IN-HOUSE TRAINING PROGRAMS AND OUTSIDE TRAINING OF PERSONNEL

In-House Biomedical Education

Training is performed continuously at AFRIMS. Laboratory personnel from WARUN, Cebu and Kamphaeng Phet have received training in new influenza primers and protocols for real-time PCR, including the new pandemic H1N1. AFRIMS also serves as a training site for US military and civilian medical students, residents, and infectious disease fellows pursuing careers in tropical medicine and research.

Outside Training Provided by Department

- Infectious Disease Surveillance – Vientiane, Laos, June 2009.
- Hosted Laos Military Personnel for Influenza training, September 2009.
- Influenza Surveillance Training, Battambang, Cambodia, September 2009.

DEPARTMENT OF ENTOMOLOGY

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

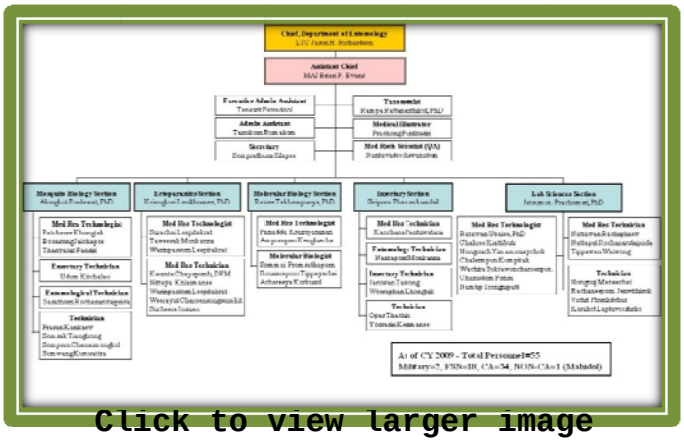
Vector Biology, Surveillance, and Control Research: Develops and evaluates mosquito surveillance traps. Evaluates control or preventive measures including insecticide barrier treatments, insecticide-impregnated bed nets and tents, and personal-use and spatial repellents. Studies are aimed at understanding basic vector biology and behavior through vector competence and oviposition assays. Taxonomic keys are continually developed and updated for the mosquitoes of Thailand.

Malaria Research: 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.

Ectoparasitic and Zoonotic Disease Research: 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, scrub typhus transmission and immunology and evaluations of candidate scrub typhus vaccines. Research is also focused on designing more effective surveillance techniques for scrub typhus vectors. Our team of veterinarians and field mammalogists conduct epidemiological and ecological studies that are aimed at better understanding the distribution and variation of vectors and their related pathogens in animal hosts.

GIS and Spatial-temporal Analysis of Vector-borne and Zoonotic Diseases: 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.

PERSONNEL



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IN-HOUSE TRAINING PROGRAMS AND OUTSIDE TRAINING OF PERSONNEL

In-House Training Programs Provided by AFRIMS

- Annual Biosurety Training
- Avian Influenza H5N1: Immunopathology and Heterosubtypic Immunity
- Occupational Health Program and Communication
- B-Virus Post-exposure Intervention and Prophylaxis and Animal Bites and other Injuries/Reports
- The Humane Care and Use of Lab Animals
- Lab training from MoPH
- Animal Biosafety Level-3 (ABSL-3) Hazard Training
- Towards Development of an Efficacious HIV-Vaccine: U.S. Military HIV Research Program Experience
- Genetics of susceptibility to Parasitic Disease
- What Really Happened during the Influenza Pandemic 1918-1919
- ELISPOT Assays for Delineation of T Cell Effector Classes and Activation States
- High-resolution Population Genetics of Hot Spring Mat Synechococcus spp: Theory, Modeling, and Reality
- Safety Training for U.S. Personnel
- Introduction to Quality System for Clinical Research
- Analysis of the Neutralizing Antibody Response to HIV Infection and Vaccines
- Hot Topic – Swine Flue
- Societal Issues Involving Laboratory
- Biosafety in Laboratory

- CHIKV: Situation and Outbreak in Thailand
- Biobanking - Large Scale Management of Biosample. A New Technology for Room Temperature Biosample Storage and Complete Solution Management
- Biosurety Introduction and BSAT Inventory SOP Training
- Fundamental GCP for Researcher
- Thailand Multicenter Diarrheal Diseases Surveillance Collaboration
- Zoonotic Foodborne Pathogens: From Farm to Fork
- AFRIMS Annual Occupational Health, Safety and Biosurety Training
- Malaria Elimination in Melanesia: What is Needed to Eliminate Malaria on Isolated Islands?
- SOPs: CMD-QP-001, AFRIMS Human Subjects Protocol Lifecycle Procedure
- CMD-AD-008, Publications CRADA, MTA, MOU, MOA, LOI and Patent Application Approval Process
- Cell Signaling Networks in Dengue Virus Infection
- Fire Fighting
- Chemical Spill Training
- Powered Air Purifying Respirator Use Training
- AFRIMS Annual Occupational Health, Safety and Biosurety Training
- Vector Biology and Vector-borne Disease Research at AFRIMS
- BSAT Training
- BSL-3 SOP Training
- BSL-3 Autoclave and Steam Generator Operation
- Freezerworks Training Workshop (English)
- Human Malarial Parasite FKBP Family Proteins and Opportunity for Novel Antimalarial Therapeutics
- GLP Basics
- Bioscavengers for the Protection against Organophosphate Toxicity, and Innovative Huperzine A Treatment in Chemical Defense and Blast Brain Injury
- Ethics Training (Mandatory Training)

In-House Training Programs Provided by Department

- ENT-MB-002-00 Oral Infection of Mosquitoes by Membrane Feeding
- ENT-MB-003-00 Assay of Virus Transmission by Mosquitoes
- ENT-GL-004-00 Diagnosis of Filarial Worm
- ENT-ML-002-00 Malaria Parasite Deoxyribonucleic Acid (DNA) Isolation from Blood Samples
- Vector Control and Pest Management
- GIS Training

Outside Training

- IACUC Training, Mahidol University, Salaya Campus

CORE ACCOMPLISHMENTS

Mosquito-borne Disease Vector Biology, Surveillance, and Control

1. Title: Evaluation of Insecticide-Impregnated Bed Nets (Permanet™) and a Barrier Treatment of Vegetation with Insecticide
Location: Mae Sot, Tak Province

a. Investigators:
- Alongkot Ponlawat, PhD
- MAJ Brian Evans, PhD
- LTC Jason Richardson, PhD

b. Objectives:
This study was designed to evaluate the long-term efficacies of insecticide-impregnated bed nets (Permanet™) and a barrier treatment of vegetation with insecticide on malaria vector densities.

c. Methods:
This study was conducted from January to September 2009 to evaluate the long-term efficacies of insecticide-impregnated bed nets (Permanet™) and a barrier treatment of vegetation with insecticide on malaria vector densities. This evaluation was carried out among three villages (10-15 km apart) of similar size (i.e.; Population, number of houses, and area). Two villages (Ban Khun Huay and Ban Pa Deh) were designated as treatment villages and one village as a control village (Ban Tham Seau). The first treatment village (Ban Pa Deh) was provided with long-lasting insecticide-treated bed nets (LLIN) containing deltamethrin (Permanet™). The second treatment village received a one-time barrier treatment of bifenthrin (Talstar One™) applied to vegetation within a 20-m swath around the perimeter of the village. The control village received no treatment. Fifteen houses from each village were selected by simple random sampling for each of the 3 collections nights as locations where collections were performed. Mosquitoes were collected over a 12-h period (1800 to 0600 hrs) for 3 consecutive nights per month per village for each of the 3 months prior to the LLIN/barrier treatments and monthly for the 9 months immediately following the treatment using CDC light traps positioned on the outside of bed ned nets. A human volunteer located inside of each bed net was used as an “attractant”. Baseline densities for each village were conducted from February 2008 to December 2008.

e. Results:
Data collection has been completed and data is being analyzed. A publication will follow.

2. Title: Development of *Aedes aegypti* Control Tools and Strategies
Location: Bangkok and Rayong

a. Investigators:
- Alongkot Ponlawat, PhD
- MAJ Brian Evans, PhD
- LTC Jason Richardson, PhD

b. Objectives:

This research was designed to develop new interventions for the control of *Ae. aegypti* populations in order to prevent transmission of dengue and/or chikungunya viruses to deployed personnel. The goal is to leverage the promising properties of pyriproxyfen to reduce *Ae. aegypti* densities in the context of a military camp.

c. Methods:

We proposed that an ovitrap/resting station device treated with pyriproxifen will reduce *Ae. aegypti* populations enough to interrupt virus transmission within a village. We have been testing the ability to eliminate productive habitat within a village and beyond the perimeter of a village based on the concept of horizontal transfer of pyriproxyfen to untreated containers. A growing body of research shows that pyriproxyfen is an extremely effective insect growth regulator which can be transferred among larval habitats by mosquitoes.

An ovitrap/resting station device treated with pyriproxifen was designed in the laboratory and evaluated in large field cage/tunnel bioassays. Field-testing of this device has been conducting in a village setting in a dengue-endemic area of Thailand. Devices have been spatially-arranged based on hypothetical *Ae. aegypti* dispersal distances and data collected in field tunnel bioassays. Their impact on *Ae. aegypti* densities will be determined. Ideally, results from this study will support the development of Ae. aegypti control products which can be added to the approved list of pesticides and vector control equipment available to deployed personnel. Products and methodology developed here will also assist local responders in the United States in emergency efforts to contain any future outbreaks of imported dengue or chikungunya.

d. Results:

Initially, we tested the hypothesis that visual contrast and select organic infusions significantly impact oviposition site selection preference of gravid *Ae. aegypti*. The influence of visual contrast on site selection was examined in mosquito cages (60 x 60 x 60 cm) under semi-field conditions. In choice-tests, significantly greater numbers of eggs were laid in a black ovitrap placed against a white paper background relative to black-black, white-white, and white-black combinations. The relative impact of select organic infusions on oviposition was evaluated in a choice experiment that involved the following infusions: lemongrass (*Cymbopogon citratus*) infusion; larval rearing water (*Ae. aegypti*), carpet shell (*Paphia undulata*) rinse water; rain water, and tap water. We discovered that significantly greater numbers of eggs were deposited in lemongrass infusion and rain water *versus* the other oviposition media. There were no significant differences in the numbers of eggs laid between the rainwater and lemongrass infusions.

In semi-field experiments conducted in 50m tunnels made of polyester netting, we evaluated the impact of pyriproxyfen exposure on egg development and followed up on earlier findings that pyriproxyfen can be transferred from a treated surface to a water substrate. Pulverized pyriproxyfen (0.05 g ai./m^2) was applied to the inner surface of a prototype black resting station (35 x 35 x 55 cm) and the resting station was subsequently placed at one end of the tunnel inside of a large cage. An oviposition container with 2 L of tap water was placed at the other end of the tunnel. A non-treated resting station placed inside of a large cage and a non-treated oviposition container were placed at opposite ends in a control tunnel. One hundred female *Ae. aegypti* (1 d post blood-fed) were released into the cage and provided with 10% sucrose for 24 h. Subsequently, the cage was opened, Mosquitoes were released into the tunnel and then permitted to lay eggs over a period of 6 d. Our results show that significantly more eggs

(2,604.4 ± 1,172.8 eggs versus 486.6 ± 360.9 eggs/container respectively) were laid in the control tunnel versus the treated tunnel ($P < 0.01$). Water from oviposition containers was used in a larval bioassay to evaluate adult emergence. Mean percent adult emergence was significantly lower and in water taken from the treated containers (74.5 ± 3.3) versus water from the control containers (90.5 ± 1.6) ($P < 0.0001$). Our data show that egg production is significantly reduced in females exposed to pyriproxyfen early in their development. Additionally, our results confirm earlier findings that the dispersal of pyriproxyfen by *Ae. aegypti* can lead to reduced adult emergence in competing containers.

Field experiments were carried out in two villages (Ban Chon and Neon Mayom) of similar size (population, number of houses, area) located 1.3 km apart in Tapong sub-district, Muang District, Rayong Province, Thailand. For the current study, inner and outer geographical circles were artificially designated for each village. The result is a village area stretching approximately 450 m in diameter and containing anywhere from 42 to 45 houses.

e. Future Plans:

Ongoing field evaluations of a prototype collapsible, ovitrap/resting station device treated with pyriproxyfen will be completed in February. The data will be statistically analyzed and reported.

3. Title: Semi-field Evaluation of a Commercially-Available Metofluthrin (SumiOne®)-Impregnated Net on Host-Seeking *Aedes aegypti* and *Anopheles dirus*

Location: Chanthaburi

a. Investigators:

- Alongkot Ponlawat, PhD
- MAJ Brian Evans, PhD
- LTC Jason Richardson, PhD

b. Objectives:

To evaluate the efficacy of a commercially-available metofluthrin (SumiOne®)-impregnated net on the entrance behavior of female *Aedes aegypti* and *Anopheles dirus* in a semi-field tunnel set-up.

c. Methods:

One-hundred fifty host-seeking mosquitoes (4-d-old) of each species are released at the mid-point of a 50-m tunnel in which either end of the tunnel are blocked by a 10.3-m^3 (2.4 X 2.4 X 1.8 m) tent containing a screened interception trap. The treatment tent will contain a metofluthrin-impregnated net (MIN) while the control tent will be without a net. Interception traps are emptied every hour over a 3-h period (0800 to 1000 hrs) for *Ae. aegypti*; and a12-h period (1800 to 0600 hrs) for *An. dirus* by a collector or human "attractant" positioned inside each of the tents. This procedure is replicated 4 times per week.

d. Results:

Preliminary data tested from October 2009-January 2010 showed nearly equal numbers of mosquitoes captured in both interception traps in the absence of metofluthrin (SumiOne®)-impregnated net.

e. Future Plans:

The actual experiment with metofluthrin (SumiOne®)-impregnated nets will be conducted in 2010. We proposed that over an 8-wk period, significantly fewer mosquitoes of either species will be captured in the treatment interception trap relative to the control trap.

Malaria Research

4. Title: Production of *Plasmodium vivax* Sporozoites to Support a Human Challenge Model

a. Investigator:

- Dr. Jetsumon Prachumsri, PhD

b. Objectives:

1) Provide mosquitoes with consistent, reproducible salivary gland infections to support *Plasmodium vivax* sporozoite challenge studies.

2) 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.

3) Provide slides of blood and exo-erythrocytic stages of *P. vivax* parasites for vaccine studies.

4) Provide qualified *P. vivax*-infected mosquitoes that can be used for human challenge studies.

c. Methods:

1) 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.

2) Refined Sporozoite Challenge System. In 2008 we prepared mosquitoes to be hand-carried to WRAIR as a first trial. The goal was to develop a system that will

i) consistently provide mosquito infection rates with >90% of blood-fed mosquitoes having +3/4 (>100 sporozoites) salivary gland infections, and

ii) provide *P. vivax*-infected mosquitoes that do not harbor concomitant pathogens. Consistency in the challenge is a critical component of any vaccine trial.

3) Parasite Characterization: This is part of routine quality assurance to obtain and use parasites with similar genetic compositions so results can be compared. In the absence of an in vitro culture system, it is necessary to feed mosquitoes on a *P. vivax*-infected volunteer or on blood from a volunteer. Since it is impossible to ensure that mosquitoes are infected with a single *P. vivax* clone (as is currently done with *P. falciparum*), it is critical that a method be in place to characterize the parasites (i.e., genetic diversity of the parasites, resistance to anti-

malarial drugs, etc.). Once mosquitoes are infected, parasites from the infectious blood meal are characterized by PCR using polymorphic gene targets, such as the nonapeptide repeat region of the circumsporozoite protein (PvCSP), and the region between interspecies conserved blocks 5 and 6 of the merozoite surface protein (PvMSP1).

4) There was an effort to develop a freezing system that can maintain viability and infectivity of *P. vivax* gametocytes. Varieties of cryoprotectant and freezing conditions were evaluated.

d. Results:

1) An updated standard operating procedure (SOP) has been prepared for the 2008-9 studies.

2) Sporozoites have been produced and used for preparation of IFA slide antigens and for the *in vitro* culture of liver stage parasites. An *in vitro* culture (HC04) of *P. vivax* parasites in human liver cells has been studied and validated for screening of antimalarial compounds. Parasite-infected liver cells were collected and prepared for the study of parasite genes and proteins.

3) Most of the cryoprotectants and freezing conditions evaluated maintained viability of asexual stage parasites with the exception of the gametocytic stages.

4) The transportation of *P. vivax*-infected mosquitoes to WRAIR proved to be successful. More than 80% of the mosquitoes survived during transport and up through 2 weeks post-feeding. Sprorozoite rates were found to be high and this method could be used for further studies at WRAIR.

e. Future Plans:

1) Continue to provide *P. vivax* parasites to support several drug and vaccine development studies. Blood collection from patients will continue in order to provide parasites for further studies.

2) We anticipate to collect 250-300 blood samples in 2009 and will provided 2-3 batches of infected mosquitoes for a human challenge study at WRAIR.

Ectoparasitic and Zoonotic Disease Vectors/Reservoirs

5. Title: Genotype Identification and Sequence Analysis of *Orientia tsutsugamushi* isolated from Scrub Typhus-Infected Chigger-Mite Colonies

a. Investigators:

- Kriangkrai Lerdthusnee, PhD
- Jason H. Richardson, PhD

b. Objective:

To determine genotype, nucleotide sequence homology and phylogenesis of *Orientia tsutsugamushi* from scrub typhus-infected *Leptotrombidium* chigger-mite colonies.

c. Methods:

O. tsutsugamushi strains isolated from 12 scrub typhus-infected chigger lines: 4 (Lc-1 to Lc-4) of *L. chiangraiensis*; 7 (Li-1 to Li-7) of *L. imphalum* and one (Ld-1) of *L. deliense*, will be identified based on the 56-kDA gene. Phylogenetic analysis will follow.

d. Results: Planning phase.

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

a. Investigators:

- Kriangkrai Lerdthusnee, PhD
- Jason H. Richardson, PhD

b. Objective:

To develop a molecular taxonomic key based on the cytochrome c oxidase I gene for the precise identification of trombiculid mites from AFRIMS colonies and from regions throughout Thailand.

c. Methods:

We will begin by first sequencing and then constructing phylogenetic trees for the non-infected scrub typhus *L. deliense,* followed by *L. imphalum, L. chiangraiensis, L. fletcheri* and *L. scutellare* chigger mite colonies at AFRIMS, followed by field-collected mites from elsewhere in Thailand, and then mites collected from other localities within Asia.

d. Results: Planning phase.

7. Title: Laboratory Evaluation of Candidate Repellent Compounds Against Larval Scrub Mites

a. Investigators:

- Kriangkrai Lerdthusnee, PhD
- Brian Evans, PhD

b. Objectives:

1) To design a laboratory bioassay that will accurately evaluate efficacies of repellent compounds against chiggers.

2) To determine the relative efficacies of candidate repellents versus (DEET) against chiggers.

c. Methods:

We are designing a laboratory bioassay based on the distance *Leptotrombodium chiangraiensis* mites walk in the presence/absence of a repellent. Determine the relative distance that chiggers walk in the presence of CO_2 (control), CO_2 and 33% DEET (control), and CO_2 and a candidate repellent (treatment). Candidate repellents include, at a minimum, IR3535 and picaridin.

d. Results: Planning phase.

8. Title: Development of a Field-Expedient Ectoparasite Trap

a. Investigators:

- Kriangkrai Lerdthusnee, PhD
- Jason H. Richardson, PhD
- Brian P. Evans, PhD

b. Objectives:

1) To determine the effects of carbon dioxide and mouse excrement on the walking distance of host-seeking chiggers towards a glue board.

2) To compare the trapping efficacy of A-Rat glue (Thailand) *versus* Stick-EM glue (USA) using various glue board modifications.

3) To evaluate the ability to retrieve mites from glue boards that have caught live rodents.

4) To compare the catching rate between the snap trap and live trap.

5) To compare the stickiness of double-sided tapes in capturing chigger mites.

6) To determine if a self-baiting (rodent) ectoparasite trap will out-compete prototype chigger traps developed in FY 08 at AFRIMS.

c. Methods/Results:

Our results show that chiggers are drawn to rodent attractants such as excrement and carbon dioxide. Based on this fact, we had attempted to devise a glue board trap that could Unfortunately, we found glue boards to be quite ineffective at collecting rodents. Secondly, even in cases where mites could be found on glue boards (presumably from the rodents), the ability to retrieve the mites was impossible due to the adhesive properties of the glue. An attempt was made to complement snap traps with a sticky tape around the perimeter so that rodents could conceivably be trapped and killed and the mites would subsequently fall off and be trapped in tape. No tape was effective in preventing the migration of mites. Based on these results, we have developed a prototype trap consisting of a 30 x 30 cm plastic board with a roof and covered on the inside by a black cloth. The trap is equipped with a snap trap and the cloth is treated with permethrin (to capture and kill mites from the dead rodent). We ran 3 field trials in central Thailand in which we compared the efficacy of this prototype chigger/rodent trap with that of a prototype trap that we developed in FY08 (our gold standard). While we continue to analyze the data, at first glance it appears that prototype self-baiting ectoparasite trap is more effective at collecting chigger mites than the gold standard from FY08.

DEPARTMENT OF IMMUNOLOGY AND MEDICINE

DEPARTMENT MISSION

To protect, project and sustain the military soldier against malaria threats produced by *Plasmodium falciparum* (*Pf*) and *Plasmodium vivax* (*Pv*). To support this mission through evaluation of new and improved vaccines, prophylactic and therapeutic drugs, rapid diagnostic kits, and the maintenance of a center of excellence focused on basic biology and epidemiology of drug resistant malaria.

To assess emerging febrile diseases along high risk regions of the Thai-Myanmar border in Southeast Asia.