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.

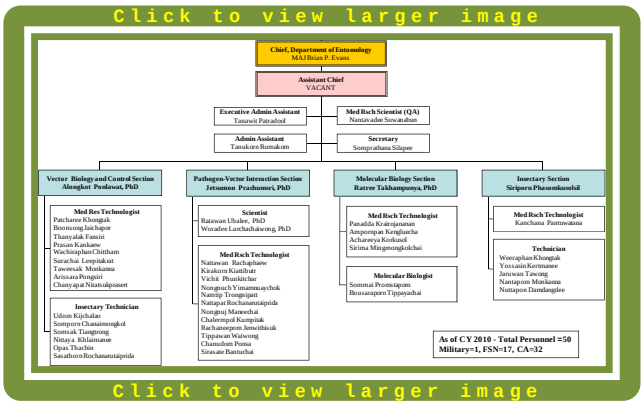
RESEARCH AREAS

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. Applies 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.

Pathogen-Vector Interactions: Conducts research on malaria biology and transmission. On-going research includes studies on malaria parasite development in liver cells and mosquitoes and the establishment of an *in vitro* system to screen antimalarial compounds and vaccines against exoerythrocytic, gametocytic and sporogonic stages of malaria parasites. Studies are also focused on gametocytogenesis in *Plasmodium falciparum* and *P. vivax* and the production of different stages of human and animal malaria parasites in order to support drug and vaccine development and transmission blocking strategies. A one-of-a-kind *Orientia tsutsugamushi*-infected *Leptotrombidium* mite colony and a chigger challenge mouse model form the basis for studies on vector-pathogen interactions and evaluations of candidate scrub typhus vaccines.

Molecular Biology and Diagnostics: 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, Plasmodium*, Chikungunya, dengue) and on relationships among the pathogen, vector/reservoir and host.

PERSONNEL



IN-HOUSE TRAINING PROGRAMS AND OUTSIDE TRAINING OF PERSONNEL

In-House Training Programs and Outside Training of Personnel

- Animal Bites and Other Injuries/Reports
- Animal BSL-3 Hazard Training
- Accident, Illness and Complaint Reporting, Records and Investigations
- B-Virus Post-Exposure Intervention and Prophylaxis
- BSL-3 Laboratory Training Program
- Bio-safety in Laboratory
- Blood-borne Pathogens Exposure Control Plan
- 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
- The Humane Care and Use of Lab Animals
- Human Subject Protection Program
- 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
- UV Meter Use

Outside Training Provided by Department

- Autophagy as Cell-autonomous Immunity against Intracellular Pathogens: a fight against Mycobacterium tuberculosis and potential mechanism for fighting against malaria
- Rickettsia Training Program
- Training on the Preparation of Scrub Typhus

ACCOMPLISHMENTS

1. Title: Development of a Molecular Taxonomic Key for the Identification of Scrub Typhus Vectors, Mites within the Genus *Leptotrombidium*

Funding Source: MIDRP U

Project Summary: Larval trombiculid mites (chiggers) are important vectors of scrub typhus within Thailand and much of Asia. Morphological identification of mite species is exceptionally challenging and few taxonomists have such knowledge and experience. Additionally, morphological identification renders a specimen unusable for pathogen identification. We, therefore, developed a molecular taxonomic key for the precise identification

of trombiculid mites using the cytochrome oxidase subunit I (COI) gene of mitochondria. Our aim was to use mtDNA barcoding to identify pre-defined species of mites collected from field sites, focusing mostly on reservoir mites for scrub typhus. Phylogenetic analysis (neighbor-joining algorithms, 1,000 bootstrap, Phylip program) of *Leptotrombidium* mites from colonies maintained at AFRIMS was performed using the full-length sequence of the mitochondrial COI gene. Four species of *Leptotrombidium* mites (*L. chiangraiensis, L. imphalum, L. fletcheri, L. deliense*), are maintained in our laboratory. Full-length sequencing of the COI gene was completed for 66 samples from 4 chigger species and compared to *Leptotrombidium* mite COI gene sequences retrieved from GenBank. Our results showed that the deduced amino acid sequence of full-length COI revealed the greatest variation and diversity which could be used to discriminate among these four chigger species. Rapid assays were developed to allow for the molecular identification of *Leptotrombidium* species from throughout the region to facilitate improved scrub typhus research in endemic regions. The PCR-RFLP assay and a multiplex assay using species-specific primers were developed to detect 3 species (*L. chiangraiensis, L. imphalum, L. deliense*) and were evaluated using unknown chiggers collected from field-caught rodents.

CY11 Plan: Expand the taxonomic key using *Leptotrombidium* mites collected from the PACOM region (i.e., Korea). Submitted MIDRP U FY12 proposal to develop a molecular taxonomic key from field collections of mites originating from Korea, Japan, and Thailand in order to distinguish among species belonging to the family Trombiculidae, the most medically-significant family of mites.

2. Title: Comparative Field Evaluation of the BG Sentinel Trap and CDC Backpack Aspirator for Sampling of the Dengue Vector, *Aedes aegypti*, in Thailand

Funding Source: MIDRP U

Project Summary: Experiments were conducted in Dong Sawong village located in Muang District, Lopburi province from December 2009 thru February 2010. Four trap configurations were evaluated against the CDC backpack aspirator: The BG Sentinel-Trap (BGS-Trap) with BG-Lure, the BGS-Trap with BG-Lure and CO_2, the BGS-Trap with CO_2, and the BGS-Trap without BG-Lure and CO_2. Traps/aspirator were randomly assigned to five sites separated by distances of at least 400m and operated from 0800 to 1600. Traps were rotated counterclockwise after each of five collection days which comprised one replicate of the study. Results from nine replicates demonstrated that the BGS-Trap with BG-Lure and CO_2 and the BGS-Trap baited with CO_2 collected significantly more *Ae. aegypti* females than the CDC backpack aspirator ($P < 0.05$). No significant differences could be found among the number of *Ae. aegypti* collected by the BGS-Trap with BG-Lure, BGS-Trap without BG-Lure and CO_2 and the CDC backpack aspirator. This was the first-ever study that evaluated the efficacy of the BG Sentinel trap in Southeast Asia. We conclude that the BGS 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.

CY11 Plan: Continue evaluations of CDC/BG Sentinel traps complemented with light-emitting diodes which would presumably render the traps to me significantly more effective. Submitted FY12 proposal to evaluate the efficacy of the commercially-available Mosquito Magnet™ Liberty Plus (MMLP) trap and the Zumba trap.

3. Title: Genotype Identification and Sequence Analysis of *Orientia tsutsugamushi* Isolated from Scrub Typhus-infected Chigger-mite Colonies

Funding Source: MIDRP WJ

Project Summary: Twelve scrub typhus-infected trombiculid mite colonies were established from chiggers collected from wild-caught rodents in Thailand. Colonies consisted of 7 *Orientia tsutsugamushi*-infected *Leptotrombidium imphalum* (Li-1 to Li-7) lines, 1 *O. tsutsugamushi*-infected *L. deliense* (Ld-1) line and 4 *O. tsutsugamushi*-infected *L. chiangraiensis* (Lc-1, Lc-2, Lc-4 and Lc-5) lines. The gene encoding a 56-kDa type-specific antigen of *O. tsutsugamushi* isolated from the 12 infected lines was amplified by nested PCR. We cloned the 0.8 kb PCR fragment into a pCR2.1-TOPO vector and analyzed the DNA sequence covering variable domains 1 to 3 in the type-specific antigen. The DNA sequences were compared with various sequences in available databases for sequence homology and phylogenetic analysis. Based on the 56-kDa nucleotide sequence data, our results showed that *O. tsutsugamushi* isolates from Lc-1, Lc-2, Lc-4 and Lc-5 were Karp-like strain with 93% homology, whereas those from Li-2, Li-3, Li-4 and Li-7 were in a group associated with a rodent source (TA763). The isolates of Li-1, Li-5 and Li-6 contained two strains, TA763 and Karp-like *O. tsutsugamushi*, co-existing within individual mites of the colonies. The presence of *O. tsutsugamushi* identified as being related to Gilliam and Karp-like strains were found from the Ld-1 line.

CY11 Plan: Submitted MIDRP WJ FY12 proposal to characterize *O. tsutsugamushi* strains maintained vertically within our laboratory-reared chigger mites and transmitted to a rodent host and to evaluate the host's immune response to infection by single/multiple strains.

4. Title: Comparative Susceptibility to *Plasmodium falciparum* and Blood-feeding Behavior of Laboratory-reared Malaria Vectors *Anopheles dirus* and *An. cracens*

Funding Source: MIDRP

Project Summary: *Anopheles dirus* and *An. cracens*, previously known as *An. dirus* A and *An. dirus* B respectively, were compared for their susceptibility to *P. falciparum* and their blood-feeding behavior. These sibling species have been separately colonized at the Department of Entomology, AFRIMS for a period exceeding 20 years. Laboratory-reared *An. dirus* requires artificial mating while *An. cracens* requires self-mating. Both species were fed a *P. falciparum* gametocyte culture. Susceptibility to *P. falciparum* was not significantly different between the species. For evaluation of pre-erythrocytic stage vaccines, malaria-infected mosquitoes were allowed to bite volunteers or an animal, either vaccinated or not vaccinated. A low percentage of *An. cracens* fed on the second blood meal if they were made to retain their eggs after the first blood meal. We found that 0 to 3.8% of *An. cracens* fed on the second blood meal after feeding on the first blood meal infected with *P. falciparum*, while 50% of *An. dirus* fed on the second blood meal under the similar conditions. If *An. cracens* were permitted to lay eggs after the first blood meal, then percent feeding on the 2nd blood meal increased, whereas egg-laying had no impact on percent feeding on the second blood meal of *An. dirus*. *An. dirus* had a higher survival rate after feeding on *P. falciparum*-infected or a malaria-naive blood meal compared to *An. cracens*.

CY11 Plan: N/A

5. Title: Development and Evaluation of Novel, Deployable, User-friendly *Aedes aegypti* Control Tools and Strategies

Funding Source: DWFP

Project Summary: Evidence from this study demonstrated that a prototype pyriproxyfen-treated ovitrap/resting station device could suppress increases in *Ae. aegypti* populations, most especially at certain time points after the devices were introduced into a village. We speculate that the observed effect was primarily the result of *Ae. aegypti* exposure to a pyriproxyfen-treated device shortly after their emergence or after taking a blood meal (i.e., exposure to pyriproxyfen while positioned on the treated resting station) resulting in decreased egg production rather than the effect being due to the horizontal transfer of the chemical by adult mosquitoes. The prototype ovitrap/resting station device that we have developed is easily collapsible and transportable and has shown moderate efficacy when deployed in a village setting. However, we believe that by streamlining the physical characteristics of the device (the ovitrap portion subtracted) and replacing the formulation, we can create a tool that is significantly more effective at suppressing increases in populations of container-breeding mosquitoes. As *Ae. aegypti* are drawn towards darker areas, this device is physically attractive as a resting station. Therefore, it is likely attractive to mosquitoes of all physiological stages (not just ovipositing mosquitoes) and exposure of mosquitoes to pyriproxyfen early in their development while resting will lead to reductions in egg productivity. We propose to continue this research (with additional funding) by streamlining the model design and evaluating an alternative formulation. We will be constructing large 18 x 13m outdoor, screened enclosures in Kamphaeng Phet, Thailand, which we will use to conduct studies under more controlled conditions. Ideally, results from future research will support the development of a prototype pyriproxyfen-treated device that could be added to the approved list of pesticides and pest control equipment available to deployed personnel. Such a device would also assist local responders in the United States in emergency efforts to contain any future outbreaks of imported dengue or chikungunya.

CY11 Plan: Submitted MIDRP U proposal to develop a pyriproxyfen-treated resting station for the control of a sylvatic dengue/chikungunya vector known as *Aedes albopictus*.

6. Title: Development of a Field-expedient Ectoparasite Trap

Funding Source: MIDRP U

Project Summary: Larval trombiculid mites (chiggers), known vectors of scrub typhus, are most commonly found in highly localized aggregations known as "mite islands". Such clustering and limited mobility hampers chigger collection efforts. We evaluated several traps, based on the standard black plate trap, and tested the utility of CO_2 as an attractant to increase trap success. Despite significantly increasing catch rates with an attractant (CO_2), static trapping is of limited use in conducting area surveillance and disease risk assessments. We designed and evaluated a novel, rodent-based trap to collect chiggers. The box-shaped device contains a snap trap and permethrin-coated interior which allows field rodents to be trapped and prevents the subsequent migration of mites from the rodent/device. Our results demonstrate this tool to be more effective at collecting chiggers relative to a prototype "fixed" device.

CY11 Plan: N/A

7. Title: Community Structure of Anopheline Mosquitoes in Kaeng Hang Maeo District, Chanthaburi Province, Thailand

Funding Source: NIH

Project Summary: Environmental changes are responsible for changes in vector community structure. Species of *Anopheles barbirostris* group have become the predominant anopheline species and potentially transmit human malaria parasites in many parts of Thailand. A study conducted in Kaeng Hang Meao district, Chanthaburi province, Thailand, from June thru September 2010 investigated the anopheline mosquito community structure in forested and deforested areas. A total of 964 anophelines comprising 19 species were captured using CDC light trap collection technique; 430 mosquitoes from forested areas and 534 mosquitoes from deforested areas. The majority of anopheline species in forested areas was *An. minimus* (71%) whereas *An. barbirostris* group (31%) was the dominant anopheline species in deforested areas. Among the six species of *An. barbirostris* group, *An. campestris* was the predominant anopheline species in deforested areas (80%). Further field evaluations on life table analysis of *An. minimus* and *An. campestris* to determine whether larval survivorship differs in habitats under different land cover conditions are clearly required.

CY11 Plan: N/A

8. Title: Laboratory Evaluation of Candidate Repellent Compounds against *Leptotrombidium* Chiggers

Funding Source: MIDRP U

Project Summary: We compared the walking distance response (olfactory response) of *Leptotrombidium chiangraiensis* chiggers exposed to candidate repellent compounds with that of chiggers exposed to 33% N,N-Diethyl-meta-Toluamide (DEET) using a walking track model bioassay. The following compounds were evaluated: (1) DEET 33% lotion; (2) IR3535 10% lotion; (3) IR3535 20% spray; (4) picaridin 20% lotion; and (5) picaridin 20% spray. All 5 compounds (augmented with CO_2) significantly shortened the walking distance of *L. chiangraiensis* chiggers relative to the control group (only CO_2) ($P < 0.05$). DEET 33% lotion was significantly more effective than both formulations of IR3535 and picaridin ($P < 0.05$) in shortening the walking distance. Results from a field bioassay demonstrated that all of the repellents evaluated prevent chigger feeding. These data indicate that additional candidate repellents containing picaridin or IR3535 can potentially be used to protect against mite-transmitted scrub typhus although DEET 33% lotion remains most effective at repelling chiggers and preventing feeding.

CY11 Plan: N/A

9. Title: Detection of *Plasmodium vivax* Pre-erythrocytic Stage Using a Rolling Cycle Amplification (RCA) Assay

Funding Source: ILIR

Project Summary: We are in the final stages of developing a sensitive and specific method to detect low numbers of *P. vivax* which will allow us to accurately evaluate the development of *P. vivax* in liver cell culture. The method involves *in situ* DNA target-primed rolling cycle amplification (RCA) of circularized probe under isothermal conditions to detect sporozoites. This proposed technique will allow for a more sensitive, *in situ* approach for detecting parasites in infected cells and can be used as a tool for scientists to expand their knowledge of the liver-stage biology of the malaria parasite. The RCA assay is being optimized using *P. vivax* sporozoites and the sensitivity is being tested with a known number of sporozoites to determine the limit of detection relative to the currently used real-time RT-PCR assay. A successful result from

this study can be applied toward other malaria research such as the development of an *in vitro,* anti-malaria drug screening assay to find a potential candidate for liver stage malaria treatment or perhaps towards the discovery of molecular markers for *P. vivax* maturation in liver cells.

10. Title: Oviposition Responses of Gravid *Aedes aegypti* to Phytochemicals and Aromatic Organic Infusions

Funding Source: MIDRP U

Project Summary: We determined the oviposition responses of gravid *Aedes aegypti* to phytochemicals and aromatic organic infusions. Gravid *Ae. aegypti* were released into mosquito cages (60 x 60 x 60 cm) under semi-field conditions in Kamphaeng Phet province, Thailand. In a binary choice assay, the oviposition response of *Ae. aegypti* to a plastic cup with a plant infusion of lemongrass (*Cymbopogon citratus*), leech lime (*Citrus hystrix*), basil (*Ocimum tenuiflorum*) or a phytochemical (camphor and borneol) infusion was determined relative to a cup containing drinking water (control). Results revealed that gravid mosquitoes most preferred to lay eggs in a lemongrass infusion ($p < 0.05$, t-test). There were no significant differences between the number of eggs in the control cup and the cup containing leech lime, basil, camphor, or borneol infusions. We propose lemongrass as a candidate attractant/stimulant that can be mixed with a toxicant to manage container-breeding mosquitoes such as *Ae. aegypti*.

CY11 Plan: N/A

11. Title: Efficacy of Primaquine against Liver Stage *Plasmodium vivax* Development in Human Hepatma Cell Lines

Funding Source: MIDRP Q

Project Summary: Development of effective drugs against liver-stage malaria parasites requires an efficient *in vitro* culture system that allows for parasite survival and facilitates drug efficacy evaluations. The lack of an efficient in vitro culture system for liver-stage malaria parasites has presented a major obstacle to drug development efforts targeting the liver stage of the parasite. Invasion and development of *P. berghei* and *P. vivax* sporozoites in hepatic immortalized cell lines, HepG2, have been demonstrated previously *in vitro*. However, the culture system has not been validated for anti-malarial drug efficacy testing against liver-stage parasites. We validated the *in vitro* culture system for liver-stage *P. vivax* and *P. berghei* development and evaluated the efficacy of the known drug, primaquine (PQ), against liver-stage parasite development in this system.

CY11 Plan: N/A

12. Title: Semi-Field, Tunnel Assay for the Evaluation of a Commercially-available Metofluthrin (Sumione®)-impregnated Net on Host-seeking *Aedes aegypti*

Funding Source: MIDRP U

Project Summary: The efficacy of a commercially-available metofluthrin (SumiOne®)-impregnated net (MIN) on the entrance behavior of female *Ae. aegypti* was determined using a semi-field tunnel set-up. One hundred fifty host-seeking (4-8 d old) *Ae. aegypti* were released at the mid-point of a 50-m tunnel in which either end of the tunnel was blocked by a 10.9-m3 (2.4 X 2.4 X 1.9 m) tent containing a screened interception trap. *Ae. aegypti* were released for 2 hours between 0800 and 1000 hrs and interception traps were emptied each hour by a human "attractant" positioned inside each of the tents. Human "attractants" were rotated within and between tunnels after each replicate. This procedure was replicated 4 times per week. When a human “attractant” was placed at both ends of the tunnel, *Ae. aegypti* showed relatively equal

preference for either end of the tunnel. When a human "attractant" was placed at only one end of the tunnel while the other was attractant-free, the number of mosquitoes collected from each trap within and between tunnels was not significantly different for *Ae. aegypti*. For the spatial repellent evaluation, the treatment tent contained a MIN while the control tent was without a net. A spatial activity index (SAI) was used to evaluate the responses of mosquitoes in the tunnel assay. The SAI ranges from -1 to 1, with zero, negative, and positive values representing no preference to either control or treatment tents, preference to treatment tent, and preference to control tent, respectively. The overall mean SAI for *Ae. aegypti* was slightly less than 0 and greater than > -0.1 after 1 week of MIN application and then decreased (less than -0.1) for the last 4 weeks. Such a result suggests that the spatial repellent properties of a commercially-available MIN may not be a promising approach to reducing disease transmission caused by vectors of dengue fever and chikungunya virus.

CY11 Plan: Received $300K+ for FY11/12 to develop and evaluate the long-term efficacy of a push-pull strategy (combination of spatial repellent and attractive toxicant) for the control of females and males of disease-relevant mosquito species in order to minimize human-vector contact. Large outdoor enclosures are being constructed in Kamphang Phet to conduct push-pull evaluations.

13. Title: *Leptospira* Pathogenic Species JBAIDS Assay Development
Funding Source: MIDRP L

Project Summary: We developed a highly sensitive and specific, dual-fluorogenic, hydrolysis probe (TaqMan), polymerase chain reaction assay for the detection of *Leptospira* human pathogenic species. The limit of detection (LOD) was established at 100 fg or 20 genomic equivalents (ge). Assay *in vitro* sensitivity was 100% and specificity 100% in testing using a broad panel of well characterized reference strains. Sensitivity and specificity testing were conducted using a diverse panel of pathogenic and non-pathogenic *Leptospira* reference strains, genetic near neighbors, broad cross-reactivity panel, and environmental/matrix panel to include vector, primary reservoirs, and human DNA. In blind testing with a panel of infected rodent kidney tissue (n = 50) assay results were 100% concordant with the comparator test. In field-evaluations using wild-collected rodents (n = 36), results of testing of extracts from spleen and kidney were 100% concordant with the comparator test (4 true positives, 0 false negatives). The associated *Leptospira* pathogenic species infectivity rate in the sampled rodent population indicated significant potential for leptospirosis transmission. Sample processing and analyses were completed in less than two hours. The intent of the assay is for use in environmental surveillance using DoD approved analytic instrumentation, the "Ruggedized" Advanced Pathogen Identification Device (R.A.P.I.D). The assay provides a promising candidate for potential clearance and use in human diagnostics.

CY11 Plan: Continue the development and validation of a diagnostic assay for detection of the etiologic agent of leptospirosis from clinical samples using the JBAIDS by developing a second Target PCR Assay and an internal positive control.

14. Rodent-borne Disease Surveillance
Funding Source: GEIS

Project Summary: Before 1996, leptospirosis cases reported to the Department of Disease Control (DDC) in Thailand were minimal in number, averaging 200 cases/year. However, Thailand experienced a marked increase in the number of annual cases beginning with 398 in 1996 and reaching a peak in 2000 with 14,285 cases. The vast majority of cases (90%) originated from the Northeast region of Thailand. The number of cases fell to 2,868 in

2005 and over the last 3 years case numbers have steadily increased with 5,278 cases reported in 2009. DDC data consistently show a clear difference in the annual incidence of leptospirosis between the Northeast and Central regions. In this study, we are looking into parameters that differ between these two regions (i.e., rodent species, pathogen strains, environment) which would hitherto result in one region becoming more severely affected by this disease relative to a neighboring region. Scrub typhus and bartonellosis are two additional rodent-borne diseases found in Thailand that are widely agreed to be underreported. 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. We will better understand regional differences in the epidemiology of these diseases and determine whether there are seasonal impacts on transmission. Collections were performed from September thru November 2010 (wet season) and all samples were evaluated for leptospirosis, scrub typhus, rickettsia, and bartonella. For each month, sampling was performed for one week in a province in the Northeast and for one week in a province in the Central region. The percentage of rodents collected from Sing Buri that was positive for *Leptospira* (37.5%) was more than triple that of Buri Ram, the second highest percentage among the 6 provinces (11.11%). Ironically, according to human case data from 2010, there were no human leptospirosis cases found in Sing Buri. It is possible that the primers used for our analysis were detecting a non-pathogenic strain of *Leptospira* although they were developed for the detection of pathogenic strains. However, if the high percentage actually represents a pathogenic strain(s), this finding would have significant implications with regard to medical surveillance activities in Sing Buri. Further analysis will determine the pathogenicity of the samples from Sing Buri. Twice as many rodents were collected in the Central region versus the Northeast region (170 versus 82 rodents). Overall, the percentage of rodents positive for leptospirosis in the Central region was twice that found in the Northeast region (12.94% versus 6.1%). All totaled, *Rattus argentiventer* represented the species having the highest rate of infection with *Leptospira* relative to the other species that were collected. Interestingly, *R. argentiventer* was not collected from within the other 5 provinces. Bartonella and scrub typhus were found in only a small percentage of the rodents. None of the rodents that were collected harbored rickettsia.

CY11 Plan: Sampling will continue from January thru March 2011 (dry season) and again in September thru November 2011 (dry season).

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

Funding Source: GEIS

Project Summary: Rayong and Surat Thani 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.

CY11 Plan: Complete vector competence experiments and discontinue study as no GEIS funding was provided for continuation of this project.

16. Title: Maintain Entomological Support for the Malaria Challenge Model
Funding source: MIDRP F

Project Summary: We continue to provide 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 continue to 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.

CY11 Plan: Continue to provide *P. vivax* parasites to support several drug and vaccine development studies. We anticipate to collect 250-300 blood samples in 2011 and will provide 2-3 batches of infected mosquitoes for a human challenge study at WRAIR.

17. Title: Develop a Rapid Diagnostic Lamp Assay for the Detection of Chikungunya Virus in Human Serum
Funding Source: MIDRP L

Project Summary: We will develop a rapid diagnostic assay for the detection of CHIKV in human serum samples to achieve sensitivity and specificity values that are comparable to existing diagnostic methods and to significantly reduce the amount of time required to obtain a clinical diagnosis relative to established methods. Project will commence in CY2011.

18. Title: Arbovirus Surveillance in Korea
Funding Source: Collaboration with USUHS (GEIS-funded)
Project Summary: In CY10, evaluated over 3,000 pools of mosquitoes from Korea for arbovirus detection. Results were provided to USUHS.
CY11 Plan: No future funding is expected.

19. Title: Performance of *Plasmodium Vivax* (PV) Transmission-blocking Assays In Support of PV Transmission-blocking Vaccine Studies
Funding Source: NIH
Project Summary: Evaluated blocking efficacy of sera samples taken from humans/ animals administered with a candidate vaccine.
CY11 Plan: Continue to support efforts to evaluate blocking efficacy of candidate vaccines.

20. Title: Dengue Vaccine and Viral Evolution in Rural Thailand
Funding Source: NIH RO1 (Multi-center collaboration)
Project Summary: Initiated clusters and collected mosquitoes from areas already mapped in the GIS for the current Kamphaeng Phet project (8,188 houses in 34 villages). Updates and expansion of the GIS will be carried out as necessary. 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. 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 will be determined and vector competence similarly will be 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.
CY11 Plan: Supportive efforts will continue thru the next 4 years.

DEPARTMENT OF IMMUNOLOGY AND MEDICINE

DEPARTMENT MISSION

To support medical product development to protect the war fighter, and conduct surveillance of diseases of military importance in Southeast Asia on behalf of:

- U.S. Army Medical Research and Materiel Command
- Walter Reed Army Institute of Research
- Other Department of Defense and U.S. Government entities
- Extramural collaborators