infected monkeys or *P. berghei* infected mice will be maintained at Entomology Department. Approximately 6-9 days or 9-11 days, 5-10% of the mosquitoes infected with *P. cynomolgi* or *P. berghei* are checked for the presence or absence of oocysts respectively. Approximately 12-14 days or 19-21 days, the sporozoites will be harvested from infected salivary glands and provided to Department of Veterinary Medicine for monkey or mice injection. The malaria infected animals are used for blood schizontocidal activity and causal prophylactic activity screening.

CY12 Plan: Continue to provide *P. cynomolgi* and *P. berghei* sporozoites to support several drug and vaccine development studies.

14. Title: Monitoring Drug Efficacy and Susceptibility of *Plasmodium vivax* to Chloroquine in Western Thailand.

Funding Source: GEIS

Project Summary: This surveillance is a prospective evaluation of the clinical and parasitological response to directly observed treatment for *P. vivax* malaria and evaluation of the susceptibility of the parasite to chloroquine *in vitro*. Individuals with *P. vivax* positive blood film examination and meeting appropriate criteria will be enrolled. The patients will be treated with chloroquine following a standard regimen and monitored for a period of 28 days. In collaboration with the Bureau of Vector-Borne Diseases, Department of Disease Control, Thai Ministry of Public Health (MOPH), the clinical study and follow-up will be conducted by Thai MOPH staff. The follow-up will consist of a fixed schedule of check-up visits and corresponding clinical and laboratory examinations. Patients will be classified as therapeutic failures (early or late) or adequate responders based on the results of these assessments. Patients experiencing a therapeutic failure during the follow-up period will be used to estimate the efficacy of the drug. Polymerase chain reaction (PCR) will be used to determine a true recrudescence due to treatment failure or re-infection. For drug susceptibility testing, *P. vivax* infected blood from patients will be collected prior to treatment for *in vitro* drug susceptibility assay. The *P. vivax*-infected blood will be incubated with different concentrations of the drug for 48 hours. Susceptibility to the drug will be determined from schizont maturation inhibition efficacy.

CY12 Plan: The human use protocol has been being prepared and will be submitted to the Thai Ministry of Public Health (MoPH) and the WRAIR Commander within February 2012. Collect blood samples from *P. vivax* infected patients and evaluate the susceptibility of the parasite to chloroquine *in vitro*. Monitor the therapeutic efficacy of chloroquine for the treatment of *P. vivax* infected patients.

15. Title: Maintain Entomological Support for the Malaria Challenge Model

Funding Source: MIDRP F

Project Summary: We provided mosquitoes with consistent, reproducible salivary gland infections to support *Plasmodium vivax* sporozoite challenge studies; provide live *P. vivax* sporozoite-infected mosquitoes and/or harvested, purified *P. vivax* sporozoites (on wet or dry-ice) to WRAIR/NMRI investigators or collaborating institutions; and provide slides of blood and exo-erythrocytic stages of *P. vivax* parasites for vaccine studies. We collect blood samples from patients after blood smears are confirmed by Ministry of Public Health (MOPH) microscopists as malaria-positive samples. As part of an approved Human Use Protocol, Department of Entomology personnel are allowed to draw 20 ml of a patient's blood to feed mosquitoes using a membrane feeding technique. Studies are conducted weekly at Mae Kasa and Mae Sot malaria clinics. Aliquots of each blood sample are spotted on filter paper and smeared on glass slides. Confirmation of parasite species is accomplished by PCR of filtered blood and by microscopic examination of blood smears. Infected mosquitoes are returned to AFRIMS and maintained in the AFRIMS insectary. Five to 10% of mosquitoes from each mosquito feed are checked for the presence or absence of oocysts approximately 7-10 days after infection. These mosquitoes are thereafter available for use in malaria sporozoite challenge studies. In October 2010, we provided *Plasmodium vivax* -infected mosquitoes for the first-ever human challenge study performed by the Department of Defense involving a candidate malaria vaccine.

CY12 Plan: Project Pending.

16. Title: Surveillance of Pathogenic *Leptospira* Strains in Rodent and Human Populations Responsible for the Observed Trends in Human Cases of Leptospirosis in Northeast and Central Thailand and GIS/Modeling for Determination of Transmission Risk Factors.

Funding Source: GEIS

Project Summary: Responsible for establishing a niche capability (over the long-term) to conduct research on the molecular epidemiology of leptospirosis in Thailand and throughout the region. In the near-term, collaborate with partners within Thailand and at Brooke Army Medical Center in San Antonio to address epidemiological questions revolving around the transmission of this disease. Research will serve to better identify risk factors associated with this disease and means of prevention. Our expectation was that there would be a high incidence of *Leptospira*-positive rodents in the Northeast region relative to the Central region as numbers of human cases in the former region are historically and currently much greater relative to the Central region. Our results from the Central region proved this hypothesis to be false based on the surveillance conducted in the wet/dry seasons (2010 and 2011) and in the wet season 2011. However, we are assuming that the quality of medical surveillance is the same across all of the provinces and that the human case numbers from the Ministry of Public Health Thailand (MoPH) are real and accurate. Indeed such an assumption may be false. In Thailand, leptospirosis is a reportable disease that is most commonly observed during the rainy season. However, leptospirosis is also often misdiagnosed in Thailand as symptoms typically resemble those of other diseases such as dengue, scrub typhus, and malaria. An overview of the national surveillance system for leptospirosis in Thailand conducted by the Centers for Disease Control and Prevention found that there was no clear case definition for the disease, laboratory confirmation was infrequently used, and there was an inability to link clinical, epidemiologic, and laboratory data.

CY12 Plan: We plan to conduct molecular epidemiological investigation to determine the sequence types associated with rodent and human *Leptospira* infections in the Northeast and Central regions in order to determine what is different about these regions. Through sequence typing, one could gain a better understanding of the epidemiology of leptospirosis in Thailand. AFRIMS-Entomology will be collaborating with the RTA, NIAH, and Brooke Army Medical Center to gain a better understanding of the transmission of this disease in Thailand.

17. Title: Maintenance of *Leptotrombidium* Larval Mite Colonies: Chigger-Feeding on ICR Mice (*Mus musculus*)

Funding Source: MIDRP WJ (core project)

Project Summary: Scrub typhus is an acute febrile vector-borne disease caused by *Orientia tsutsugamushi*. Larval trombiculid mites (chiggers) are important vectors of scrub typhus within Thailand and throughout the Pacific. In the chigger mite, *O. tsutsugamushi* is transmitted transovarially (from adult female to eggs) as well as trans-stadially (from egg to larva to adult stages of its life cycle). The department of Entomology currently maintains 7 genetically distinct strains of *O. tsutsugamushi*, each in unique population of chiggers: *Leptotrombidium chiangraiensis* (Lc1 and Lc5), *Leptotrombidium imphalum* (L1, L2, L3 and L4) and *Leptotrombidium deliense* (Ld1). The life cycle of the *Leptotrombidium* chigger begins with the adults laying eggs. Eggs will develop to the deutovum stage. After emerging from the eggs, the deutovum develop into larval mites (chiggers) which require tissue fluids from an animal host to develop into the nymphal stage. Finally, the nymphs become adults. Our scrub typhus infected chigger colonies were established with wild caught *O. tsusugamushi* infected *Leptotrombidium* mites. Infected and uninfected *Leptotrombidium* feed on tissue fluids from ICR mice. Research is

focused on mite biological transmission; interactions among pathogen strains (*O. tsutsugamushi*); vector competency; interactions between vectors and host; effects of multiple pathogen strains on transmission and infection rates; potential transmission blocking; and vaccine challenge trials.

CY12 Plan: Continue with current research is support of MIDRP, and GEIS goals. Expand support of collaboration project with MORU to establish a non-human primate model for scrub typhus investigations.

18. Title: Establishment of a Chigger Challenge Model. Determination of Optimum Inoculation Doses of Two *Orientia tsutsugamushi* Strains in ICR Mice. Evaluation of Scrub Typhus Vaccine Candidates by Natural and Artificial Challenge Models in ICR Mice (*Mus musculus*)

Funding Source: MIDRP WJ

Project Summary: *Orientia tustsugamushi* is an obligate intracellular parasite of human and other vertebrate hosts. Chigger is the parasitic stage of *Leptotrombidium* mites. Chiggers transmit *O. tsutsugamushi* to hosts. *Leptotrombidium chiangraiensis*, particularly the Lc1 strain, has a high transmission rate and virulence as compared with others strans. Lc1 lines and ICR mice model were selected to establishment chigger challenge model. Two *O. tsutsugamushi* strains, Karp and Lc1, were studied to determine their LD50 using a ICR mouse model. These two LD50's will be used to evaluate a protein vaccine (56KDa) using different challenge methods between artificial and natural pathways.

CY12 Plan:

A. Utilize LD50 of Lc1 and Karp strains to evaluate a candidate vaccine using artificial and naturaly challenge methods. This is collaboration with the Navy Medical Research Center (NMRC).

B. Scrub typhus Immunopathophysiology model in nonhuman primate (STIMP)

Funding Source: National Institute of Health-IAA

Project Summary: Overall objective is to establish a non-human primate model for studying the immunopathophysiological parameter of natural infection and vaccine-induced protection in scrub typhus. This project is a collaboration betwen AFRIMS, Navy Medical Research Center (NMRC) and Mahidol Oxford Research Unit (MORU). Department of Entomology, AFRIMS is responsible for establishing effective mechanical injection of mites using Karp human strain. The objective is to determine the most suitable position on the primate to achieve transmission using optimum mite infection rates established in the mouse model. The advantage of this study is the nonhuman primate pathophysiology more closely mimics humans, and will produce more accurate conclusions and decision points for vaccine challenges.

19. Title: Mosquito Rearing for Experiment Support

Funding Source: MIDRP

Project Summary: Rear and maintain mosquito colonies of *Anopheles dirus*, *An. cracens*, *An. minimus*, *An. sawadwongporni*, *An. campestris*, *Aedes aegypti*, *Ae. albopictus*, and *Toxorhyncites splendens* which are required to support malaria,dengue, and Japanese Encephalitis research studies at the Department of Entomology.

Laboratory-reared mosquitoes are disease-free mosquitoes of the same age in an impeccable condition. The first step of rearing starts when eggs are immersed in water in larval rearing trays. After 1-2 days, eggs will hatch. On day 2 after hatching, larvae will be fed in the rearing tray with ground fish food. When pupation starts, pupae will be collected and kept in a tub of fresh water until emergence. Adults will be fed with vitamin syrup until 5-7 days old when females are needed for experiments or for natural/artificial mating to maintain the colony. A blood meal will be fed to females to provide protein for egg production.

Numbers of mosquito females produced to support experiments in 2011 are recorded in the table below;

Mosquito sp.	Number of females produced to support experiment in 2012
An. dirus	312,000
An. sawadwongporni	26,000
An. campestris	15,600
An. minimus	83,200
An. cracens	46,800
Toxorhynchites splendens	31,200

CY12 Plan: In 2012, establish colonies of 2 new species from Korea, *Anopheles kleini* and *An. sinensis*, to support malaria prevention and or reduction efforts in Korea.

20. Title: Maintenance of the Scrub Typhus Chigger Challenge Model Utilizing *Orientia tsutsugamishi* Infected *Leptotrombidium* Chigger-Mite Colonies

Funding Source: MIDRP WJ

Project Summary: This is the onlyDoD laboratory which maintains *O. tsutsugamushi* infected mite colonies and, to our knowledge, is the only *O. tsutsugamushi* infected mite colony in the world. We currently maintain four species of *Leptotrombidium* mites with12 infected lines: *L. chiangraiensis* (Lc) (4 lines); *L. deliense* (Ld) (1 line); *L. imphalum* (Li) (7 line); and one non-infected line, *L. fletcherie* (Lf).

O. tsutsugamushi infected mite colonies are maintained in an Animal Biosafety Level-3 (ABSL-3) ontainment facility. The procedures are conducted in the BSL-3 facility for the infected colonies as well as in the BSL-2 laboratory for the non-infected colonies. These mites have a complex 3-month life cycle during which *O. tsutsugamushi* is maintained transovarially and transtadially. Only the larvae (chigger) are parasitic, feeding on extra-cellular fluid of their mammalian hosts in order to develop to the next stage. We are rearing 80,000 mites/year. Standard protocols are used to raise both *O. tsutsugamushi* infected and non-infected *Lepto-trombidium* mites to support:

1. Chigger challenge model
2. *Orientia tsutsugamushi* transmission studies
3. Development of a molecular taxonomic key for *Leptotrombidium* mites
4. Chigger behavior and its relevance in trapdevelopment and evaluation.
5. Treatment of scrub typhus-infected adult mite (*Leptotrobidium*) with doxycycline.