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Award Number: DAMD17-01-2-0005

TITLE: Research and Operational Support for the Study of
Militarily Relevant Infectious Diseases of Interest to
United States and Royal Thai Governments

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REPORT DATE: January 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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20040329 034

REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE January 2004	3. REPORT TYPE AND DATES COVERED Annual (1 Jan 2002 - 31 Dec 2003)	
4. TITLE AND SUBTITLE Research and Operational Support for the Study of Militarily Relevant Infectious Diseases of Interest to United States and Royal Thai Governments			5. FUNDING NUMBERS DAMD17-01-2-0005	
6. AUTHOR(S) MG Suebpong Sangkharomaya, M.D. COL Sorachai Nitayaphan, M.D., Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Armed Forces Research Institute of Medical Sciences Bangkok 10400 Thailand <i>E-Mail:</i> snitayap@mozart.inet.co.th			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) Cooperative agreement # DAMD17-01-2-0005 was implemented January 1, 2001 to provide funding support for Royal Thai Army at Armed Forces Research Institute of Medical Sciences (AFRIMS) engaged in research activated in collaboration with US Army. Administrative, logistical, and scientific personnel required to support the ongoing US Army AFRIMS research efforts, and utilities and maintenance required to support the US Army AFRIMS research effort.				
14. SUBJECT TERMS No Subject Terms Provided.			15. NUMBER OF PAGES 64	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

FOREWORD

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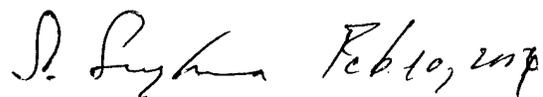
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 Feb 10, 2018

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I. INTRODUCTION

A. General

Collaborative studies into infectious diseases of military importance have been conducted at the Armed Forces Research Institute of Medical Sciences (AFRIMS) by both the US Army Medical Component (USAMC) and the Royal Thai Army Medical Component (RTAMC) for 4 decades. Studies leading to develop drugs and vaccines to combat tropical diseases of military relevant importance.

B. Statement of work

Administrative, logistical and scientific personnel required to support the ongoing US Army AFRIMS research efforts, and utilities and maintenance required to support the US Army AFRIMS research effort.

C. US ARMY AFRIMS research efforts at Department of Entomology

Department of Entomology research efforts are the following:

1. Transmission-dynamics of anti-biotic resistant scrub typhus.
2. Field and laboratory evaluation of novel arthropod repellents against the vectors of malaria, dengue and scrub typhus in Thailand.
3. Use of a Geographic Information System (GIS) to implement and evaluate the efficacy of targeted vector control as a means of reducing malaria transmission.
4. Development of a chigger-challenge model for the evaluation of candidate scrub typhus vaccines.
5. Optimization of sporozoite production to support a human Plasmodium vivax sporozoite-challenge model.

D. US ARMY AFRIMS research efforts at Department of Immunology

Department of Immunology research efforts are the following:

See page 26-27

E. US ARMY AFRIMS research efforts at Department of Enteric Diseases

Department of Enteric Diseases research efforts are the following:

1. Surveillance of Diarrheal Diseases in Adult Travelers and Residents from Developed countries and Thai Adults: Etiology and Antibiotic Susceptibility Pattern Transmission-dynamics of anti-biotic resistant scrub typhus.
2. Surveillance of Diarrheal Diseases in Travelers and Resident Expatriates in Nepal: Etiology and Antibiotic Susceptibility Pattern.
3. Characterization of Enteric Pathogens isolated from Children in Hanoi.

4. Case-Control Study of Endemic Diarrhea in Children in Sangkhlaburi; along the Thai-Myanmar border.

5. Development and standardization of realtime PCR assays for detection and characterization of enteric pathogens.

6. Amplified Fragment Length Polymorphism (AFLP) fingerprint method to characterize enterotoxigenic *E. coli* (ETEC) colonization factors (CFs) and to study the genetic relationship among serotypes of *Shigella flexneri*.

7. Mechanisms of Antibiotic Resistance in *Campylobacter jejuni*

8. Establishment of a non-human primate *Campylobacter* disease model prior to the pre-clinical evaluation of *Campylobacter* vaccine formulations.

9. Travelers' Diarrhea Among US Forces Deployed for Operation Cobra Gold

10. "Safety, dose, immunogenicity, and community transmission risk of a candidate *s.flexneri* 2a vaccine among young children in rural Bangladesh".

F. US ARMY AFRIMS research efforts at Department of Veterinary Medicine

Department of Veterinary Medicine research efforts are the following:

1. Antimalarial Drugs Efficacy Testing in the Rhesus Monkey (*Macaca mulatta*)/*Plasmodium cynomolgi* Relapsing Malaria Model

2. Pharmacokinetics of Novel Antimicrobial Drugs in Cynomolgus Monkeys

3. Care and Maintenance of Rhesus and Cynomolgus monkeys and Management of Breeding Colonies in support of USAMC-AFRIMS animal research needs

4. Care and Maintenance of Laboratory Rodents and Rabbits, Maintenance of Rodent Breeding Colonies, and Quality Assurance/Quality Surveillance Program in support of USAMC-AFRIMS animal research needs

5. A *Plasmodium berghei*-Mouse Model for Screening Blood-stage Antimalarial Drugs

G. US ARMY AFRIMS research efforts at Department of Virology

Department of Virology research efforts are following:

1. The Dengue Hemorrhagic Fever Project II: Continued Prospective Observational Studies of Children with Suspected Dengue

2. A Recombinant Hepatitis E Vaccine Efficacy Study In Nepalese Volunteers

3. Prospective Study of Dengue Virus Transmission and Disease in Primary School Children

4. Training and Workshops

5. Febrile Disease Surveillance, Kathmandu, Nepal

6. Hospital-based EID Surveillance, Kamphaeng Phet, Thailand

7. Influenza Surveillance in Southeast Asia

H. US ARMY AFRIMS research efforts at Department of Retrovirology, AFRIMS FY03 Research Efforts

1. Title of Research Project: Screening and evaluation of potential volunteers for a preventive HIV-1 vaccine trial in Thailand (RV148, HSRRB Log No.).

2. A Phase III Trial of Aventis Pasteur Live Recombinant ALVAC-HIV (vCP1521) Priming With VaxGen gp120 B/E (AIDSVAX[®] B/E) Boosting in HIV-uninfected Thai Adults (RV144, HSRRB Log No. A-11048, BB-IND 8795).

I. Space and Utilities Required

Funding under the cooperative agreement is also directed by the Principal Investigator to the provision of site maintenance including space and utilities management for both the RTAMC and the USAMC in support of research activities.

II. BODY

A. Department of Entomology, AFRIMS FY03 Research Accomplishments

1. Title of research project: Field and laboratory evaluation of novel arthropod repellents against the vectors of malaria, dengue and scrub typhus in Thailand.

a. Investigators:

LTC James W. Jones, PhD;
LTC Mustapha Debboun
Kriangkrai Lerthusenee, PhD
Ratana Sithiprasasna. PhD

b. Objectives:

1. Evaluate new repellent compounds against mosquito vectors of malaria and dengue using the human-skin (Klun and Debboun) bioassay. Determine ED50 and ED95 concentrations of each repellent against selected mosquito species (*Anopheles dirus*, *An. minimus*, *An. sawadwongporni*, *Aedes aegypti*, and *Ae. albopictus*).

2. Evaluate new repellent compounds against chigger vectors of scrub typhus using an in vitro assay. Determine ED50 and ED95 concentrations of each repellent against selected chigger species (*Leptotrombidium deliense* and *L. imphalum*).

3. Select sites for field evaluation of candidate repellents and gather base-line data on species composition and biting densities.

c. Methods:

1. We are able to evaluate up to 5 experimental compounds that have been approved for testing on human volunteers. Deet serves as the appropriate reference compound. ED50 and ED95 concentrations of each repellent can be determined against each of 5 selected mosquito species (*Anopheles dirus*, *An. minimus*, *An. sawadwongporni*, *Aedes aegypti*, and *Ae. albopictus*).

2. We can determine ED50 and ED95 concentrations of up to 10 experimental compounds against *Leptotrombidium deliense* and *L. imphalum*. Deet serves as the appropriate reference compound. Narrow bands of filter paper are each treated with a given concentration of a each repellent, with the control filter paper treated with ethanol only. A total of 4 concentrations of repellent is required in order to calculate the ED50/95 using a PROBIT Analysis Procedure. A band of repellent-treated filter paper is placed in a small plastic tube along

with an individual chigger. The number of times that each chigger crosses the band is determined every minute for 10 mins, and ED50 and ED95 doses (dose that repelled 50% and 95% of chiggers, respectively) calculated. The test is replicated a minimum 10 times for each tested dilution of each repellent.

3. Select field sites and obtain ethical approval for human testing at these sites.

d. Results (accomplishments during the period of January 2003 - December 2003):

Completed evaluation of repellent efficacy for all compounds supplied by the WRAIR Repellent Manager this year. We identified field sites for field repellent evaluation.

e Future plans:

Continued with repellent evaluations in FY04.

2. Title of research project: *Use of a Geographic Information System (GIS) to implement and evaluate the efficacy of targeted vector control as a means of reducing malaria transmission.*

a. Investigators:

LTC James W. Jones, Dr. Jetsumon (Sattabongkot) Prachumsri, Dr. Ratana Sithiprasasna, Dr. Benjawan Khuntirat, LTC Robert Scott Miller

b. Objectives:

This project was a continuation of the Ban Kong Mong Tha study in 2002. The focus is on evaluation of the efficacy of vector control techniques to interrupt malaria transmission. Established control strategies that were evaluated include use of permethrin-treated bednets and use of residual deltamethrin spray. Novel strategies that were evaluated include use of Mosquito Magnet trap to eradicate mosquitoes within a specific area, with impact on vector populations and malaria prevalence determined. We will continue to use GIS to evaluate mosquito (adult and larval), human, and parasite populations. This site will serve as a core site for development of a malaria vector control system.

c. Methods:

1. Site Mapping: LANDSAT images (30 meter resolution) of the village and surrounding area have been acquired and used to establish the GIS using ERDAS software. IKONOS images (1 meter resolution) of the study site are on order.

2. Adult Mosquito Collections: Each month, adult Anopheles mosquitoes are collected while attempting to bite collectors at selected sites throughout the village. The village has been divided into 11 grids (A-I, School, and Wat) -- monthly collections are made at 8

separate sites (in each in Grids A-H). Mosquitoes are identified to species, parity determined, and the abdomen and thorax of each individual mosquito tested by ELISA for *P. falciparum*, *P. vivax*-210, and *P. vivax*-247 circumsporozoite protein. The geographic and temporal distribution of each species is mapped out. A total of 9,618 adult anophelines representing 29 species have been collected during landing/biting collections. A total of 8,457 mosquitoes have been tested by ELISA, with 17 positive mosquitoes (0.2% infection rate). Mosquitoes were infected with *P. falciparum*, *P. vivax* 210, *P. vivax* 247, and mixed *P. vivax* 210/247.

3. Larval Mosquito Collection: Each month, larval Anopheles mosquitoes are collected within the village and surrounding areas to determine if there is an association between larval mosquito habitat and adult mosquito distribution in the town. GPS is used to mark the site of each larval mosquito collection. Larvae are returned to AFRIMS and emerging adults are identified to species. To date (Jun 99-Sep 01), a total of 7,203 larval Anopheles mosquitoes representing 32 species have been collected, reared to adults, and identified.

4. Human Malaria Surveillance: A human use protocol for this project received final approval from the US Army HSRRB and the Thai Ministry of Public Health in April, 2000. Villagers were entered into the study beginning in May, 2000, when baseline demographics and whole blood (200ul/person) from the entire volunteer population was obtained by fingerprick. During each subsequent month (currently May 2000-Sept 2001), 50-75% of the cohort was evaluated for malaria by fingerprick (thick- and thin-blood smears) and dipstick assay to determine Plasmodium prevalence. Additional blood samples were stored on filter paper and in microcapillary tubes for subsequent genetic analysis of parasites and determination of transmission-blocking antibody levels. Individuals positive for malaria in any given month have a 2.5-ml (individuals <10 years old) or 5-ml (individuals > 10 years old) blood sample drawn by venipuncture within 24 hrs of the finger-prick. Approximately 1-2ml of this blood sample is fed to mosquitoes in a membrane feeding system (this allows us to determine which particular individuals are capable of infecting mosquitoes at that point in time). Duplicate thick/thin blood smears are made at the time of the mosquito feed. An additional sample (1-2 drops) is stored on filter paper for PCR analysis for malaria. The remainder of each blood sample is separated into 0.1ml aliquots for evaluation of factors that might affect transmission. The goal is to determine the focality of transmission by tracking the spread of specific Plasmodium clones through the human population.

5. Establishment of the GIS and malaria modeling: Mapping out of the village (houses, road, building, rivers) and larval mosquito habitats has been completed. Current effort focuses on incorporating data (adult and larval mosquito collections, # of people per house, blood films collected, positive blood films, vegetation, etc.) into the GIS. Dr. Don Roberts and Mrs. Penny Masuoke in the Department of Preventive Medicine, USUHS, are collaborators on this project and are currently establishing the GIS. In addition, Dr. Richard Kiang at NASA is developing a plan for modeling of the interaction of vector mosquitoes and actual malaria.

6. Implementation of Malaria Control: Current effort has focused on evaluating the impact of the Thai Ministry of Public Health effort to spray all houses in the village with 5% Deltamethrin. Houses were sprayed in March and October 2000 and March and July 2001. In addition, permethrin-treated bednets were provided to 123 families in the village. Analysis suggests that neither of these two control methods had a significant impact on either vector populations or malaria rates.

In FY03: Collection of human/parasite/mosquito data continued as described above. Key components of the GIS include remote sensing data (vegetation and water sources), environmental (daily temperature, relative humidity, rainfall), demographic (house information, age, sex), parasitological (parasite species distribution, gametocyte rates, etc.), and entomological (adult and larval distribution and habitats as well as entomological inoculation rates) indices. Cluster analysis was used to identify relationships between malaria cases and adult mosquito distribution, and between adult mosquito distribution and larval distribution. The GIS was then used to identify key sites for intervention (larval and adult mosquito control, as well as treatment of malaria reservoirs with transmission-blocking antimalarials). The different grids in the town are randomly selected as control or treatment sites. Control sites receive no treatment, other than that normally provided by the Ministry of Public Health (normally chloroquine or mefloquine treatment of clinically-ill *P. vivax* or *P. falciparum* patients, respectively). The impact on malaria transmission within the village was determined. Subsequent to this study, we evaluated the efficacy of using the American Biophysics Corporation Mosquito Magnet™ (also known as the Counterflow 2000) Pro Model trap to control vector populations in selected areas within the village. Use of this trap has been advocated as a means of eliminating mosquitoes within an approximately 1-acre area.

d. Results (accomplishments during the period of January 2003 - December 2003):

The design of the Geographic Information System (GIS) was completed in Apr 03. Key components of the GIS include remote sensing data (vegetation and water sources), environmental (daily temperature, relative humidity, rainfall), demographic (house information, age, sex), parasitological (parasite species distribution, gametocyte rates, etc.), and entomological (adult and larval distribution and habitats as well as entomological inoculation rates) indices. Cluster analysis is used to identify relationships between malaria cases and adult mosquito distribution, and between adult mosquito distribution and larval distribution. Primary efforts focus on the evaluation of the efficacy of the American Biophysics Corporation Mosquito Magnet Pro Model trap to control vector populations in selected areas within the village. This portion of the study was initiated in May 02 and is scheduled to continue through Dec 04. The schedule for the study was as follows: May-Jun 2002: Pre-treatment months. Surveillance conducted without any traps. Jul-Dec 2002: Treatment months (Phase I). 2 control grids (B/H) and 2 treated grids (D/F). Jan-Jun 2003: Control months (no traps set out) Jul-Dec 2003: Treatment months (Phase II). 2 control grids (D/F) and 2 treated grids (B/H). Jan-Jul 2004: Follow-up months to evaluate residual impact on vector populations. The military has expressed an interest in using this trap for vector control. Grids B, D, F and H of the village are used as sites for this evaluation. In the first year of the study, Grids D and F were selected as treatment sites, and Grids B and H served as control sites. In the second year of the study, Grids D and F serve as control sites and Grids B and H are treated sites. Three Mosquito Magnet (MM) traps are set up in each of the two treatment areas (4 traps in Grid H), located within 50 meters of each other. Traps are run continuously (using propane and octenol packets as recommended by American Biophysics Corporation) for 6 months. Landing/biting (L/B) collections (2 people/house) are conducted monthly in a total of 6 houses in each treatment and control grid (6 houses per grid x 4 grids = 24 houses total). For 2 nights each month, mosquitoes are collected

from 3 of the 6 houses in each grid on nights 1 and 3, and from the remaining 3 houses on nights 2 and 4. This means that there is a total of 12 collections made per grid every month. Collections are made from 1800 to 2400 each night (our current data set reveals that approximately 70% of all mosquitoes are collected before 2400 hours). Collections using these procedures commenced in May 2002, with the MM traps switched on in Jul 02 and switched off in Jan 03. We switched the traps on again in Jul 03 (control and treated areas reversed); the traps will be switched off in Jan 04. To date (May 02-Jul 03) L/B collections in the four grids comprise 8328 anopheline mosquitoes, of which 14 (0.2%) or 27 (0.3%) were Pf- or Pv-positive, respectively. During Phase I (Jul-Dec 02), MM trap catches comprised 560 anopheline mosquitoes, including 275 (49%) *An. kochi* and 184 (33%) *An. maculatus* mosquitoes. The prevalence (%) of *P. falciparum* (PF) cases was higher in control grids compared to treated grids in each of the paired study areas (see Attachment #1). Current efforts are underway to fully analyze the data set from the Ban Kong Mong Tha study (U-0002-02-AF) and to work with the Thai Ministry of Public Health to further evaluate the efficacy of residual house spray and the impact of the treated bed-nets on the mosquito populations. Preliminary analysis of the data set (Jun 1999-Jan 2002) suggests that neither residual house spraying nor use of permethrin treated bed nets has had a marked impact on either mosquito populations or malaria prevalence rates. Due to the importance of these 2 methods towards malaria control in Thailand and other countries in SE Asia, we will focus on determining whether the vector populations are resistant to deltamethrin or permethrin and/or whether vector populations avoid treated areas. We also conduct further analysis of the resting behavior of anopheline malaria vectors to determine whether they are avoiding treated areas.

e. Future plans:

Collection of human/parasite/mosquito data will continue through December 2004 as described for the Ban Kong Mong Tha study (U-0002-02-AF). FY04 funding for this project was not obtained. Supplemental funding from a NIH grant (#1-R01-AI48813-01A1, 2001-2004, Population Dynamics of Sporogony in Thailand, Dr. Jefferson Vaughan, Primary Investigator) that complements this project will be used to support the evaluation of malaria vector control systems.

3. Title of research project: *Development of a chigger-challenge model for the evaluation of candidate scrub typhus vaccines.*

a. Investigators:

LTC James W. Jones, PhD; Dr. Kriangkrai Lerdthusanee, Dr. Benjawan Khuntirat

b. Objectives:

1. Conduct genetic characterization of *O. tsutsugamushi* infecting 12 colonies of *Leptotrombidium* chiggers sps. maintained at AFRIMS.
2. Evaluate the ability of each of the 12 chigger colonies to transmit *O.*

tsutsugamushi to laboratory mice. Down-select 4-5 key chigger colonies for further studies. These chigger colonies should be infected with different strains of *O. tsutsugamushi* and should produce consistent, high infection rates when fed on mice.

3. Focus efforts on building up down-selected chigger colonies to the high levels required for potential vaccine studies.

4. Develop methods for assessing the efficacy of candidate vaccines using the chigger/mouse model. Criteria used to assess efficacy must include quantification of rickettsemia in the mice; however, additional methods (clinical or immunological responses) may also be assessed.

c. Methods:

1. Characterization of Strains/Isolates of *Orientia tsutsugamushi*: The goal is to characterize the 12 strains of *Orientia tsutsugamushi* infecting our 12 chigger colonies in order to determine phenotypic and genotypic relationship between different strains

2. Evaluate the efficacy of chigger colonies to transmit *O. tsutsugamushi* to mice and down-select 4-5 key colonies: Colonies of mites were characterized by PFGE and DNA amplified fingerprinting to identify colony lines with the highest rate of infectivity to laboratory mice. These colonies are being monitored over several generations to ensure a "hot" strain capable of infecting >90% of recipient animals is maintained. In addition, control lines of uninfected chiggers have been monitored. This ensures that the mite colonies that are the best vectors of scrub typhus to be used in the mouse protection model. Additional studies need to be conducted to determine if intrathoracic inoculation of *O. tsutsugamushi* into uninfected chiggers can be used as a means of developing new colonies of infected chiggers. The ability to rapidly develop colonies of chiggers infected with newly identified strains (to include antibiotic resistant strains) of *O. tsutsugamushi* allows us to evaluate vaccine candidates against a wide variety of pathogens. Finally, we attempt to characterize mechanisms by which *Leptotrombidium deliense* and *L. chiangraiensis* become infected with *O. tsutsugamushi*, to include analysis of vertical (mite to mite) and horizontal (vertebrate to mite to vertebrate) transmission. The ability to infect mites from infected hosts would allow us to evaluate the ability of potential vaccine candidates to prevent the transmission of *O. tsutsugamushi* from mites to vertebrates and subsequently back into mites. This ability of a vaccine candidate could have important epidemiological implications.

3. Build-up key chigger colonies to levels sufficient to support vaccine challenge studies: The rearing and maintenance of *Leptotrombidium* chiggers is a long, slow process. The total life cycle (from egg to egg-laying adult) requires approximately 3 months (this is in contrast to 2-3 weeks for most mosquitoes). Each female chigger will only produce about 1000 eggs over her lifetime. Once a chigger colony is selected for use in vaccine trials, it requires approximately 6 months to build it up to a level required to support the trial.

4. Develop methods for assessing the efficacy of candidate vaccines using the chigger/mouse model. Initial efforts focus on determining the course of rickettsemia over time for the 4-5 strains of *O. tsutsugamushi* selected for further study and on the development and/or confirmation of diagnostic procedures (PCR, ELISA, etc.) to quantify rickettsemia in challenged mice. We will also evaluate the effect of chigger infection with specific strains of *O. tsutsugamushi* on potential indicators of immunity, to include lymphocyte transformation, morbidity (as quantified by food consumption, weight gain/loss, activity, etc.), and mortality (time to death following chigger infection).

d. Results:

Finished the first draft of the Chigger-Challenge model's SOP. Completing suggested changes. Currently preparing an animal-use protocol for the chigger-challenge model. Continuing to sequence the 56kDa polypeptides of the Lc-5 isolate from *Leptotrombidium chiangraiensis* and the other 3 *O. tsutsugamushi* isolates of Li-3, Li-4 & Li-7 (of the *Leptotrombidium imphalum* colonies). Establishing the procedures for quantifying the rickettsemia by the Real-time (RTQ) PCR of the *Orientia tsutsugamushi* Lc-1 strain, the isolated strain from the *Leptotrombidium chiangraiensis*. This particular *O. tsutsugamushi* isolate was a Karp-like strain. No problems associated with project.

e. Future plans:

Conduct mouse-challenge evaluation of candidate vaccines in FY04.

4.. Title of research project: *Optimization of sporozoite production to support a human Plasmodium vivax sporozoite-challenge model.*

a. Investigators:

LTC James W. Jones, PhD.; Jetsumon Prachumsri, Ph.D.; LTC Robert Scott Miller

b. Objectives:

1. Collaborate with the Department of Immunology, AFRIMS, in the development a sporozoite-challenge model for use in the evaluation of candidate *P. vivax* vaccines. The specific objective is to develop methods of producing mosquitoes with consistent, reproducible salivary gland infections that will minimize variation in the course of human infection following sporozoite challenge.

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. All requests for infected mosquitoes and/or sporozoites are routed through the PDP Coordinator to the Chief, Dept of Entomology, AFRIMS.

3. Conduct studies to improve the ability to infect mosquitoes using gametocytomic blood. Emphasis is on the development and validation of a membrane-feeding method for the infection of mosquitoes using blood obtained from gametocytomic patients

rereporting to Thai malaria clinics. Secondary studies focus on ensuring that laboratory-reared *P. vivax* infected mosquitoes (infected by feeding on gametocytemic persons) are not capable of transmitting concomitant infections to challenge volunteers. Additional studies focus on the development of methods to stimulate gametocytogenesis as a means of enhancing mosquito infection rates.

4. Conduct studies in support of DoD and NIH efforts to develop malaria vaccines, with key emphasis on the evaluation of candidate transmission-blocking vaccines and vaccines directed against exo-erythrocytic (liver) stage parasites.

c. Methods:

A. Our department received FY02 funding for the production of *P. vivax* sporozoites (Project A1-0001-02-AF). This FY03 proposal was a continuation of this project. This project had several key goals, to include the following.

A.1. Develop and standardize methods for infecting mosquitoes with *P. vivax* using gametocytemic blood obtained from infected individuals reporting to Thai malaria clinics. This study is ongoing. Although we routinely infect mosquitoes using this method (227 patient feeds conducted in FY01), the consistency of infection is still highly variable (not all gametocytemic blood infects mosquitoes, and even when mosquitoes are infected the infection rates are variable). Efforts in FY03 focused on developing methods to stimulate gametocytogenesis as a means of increasing infection rates and minimizing variability.

A.2. Provide *P. vivax*-infected mosquitoes to DoD investigators and/or collaborating institutions. Currently, *P. vivax* sporozoites and/or *P. vivax*-infected mosquitoes are available to DoD investigators for malaria studies at any time. Coordination for shipment of sporozoites and/or infected mosquitoes should be through the PDP coordinator to the Chief of the Entomology Department at AFRIMS.

B. Other goals of the project will follow those identified in the FY02 proposal, to include the following:

B.1. Refined Sporozoite Challenge System: Focus on refining the membrane-feeding system in order to reduce the variability in the mosquito infections (critical for ensuring consistent challenges) and to eliminate the risk of concomitant mosquito infections. The goal is to develop a system that will i) consistently provide mosquito infection rates with >60% 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. *Plasmodium vivax*-infected patients reporting to local malaria clinics will serve as the starting point for development of the "refined system." Instead of allowing mosquitoes to feed directly on gametocytemic patients, mosquitoes are fed on venous blood provided to them in an artificial membrane feeding system. A series of carefully controlled experiments is conducted using patient blood and the membrane feeding system. Each of these experiments will provide "stand-alone" data to support the development of

the sporozoite-challenge model; however, each experiment will build upon the information derived from the previous experiment. A description of each experiment follows:

a. Dilution of Blood to a standard Gametocyte Concentration: The number of gametocytes per 500 white blood cells (WBCs) is calculated from the thick blood smear. Blood group is checked, and autologous uninfected blood is used to dilute the infected blood to several defined gametocyte concentrations (i.e. 5, 10, and 20 gametocytes per 500 WBCs). Mosquitoes are fed on each diluted blood sample, and mosquito infection rates compared to that of the original patient blood sample. This experiment is repeated with 10 different patients.

b. Pooling of Blood Samples from 5 Infected Patients: Blood is collected from 5 separate patients, pooled, and used to infect mosquitoes using a membrane feeding system. Controls will consist of blood from each separate patient. Percent of mosquitoes with oocysts, mean number of oocysts per mosquito, percent of mosquitoes with sporozoites in the salivary glands, and mean number of sporozoites in the glands are the criteria used to evaluate variability. We hypothesize that pooling blood from infected patients will reduce inherent variability in the mosquito infections. In addition, pooling blood from several patients may offer the added benefit by increasing the genetic diversity of the sporozoite challenge, and thus may more truly evaluate the efficacy of any candidate vaccine.

c. Replacement of Patient Sera with Commercial Sera: Blood is collected from patients, and packed red blood cells separated from the sera and subsequently reconstituted with commercial sera. The reconstituted blood is fed to mosquitoes in a membrane feeding system and mosquito infections quantified. This method has the advantage of removing anti-malaria antibody that may affect gametocyte infectivity (10) and replaces patient sera that is potentially infected with concomitant acellular pathogens with commercial sera that is known pathogen-free.

d. Use of Frozen Gametocyte Preparations to infect Mosquitoes: One of the key factors that limits production of *P. vivax*-infected mosquitoes is the requirement for fresh blood that contains infective gametocytes. Since there is no standardized in-vitro system capable of routinely producing infective gametocytes, this means that we must either bring the mosquitoes to the gametocytemic patient or collect fresh blood from the gametocytemic patient and allow mosquitoes to feed on it within 24 hours. A thorough review of literature back to 1966 found no published studies that have evaluated the ability of cryopreserved gametocytemic blood to infect mosquitoes. However, erythrocytic stages *Plasmodium* are routinely cryopreserved, while Collins et al. (18) were able to use frozen sporozoite preparations to infect non-human primates with the Salvador I strain of *P. vivax*. In this portion of the study we propose to evaluate the ability of cryopreserved gametocytemic blood to infect mosquitoes. Effort will focus on establishment of cryopreservation techniques that will maximize gametocyte viability and on procedures to maximize the percentage of mosquitoes that will feed on the frozen blood. Development of procedures to infect mosquitoes using cryopreserved blood would allow for production of sporozoites from *P. vivax* specimens obtained from throughout the world. These

sporozoites could then be used in a variety of experimental models (i.e., we could use sporozoites obtained from East Timor in our hepatoma cell model to evaluate resistance of exo-erythrocytic stage parasites to primaquine and tafenoquine).

B.2. Parasite Characterization: In the absence of an in vitro culture system, it is necessary to feed mosquitoes on a *P. vivax*-infected volunteer or the 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 we develop a method of characterizing the parasites (i.e., genetic diversity of the parasites, resistance to antimalarial 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).

d. Results (accomplishments during the period of January 2003 - December 2003):

1. Enrichment of reticulocytes from normal blood did not yield enough reticulocytes for long term culture thus comparison of parasite growth in reticulocytes prepared from normal and cord blood was not possible. An alternative approach is to use stem cell culture as a source of reticulocytes.

2. PV infected blood collected and frozen at field sites did not yield good quality and quantity of parasites for long term culture and for gametocyte production compared to parasite culture prepared from fresh blood. A search for new reagents to use to improve blood cryopreservation will be accomplished in FY04.

3. Separation of old RBC cells by Percoll reduced number of parasites that can invade new red cells.

4. Short term culture at field clinics increased maturation and/or number of infective gametocytes. From 51 PV isolates, 15 of them were not infective for mosquitoes by membranae feeding before short term culture. After short term culture (6-36 hr), 6 of those isolates provided infective gametocytes that infected mosquitoes. Mosquito infection rate was increased for 40% by this method.

5. Characterization of PV gametocyte maturation will be accomplished in FY04.

e. Future plans:

Continue in FY04 to search for new reagents to use to improve blood cryopreservation.

B. Department of Immunology AFRIMS FY03 Research Accomplishments

1. Title of research project:

Number	Projects	Status
1	MRDD Phase III (687-2001)	In life completed 2002; Analysis
2	MRDD Phase II (818)	Completed; manuscript
3	MRDD Phase IIIb – Venous vs. Fingerstick	In life completed 2003
4	Binax MRDD – effect of treatment on performance	Completed; analysis
5	MRDD Comparison to LC-MS and PCR analysis	Protocol approved
6	Establishment of Scrub Typhus Gold Standard	Analysis complete; report
7	Human Malaria Vivax Challenge	Protocol dev't
8	RTS,S/TRAP Vaccine in Rhesus Neonates	completed; paper submitted
9	Rhesus PfMSP-1 Vaccine –Safety/Immunogenicity	completed; paper submitted
		Phase I completed; amendment in progress
10	Rhesus Pf AMA-1 Combinations Vaccine	
11	ELISPOT Automated Reader Validation	Completed
12	MSP-1 and Innate Immune cells	ILIR completed
13	PvDBP Polymorphisms	PhD project in progress
14	Dengue activates plasmacytoid DCs	Completed and published
		Completed; manuscript submitted
15	Schizont activates plasmacytoid DCs	
16	Tafenoquine Radical Cure Dose Ranging Part II	Manuscript in progress
17	Tafenoquine Measurement – in Prophylaxis	Completed; published
18	Tafenoquine Cure/Radical Cure Dose Ranging NIH	Study in progress- Mahidol U.
		Completed; Manuscript submitted
19	Phase II Assessment of Azithro-Quinine Combos	
20	Azithro-Quinine and Azithro Artesunate in Pf Rx	Protocol approved; start in 2004
21	Rhesus Artelinate (AL)/Artesunate (AS) Efficacy	Completed; Report submitted
22	Rhesus AL/AS Toxicology	Completed; Report submitted
23	Rhesus AL/AS PK/PD	Completed; Report submitted
		AS complete; LC-MS In progress
24	Bioassay/HPLC/LC-MS Validation - FDA	
25	Artesunate Phase I Protocol Development	In progress
26	IND and Investigator Brochure Completeion	In progress
		Completed; analysis; new analogs in synthesis
27	Assessment of Febrifugine Analogs	
		Completed; further development stopped
28	Assessment of Plasmepsin/MAK Kinases	

29	IV Artesunate/Methylene Blue Efficacy in Rhesus P. cynomolgi model	Protocol approved; PK completed
30	Fever Surveillance in Sangkhalburi	Ongoing
31	Flavivirus Surveillance	In progress
32	Leptospirosis in Sangkhalburi	Manuscript published; second in progress
33	Field validation of New In Vitro Sensitivity Assay	Completed; manuscript submitted
34	Molecular Assessment of Nepal Malaria Isolates	In life completed; in data analysis
35	Bangladesh In Vitro Pf Resistant assessment	Protocol approved; First of 5 year project completed
36	Cambodia In Vitro Pf Resistant assessment	Planning initiated
37	Thailand In Vitro Pf Resistant assessment	Ongoing
38	PF MSP-1 Genotyping – assay development	In progress;
39	Vivax Genotyping - PV Mahidol	Data analysis
40	Gametocyte Production for Entomology	In Progress

a. Investigators:

Dr. R. Scott Miller, MD; Dr. Mark Fukuda, MD; Dr. Victor Melendez Ph.D.; Dr. Sathit Pichyangkul, Ph.D.; Dr. Paktiya Teja-Isavadharm Ph.D.; Dr. Krisada Jongsakul, MD; Dr. Harald Noedl MD, PhD; Dr. Ruth Ellis MD.

Left in 2003: Dr. Suping Jiang, Ph.D., Dr. Chansuda Wonsrichanalai MD, Ph.D.

b. Objectives:

To protect, project and sustain the military soldier against disease threats produced by the 2 major species of malaria, *Plasmodium falciparum* (Pf) and *Plasmodium vivax* (Pv). To support this mission through the evaluation of new or improved vaccines, prophylactic and therapeutic drugs, rapid diagnostic kits, and the maintenance of a center for excellence focused on the basic biology and epidemiology of malaria. Secondly, to assess emerging febrile diseases along high-risk regions of SE Asia, particularly the Thai-Myanmar borders.

c. Methods:

The Department of Immunology and Medicine has applied as many kinds of classical and state-of-the-art technologies as possible to the above multi-faceted research. Clinical research included mobile epidemiology team able to work in adverse conditions where malaria is present, including field sample collection and processing screening, referencemicroscopy, assessment of rapid diagnostics, and a staff well-versed in conduct of clinical trails to GCP and ICH standards. The animal resrach teams are all trained in laboratory

animal research and regulations, current AALAAC requirements, and laboratory animal test and observation methods. State-of-the art methodologies are available for the studies of vaccine and drugs to include advanced molecular biology methods such as sequencing and SNP analysis, real-time pCR. Cellular immunology techniques are available which include flow cytometry and sorting technologies, ELISPOT and molecular methods. Pharmacology assays include HPLC, LC-MS, malaria bioassay, sustained malaria cell culture and radioisotopic uptake methods.

d. Results (accomplishments during the period of January 2003 - December 2003):

1. Malaria Vaccines STEP F/STO AF/STO A1

- Initiated development of a complex, multi-year project to develop a *P. vivax* human malaria challenge model. This model will allow better understanding of immunologic processes early in parasitemia, phase IIa pilot efficacy studies of candidate Pv vaccines, and testing of drugs with causal prophylactic and radical cure properties of Pv in a controlled setting. Efforts continuing in to FY04 to complete the validation of the model. MIDRP funded.

- Completed the analysis of a rhesus safety and immunogenicity protocol for a candidate Pf malaria multi-antigen, multi-stage vaccine. The vaccine involves combinations of RTS,S, MSP1₄₂, and AMA-1 candidate vaccines, adjuvanted with our lead agent, AS02A. 46 rhesus monkeys were immunized over a 3-month schedule, and the vaccine is safe and well tolerated. Antibody and cellular immunology assays suggest immune competition may occur at the doses chosen. Further testing of two groups was approved and funded by USAID, and in-life portions are now completed. This MIDRP effort is partnered with GlaxoSmithKline and USAID.

Completed evaluation of candidate Pf vaccine, MSP1₄₂, in rhesus, which is now in early phase human studies. MIDRP/USAID funded. Publication submitted.

Continued assessment of safety and immunogenicity of RTS,S/TRAP vaccine candidate in neonatal rhesus monkeys. GSK and WHO funded. Publication is accepted.

Investigated interplay of MSP-1 with the innate immune system and how this may cause aspects of severe malaria. The 33-kD component of MSP-1, which is cleaved into the blood stream upon parasite entry in the red cell, is a potent trigger of the innate immune system. Impact on vaccine development is being investigated, and this may be a target in prevention of severe malaria. ILIR funded.

Two new studies have been funded for FY04:

- GlaxoSmithKline will fund a safety and immunogenicity study of a prime boost strategy for SIV gag protein in rhesus monkeys using prime boost strategies with an adenovirus construct. This technology, which promises an enhanced CD-8 T cell

cellular responses may have utility in future malaria vaccine designs. Due to start in March 2004.

- Testing safety and immunogenicity of LSA-1 vaccine constructs in rhesus monkeys in summer 2004. MIDRP funded.

2. Malaria Drugs STEP Q, STO-AQ, STO-A4, STO-A5

- Reports completed of complex studies of a severe malaria model using *P. coatneyi* in splenectomized rhesus for efficacy testing of candidate intravenous artemisinins (lead agents for the new drug to replace quinidine). Presented for down-selection meeting in October 2002 and a pre-IND meeting to the FDA for IV artesunate in March 2003. Data presented at ASTMH in 2003. This MIDRP effort is partnered with the Medicines for Malaria Venture (MMV). Planned IND submission in mid 2004.
- Reports completed for the acute and subacute administration of intravenous artemisinin candidates (artelinate and artesunate) head-to-head in standard toxicology protocol (including neurotoxicity studies) in rhesus. Presented for down-selection meeting in October 2002 and a pre-IND meeting to the FDA for IV artesunate in March 2003. Data presented at ASTMH in 2003. Planned IND submission in mid 2004.
- Added LC-MS at AFRIMS for the primary purpose of high-throughput GLP testing of artesunate and its metabolites in the clinical development program. GLP validation in progress for planned Phase I human studies in FY04. MIDRP and MMV funded.
- Dr. Miller is lead on the clinical development team for IV artesunate. He is evaluating clinical trials sites in SE Asia, and completing the phase I protocols planned for execution outside Washington DC in summer 2004.
- Based on in vitro and rodent malaria data, a new study of the efficacy of IV artesunate and methylene blue combinations against *P. cynomolgi* malaria in rhesus was initiated. Protocol was approved in 2003, and PK curves of methylene blue in rhesus completed. Challenge start in early FY04. ILIR funded.
- Secured funding and developed a new Phase II dose ranging protocol of azithromycin/quinine and azithromycin/artesunate combinations for the treatment of uncomplicated falciparum malaria. Due to start in March 2004 in collaboration with the Hospital of Tropical Diseases, Mahidol University. Efforts are partnered with Pfizer and the NIH.
- Developed and approved a protocol to test tafenoquine (WR238605) in adults for evaluation of radical curative ability and pharmacokinetics in *P. vivax* malaria. Funded with NIH co-development grant with GSK, and partnered with Hospital of Tropical Diseases, Faculty of Tropical Medicine, Mahidol University. Phase II trial

initiated in October 2003, and expected to be completed by September 2004. Publication of previous dose-ranging studies of tafenoquine completed. Publication of prophylaxis study in the Royal Thai Army has been submitted.

- Screened derivatives of the febrifugine for antimalarial activity and toxicity in mouse models and cytotoxicity assays. Febrifugines are candidate drugs in the discovery phase of development, extracted from the Chinese herb, Chang Shan (*Dichroa febrifuga* Lour). This has led to renewed interest in the class and synthesis of new analogs at WRAIR.
- Supported parasitology requirements for continuing efforts to develop a hepatocyte cell line to screen activity in the liver of antimalarial drugs and vaccine candidates. Collaboration with Department of Entomology. MIDRP funded.

3. Diagnostics/Rapid Diagnosis of Malaria STEP-L/STO-L

- Completed the in-life and microscopic assessment portions of the definitive multi-center trial of a second-generation malaria rapid diagnostic kit (NOW ICT Pf/Pv) in Mae Sod (n=2400), in association with WRAIR, NAMRID Peru, USAMMDA and Binax Inc. Now in final data analysis and report is being developed.
- Developed protocol, and completed the in-life portion of the phase IIIb study of the same malaria rapid diagnostic device comparing its accuracy from samples collected from both fingerstick and venous samples. In life phase completed in August 2003, and final microscopic analysis in progress.
- Evaluated available scrub typhus tests to develop a gold standard for comparison to immunochromatographic scrub typhus (*Orientia tsutsugamushi*) rapid diagnostic tests in development.
- Tested two commercially available leptospirosis diagnostic tests at the Sangkhlaburi febrile diseases study site for preliminary assessment by the STO L committee. Analysis in progress.

4. Emerging Infectious Diseases (GEIS)

Epidemiology of Falciparum Malaria Drug Resistance Patterns in Asia:

- Continued surveillance activities throughout Southeast Asia (Bangladesh, Myanmar, Thailand and Vietnam) for threat assessment of multi-drug resistant malaria. Looked at new field sites in eastern Bangladesh, Nepal and northern Thailand (Chiang Dao). A new five-year effort was launched in Bangladesh in coordination with the ICDDR,B. No emergence of artemisinin resistance has been detected, but evidence that mefloquine resistance has spread across Myanmar and into Bangladesh. Data

presented in numerous forums and samples archived in the cryobank for further analysis as needed. GEIS funded, and coordinated with Public Health departments in the various countries.

- Completed an *in vivo* trial of efficacy of mefloquine along a high-risk area of the Thai-Myanmar border, Sangkhlaburi district. Results revealed mefloquine failure rates of 45%, prompting the government to change the treatment paradigms in this region. A follow-on study of the new regimen (mefloquine and artesunate) has revealed 100% efficacy. Funded by GEIS and an NIH R01 grant with University of North Carolina. Final publication submitted.
- Validated under field conditions a new non-isotopic method for *in vitro* drug resistance assays, which is simpler, as robust, and avoids radioisotopes. The method has been made available free of charge to the malaria research community as a public service (see <http://malaria.farch.net>). The test shows very reliable comparisons to the WHO microtest using a much simpler methodology. Funded by GEIS with support from Mahidol University and University of Vienna.
- Developed and executed a fever surveillance trial in the southeastern Terai region of Nepal with the objectives to ascertain the rates of malaria and assess Pf isolates for the severity of sulfadoxine pyrimethamine resistance by molecular methods. Genetic analysis is completed and in analysis. Funded by USAID and GEIS.

Surveillance of Febrile Diseases along the Thai-Myanmar Border:

- Continued a multi-year effort to establish infectious etiologies to undifferentiated fevers along the Thai-Myanmar border in Kanchanaburi province. Over 1000 persons enrolled. Malaria accounts for approximately 25% of adults with fever. Leptospirosis appears to be a frequent, but previously unrecognized cause of morbidity and mortality, as is spotted fever rickettsia. Our data has led to a change to local health treatments.
- Serological evidence two Spotted Fever rickettsias not described in Thailand has been made. Prospective study for rickettsial diseases including rodent isolation techniques has not yet yielded a pathogen. Tick and flea collection have yielded numerous new *Rickettsia*, and *Ehrlichia* organisms of unknown pathogenic potential. Data all published in 2003.
- Serologic studies suggest an unusual flavivirus infection in this population. Mosquito collections have revealed extensive local Tembusu virus transmission, and unusual JE-like viruses currently under evaluation at USAMRIID. Joint effort with Entomology, Vet Med and USAMRIID. Data presented in 2003 at ASTMH.

e. Future plans:

We plan to continue our multi-faceted emphasis on support for malaria product development in diagnostics, new drugs, and new vaccines. We anticipate involvement in the malaria rapid diagnostic testing until eventual US FDA licensure in late 2004, and development of a DoD wide effort on malaria microscopy QA procedures. Furthermore, we anticipate being the lead overseas lab for field-testing intravenous artesunate in phase I and II, as possibly phase III testing. We will continue efforts for tafenoquine development, especially towards an indication of radical cure for *Plasmodium vivax*. We will continue safety and immunogenicity testing of candidate malaria vaccines in rhesus, and progress towards vivax challenge studies for eventual human testing of vivax vaccines in Thailand. Emerging infection work in Sangkhlaburi will continue with emphasis on flaviviruses, leptospirosis and typhoidal illnesses, and this study will be expanded to another targeted site in Nepal. Lastly, we anticipate an expand role in regional malaria surveillance with a combination of in vivo, in vitro and genetic methods to define expanding malaria drug resistance.

C. Department of Veterinary Medicine AFRIMS FY03 Research Accomplishments

1. Title of research project: Antimalarial Drugs Efficacy Testing in the Rhesus Monkey (*Macaca mulatta*)/*Plasmodium cynomolgi* Relapsing Malaria Model.

a. Department:

Veterinary Medicine

b. Investigators:

Dr. Montip Gettayacamin

c. Objectives:

1) Use the rhesus monkey/*P. cynomolgi* *bastianellii* model to determine the effectiveness of new causal prophylactic and readical curative compounds which are being synthesized and developed by the US Army antimalarial drug development program.

d. Methods:

Malaria is one of the most important parasitic diseases worldwide. Traditional treatment for malaria includes drugs used to prevent disease (prophylaxis) and to cure the infection (therapeutic). Antimalarial drug screening in the rhesus monkey model is very effective for making comparisons between drugs. It is fairly rapid, relatively inexpensive, and makes

reliable predictions of how drugs will in act in man. Antimalarial drug screening in the rhesus monkey has played a key role in the development of every antimalarial drug licensed in the the US for the past 30 years. This model provides a mechanism to identify effective new drugs for the enhanced prevention and treatment of malaria infections.

e. Results:

The protocol successfully tested 4 new potential antimalarial compounds received from WRAIR. Two pyrroloquinazoline compounds showed partial prophylactic and radical curative activity at 3-12 mg/kg when administered orally.

f. Future plans:

We anticipate testing at least two new compounds over the next calender year.

2. Title of research project: Pharmacokinetics of Novel Antimicrobial Drugs in Cynomolgus Monkeys

a. Department:

Veterinary Medicine (in collaboration with WRAIR-ET)

b. Investigators:

MAJ Lloyd T. Phinney (AFRIMS), MAJ Michael Kozar (WRAIR)

c. Objectives:

The objective of this protocol is to assess the plasma concentrations of novel antimicrobial drugs after a single dose in a non-human primate, the cynomolgus monkey. We hypothesize that plasma concentrations of the drug occurs in non-human primates similar to the way it does in rats and mice. Serum chemistries will be assessed at the end of the study to evaluate for safety. Data from this study will be used in efficacy studies at United States Army Medical Institute of Infectious Diseases (USAMRIID). Five novel antimicrobial compounds will be tested under this protocol.

d. Methods:

DNA minor-groove binding ligands (antigenomics) are a novel class of compounds that demonstrate antimicrobial activity. Genesoft's GSQ7302 is a member of this class of compounds that bind to AT-rich regions of DNA which are common in bacteria and viruses, but rare in humans. This compound has demonstrated in vitro efficacy against Staphylococcus, Streptococcus, Anthrax, Small Pox, and Malaria. The research proposed here is

intended to measure the pharmacokinetics in nonhuman primates. Data collected here will be used in future efficacy studies.

e. Results:

One compound has been successfully administered to monkeys and pharmacokinetic analysis performed on blood samples.

f. Future plans:

We anticipate testing four additional compounds in the next year.

3. Title of research project: Care and Maintenance of Rhesus (*Macaca mulatta*) and Cynomolgus (*Macaca fascicularis*) monkeys and Management of Breeding Colonies.

a. Department:

Veterinary Medicine

b. Investigators:

Dr. Montip Gettayacamin, Mr. Sravuth Komcharoen

c. Objectives:

Maximize the production of specific pathogen-free rhesus and cynomolgus monkeys in the USAMC-AFRIMS production colony, using the best and most humane husbandry care, maintenance procedures, veterinary care, and disease surveillance and environmental enrichment procedures available.

d. Methods:

USAMC-AFRIMS maintains a breeding colony of rhesus and cynomolgus macaques using a closed colony system. Approximately 250 rhesus and 50 cynomolgus monkeys are used in the breeding program. Two types of breeding is managed: compatible male and female pairs are housed in special paired-type caging, and multiple harem groups are established and maintained in large gang cages. Harems consist of one breeding sire and 5-15 adult females. Newborn monkeys are weaned at approximately 6 months of age, and then are reared to adulthood in gang cages with other weanlings. All colony primates are tested routinely for the presence of infectious diseases that pose a threat to either the health of the colony or to personnel working with the primates. Humane use of the animals is assured by the intense oversight of the

Institutional Animal Care and Use Committee. Veterinary and technical care is extensive and continuous.

Whenever possible, animals are re-utilized in multiple protocols in order to optimize the use of this limited and essential resource.

e. Results:

Approximately 71 rhesus and 2 cynomolgus macaques were produced in the previous year.

f. Future plans:

Maintain and expand the colony by obtaining 20 new breeding males, increasing the number of paired housing cages, and placing breeding pairs in these new cages into additional animal rooms in the vivarium.

4. Title of research project: Care and Maintenance of Laboratory Rodents and Rabbits, Maintenance of Rodent Breeding Colonies, and Quality Assurance / Quality Surveillance Program.

a. Department:

Veterinary Medicine

b. Investigators:

Dr. Montip Gettayacamin, Ms. Anchalee Tungtaeng

c. Objectives:

Maintain a breeding colony of specific pathogen-free laboratory rodents to meet the scientific research needs of the USAMC-AFRIMS, using state-of-the-art knowledge, equipment, and facilities.

d. Methods:

USAMC-AFRIMS maintains breeding colonies of laboratory rodents to meet the needs of AFRIMS research. Using state-of-the-art equipment, knowledge, and facilities, production is matched to the anticipated needs of individual research projects. Extensive and thorough recordkeeping ensures that outbred strains remain outbred, and that inbred strains remain truly inbred. An extensive quality assurance/quality surveillance program, which includes serologic assessments as well as necropsy/histopathologic analysis, ensures that the

colony produces only high-quality disease-free animals. When necessary, new breeder stock is procured from a reliable vendor in the United States or Japan. Veterinary and technical care is extensive and continuous.

e. Results

Approximately 8,400 ICR mice (*Mus musculus*) and 1,000 hamsters (*Mesocricetus auratus*) were produced in support of USAMC-AFRIMS research mission. Quality assurance evaluations have verified the highest quality rodent colony.

f. Future plans:

These breeding colonies will continue to be maintained in order to provide a cost-effective means of supply of specific pathogen-free rodents to support USAMC-AFRIMS research needs.

5. Title of research project: A Plasmodium berghei-Mouse Model for Screening Antimalarial Drugs.

a. Department:

Veterinary Medicine

b. Investigators:

Dr. Montip Gettayacamin, Pranee Hansukjariya, Anchalee Tungtaeng

c. Objectives:

To evaluate potential antimalarial chemotherapeutic agents in the *P. berghei* ICR mouse - the modified Thompson Test model.

d. Methods:

The test system used for the determination of antimalarial activity of the compounds is a modification of the suppressive test known as the Thompson Test. Typically in this test, up to 22 groups of 8 mice are inoculated intraperitoneally (IP) with *P. berghei*-infected erythrocytes then treated with candidate drugs to determine the antimalarial activity. Infected erythrocytes are provided from donor mice. On experiment day 0, the donor mice are anesthetized then exsanguinated via cardiac puncture, the blood pooled and the level of parasitemia determined. The pooled blood is then diluted with normal mouse serum to a concentration of 1×10^6 *P. berghei*-infected erythrocytes per inoculum (0.1 ml). The groups of experimental and control mice are inoculated with this parasitized blood on day 0. On day 3, 4, and 5 mice are treated with either the

candidate antimalarial drug or with vehicle alone, to serve as the negative control. The drug is administered orally (PO), subcutaneously (SC), intramuscularly (IM), and/or intraperitoneally (IP) up to three times a day, based on the individual and unique pharmacodynamics of the test compound. Each experimental group receives a different dose level, with up to 7 different dose groups per compound. A standard antimalarial drug may be tested along with the candidate drug for structure-activity determination and for quality assurance of the model. Blood films and body weights are taken on the third and sixth days post-infection, then at weekly intervals through day 60. Blood films are stained, examined by light microscopy, and the percent parasitemia determined. All mice are observed twice a day to assess their clinical signs. All mice with negative smears at 60 days are considered cured.

d. Results:

A total of 11 compounds were tested in 9 experiments (Exp. 0080 to 0088).

e. Future plans:

This mouse model for screening new candidate antimalarial compounds has been used for over 30 years and is very effective for making comparisons between drugs. It is rapid, relatively inexpensive, and makes reliable predictions of how drugs will act in higher mammalian hosts, including humans. This is a core capability of the USAMC-AFRIMS Department of Veterinary Medicine and will be maintained so that many more compounds can be tested.

D. Department of Virology, AFRIMS FY03 Research Accomplishments

1. Title of research project: The Dengue Hemorrhagic Fever Project III: Continued Prospective Observational Studies of Children with Suspected Dengue

a. Investigators:

1. Principal Investigators:

1. Siripen Kalayanarooj, MD (Queen Sirikit Institute of Child Health, Bangkok)
2. Stephen J. Thomas, MAJ, MC (USAMC-AFRIMS)

2. Associate Investigators:

1. Mammen P. Mammen, Jr. LTC, MC (USAMC-AFRIMS)
2. Chunlin Zhang, DVM, PhD, MAJ, MS (USAMC-AFRIMS)
3. Chuanpis Ajariyakhajorn, DVM, Research Coordinator (AFRIMS)
4. Ananda Nisalak, MD (AFRIMS)
5. Pra-orn Supradish, MD (QSNICH)
6. Anchalee Krautrachue, MD (QSNICH)

7. Lawan Wongtapradit, MD (QSNICH)
8. Narong Nithipanya, MD (QSNICH)
9. Warangkana Ratanaprakarn, MD (QSNICH)
10. Anon Srikiatkachorn, MD, Assistant Professor (UMMS)
11. Daniel H. Libraty, MD, Assistant Professor (UMMS)
12. Irene Bosch, PhD, Assistant Professor (UMMS)
13. Alan L. Rothman, MD, Associate Professor, (UMMS)
14. Sharone Green, MD, Associate Professor, (UMMS)
15. Francis A. Ennis, MD, Director (UMMS)
16. Henry A. F. Stephens, Ph.D., Clinical Scientist and Head of Tissue Typing (University College London)

b. Objectives:

This study continues to investigate pathophysiologic mechanisms of illness resulting from dengue infections. Information gained from this study provides important insight into the methods of preventing and intervening in severe dengue disease. The project encompasses studies from 2003 to 2007.

c. Study Specific Objectives:

1. Characterize genetically and functionally the dengue virus-specific T lymphocyte response during, and after dengue virus infections (intracellular cytokine staining, HLA tetramers, T cell receptor gene usage);
2. Analyze interactions between dengue virus, virus-specific antibodies, and target cells in PBMC during acute dengue virus infections (quantify and characterize immune complexes, define the major cellular compartments in PBMC supporting dengue viral replication);
3. Determine if ultrasound or interstitial fluid albumin levels can predict early plasma leakage and shock. The ability to detect these shifts early in disease progression may help in prediction algorithms for DHF and permit early intervention with new therapies in the at-risk population
4. Assess the utility of plasma sNS1 levels in predicting disease severity for subjects with primary or secondary infection due to any of the four dengue serotypes
5. Analysis of the activation of innate immune responses in vivo during acute dengue virus infections (chemokine gene expression, inhibitory and activating NK receptor expression);
6. Identification of polymorphisms in immune response genes associated with disease manifestations and cellular immune responses during dengue virus infections (MHC class I and II, Fcγ receptor gene, KIR genes, NK receptors) and MHC class I chain-related (MIC) genes (ligands for lectin-like receptors);
7. Quantitation of viral burden in plasma and cell subsets of peripheral blood mononuclear cells (PBMC) for all four serotypes in primary and secondary dengue virus infections and

determine if there is a correlation between viral load in these compartments and disease severity;

8. Measurement of neutralizing antibody elicited by primary infections, over an extended period of time. Few long-term studies of antibody titer following dengue infection have been performed previously. Neutralizing antibody will be measured on study day 1, 6 months, 1 year, and annually thereafter. Understanding wild type responses will help to set realistic standards for vaccines. Mature secondary responses determined by neutralization six months or more after infection will be correlated with class II HLA type;
9. Determination of memory T-cell responses following primary and secondary dengue infections, over an extended period of time. Understanding wild type responses and the durability of these responses over time will be crucial in setting standards for testing of candidate dengue vaccines;
10. Continue sequencing portions of the dengue genome from patients with mild dengue fever and those with severe DHF/DSS to test a hypothesis that severity of disease is strain related. In addition, compare the kinetics of plasma viral load and immune responses in primary and secondary infections with different DV serotypes;
11. Evaluate the accuracy of sequentially measured semi-quantitative d-dimer assay, as compared to standard clinical parameters, at predicting the clinical progression to severe clinical dengue.

d. Methods:

Children were enrolled if they were suspected of having an early DV infection (without evidence of DHF) or a fever without an identifiable source. Inclusion criteria included an oral temperature $\geq 38.5^{\circ}\text{C}$, fever onset not longer than 72 hours prior to the initial evaluation, weight $> 6\text{kg}$, flushed face, signed consent by parent or guardian. After informed consent is obtained, subjects are admitted to the hospital and a blood specimen obtained. The result of the plasma test for DV RNA by RT-PCR is available the morning of study day 2. Children who are DV RT-PCR-negative are given the opportunity to leave the study, or to continue in the study for clinical observation. Those children remaining in the hospital undergo inpatient observation until one day following defervescence (fever day +1). Clinical information is collected and recorded daily. Radiographic studies are performed as outlined in the protocol. Serial blood samples are collected and analyzed for routine and dengue-specific blood and plasma tests were conducted to include, but not limited to:

1. CBC, WBC differential, AST, Albumin
2. Hemagglutination inhibition (HAI) assay for dengue
3. Antibody-capture DV IgM/IgG enzyme immunoassay (EIA)
4. RT-PCR for dengue, Plasma viremia titers
5. Dengue virus isolation in *Toxorhynchites splendens* and typing
6. IL-15, IL-18, MIP-1a, MIP-1b, and MCP-1, CD69, CD38, and Ki-67
7. Labeled antibodies to identify T cell subsets, NK cells and B cells
8. NS1 (soluble NS1 and anti-NS1 antibodies)
9. Complement assays

e. Results (January 2003- December 2003):

The logistical and scientific framework required to support the DHFIII Project has been created. The study protocol and supporting documents have been composed and reviewed by all required IRBs.

f. Future plans:

1. Study Subject enrollment is slated to begin in FEB 2004 following review and approval by all IRBs.

2. Title of research project: A Recombinant Hepatitis E Vaccine Efficacy Study In Nepalese Volunteers

a. Investigators:

1. Principal Investigators:

1. M. P. Shrestha (WARUN)
2. R. M. Scott (WARUN)

2. Associate Investigators:

1. G. B. Thapa (SBH)
2. K. S. A. Myint (AFRIMS)
3. R. A. Kushner (WRAIR)
4. D. M. Joshi (SBH)
5. M. P. Mammen (AFRIMS)
6. B. L. Innis (GSK)

b. Objectives:

To evaluate the protective efficacy for the prevention of hepatitis E disease provided by the candidate hepatitis E vaccine administered according to a 0, 1 month schedule with a booster dose at month 6.

c. Results (January 2003 - December 2003):

A candidate recombinant baculovirus expressed hepatitis E virus (HEV) vaccine was found to be safe and immunogenic in 88 American and 44 Nepali volunteers. A 20µg formulation was selected for further evaluation in a randomized double blind placebo controlled efficacy trial in susceptible, active duty Royal Nepal Army volunteers. Of 5,571 consenting

volunteers screened, 3,113 were susceptible to HEV. Two thousand volunteers (5 females, 1,995 males) were enrolled, receiving either placebo or 20µg of active vaccine. Volunteers were vaccinated at 0, 1, and 6 months with sera collected at months 0, 1, 3, 6, 7, 13, and 24. One tenth of the volunteers were followed on days 1, 3, 5, and 7 after each vaccination for local and general solicited adverse events (SoAE). Non-serious adverse events (NSAE) were recorded for 30 days after each vaccination and serious adverse events (SAE) were to be collected throughout the 2-year study period. Sera and stool from cases meeting clinical and biochemical criteria compatible with viral hepatitis, were examined for HEV RNA by a reverse transcriptase-polymerase chain reaction, and serologically for HEV IgM and Ig, HAV IgM, HBsAg, HBeIgM and HCV IgG.

One thousand eight hundred and twenty five (1,825) volunteers received a three vaccine series. >86 HEV cases occurred during the two year period. Clinical phase closure was on 16 DEC 03 and unblinding these cases will occur in early 2004 and allow for determinations of vaccine efficacy.

Preliminary Adverse Event Data:

ADVERSE EVENT	Dose 1 N=2000	Dose 2 N=1890	Dose 3 N=1828
Local	109 (5.4%)	84 (4.4%)	70 (3.8%)
Redness	24 (1.2%)	9 (0.5%)	20 (1.1%)
Swelling	19 (0.9%)	9 (0.5%)	11 (6.0%)
Pain	106 (5.3%)	82 (4.3%)	64 (3.5%)
Generalized	84 (4.2%)	59 (3.1%)	46 (2.5%)
Fever	6 (0.3%)	1 (0.01%)	5 (0.3%)
Headache	56 (2.8%)	45 (2.4%)	39 (2.1%)
Fatigue	56 (2.8%)	35 (1.9%)	31 (1.7%)

d. Future plans:

Additional clinical studies planned on safety and immunogenicity in adult females, adolescents and in chronic liver disease patients.

3. Title of research project: Prospective Study of Dengue Virus Transmission and Disease in Primary Schools and Villages in Kamphaeng Phet, Thailand

a. Investigators:

1. Principal Investigators:

1. Suwich Thampolo, MD, MPH, Dengue Office, Division of Vector-Borne Diseases, Ministry of Public Health (MOPH)
2. Mammen P. Mammen Jr, LTC, MD, MC (USAMC-AFRIMS)

2. Associate Investigators (by institution):

Armed Forces Research Institute of Medical Science (AFRIMS):

Department of Virology

1. Ananda Nisalak, M.D., Consultant in Arbovirology
2. Stephen Thomas, M.D., MAJ, MC, Assistant Chief and Head of Field Operations
3. Chunlin Zhang, DVM, Ph.D., MAJ, MS, Head, Laboratory Operations
4. Chuanpis Ajariyakhajorn, DVM, Research Coordinator

Department of Entomology

1. James Jones, Ph.D., MAJ, MSC, USA, Departmental Chief
2. Ratana Sithirprasana, Ph.D. Candidate and Head, Mosquito Biology Section

Thai Ministry of Public Health (MOPH):

1. Virat Puthimethee, M.D., Office of the Provincial Public Health, KPP
2. Supamit Chunsuttiwat M.D., Senior Medical Officer, Ministry of Public Health
3. Somsak Prajakwong, M.D., Director of Vector-borne Disease Control Office

Institute of Urology and Nephrology, University College London, The Middlesex Hospital,

1. Henry A. F. Stephens, Ph.D., Clinical Scientist and Head of Tissue Typing

Center for Infectious Disease and Vaccine Research, University of Massachusetts Medical School (UMMS):

1. Anon Srikiatkachorn, M.D., Assistant Professor, e-mail: anons@thai.amedd.army.mil
2. Daniel H. Libraty, M.D., Assistant Professor, e-mail: daniel.libraty@umassmed.edu
3. Alan L. Rothman, M.D., Associate Professor, e-mail: alan.rothman@umassmed.edu
4. Sharone Green, M.D., Associate Professor, e-mail: sharone.green@umassmed.edu
5. Francis A. Ennis, M.D., Director, e-mail: francis.ennis@umassmed.edu .

Department of Entomology, University of California, Davis:

1. Thomas W. Scott, Ph.D., Professor of Entomology and Director, Davis Arbovirus Research Unit
2. John D. Edman, Ph.D., Professor of Entomology and Director, Center for Vector-Borne Diseases
3. Amy C. Morrison, Ph.D., Assistant Research Entomologist

Department of Geography, San Diego State University:

1. Arthur Getis, Ph.D., Stephen and Mary Birch Chair of Geographical Studies

b. Objectives:

The goal of the proposed study is to identify those factors that have the strongest influence on determining the early events in acute DV infections, and the eventual clinical manifestations of disease. An equally important goal is to characterize protective immune responses (e.g. CD4⁺ and CD8⁺ T-cell responses, neutralizing antibody responses) as we have found that low levels of pre-existing neutralizing antibodies to a subject's own infecting virus isolate do not necessarily protect from symptomatic DV infection. We plan to prospectively identify host-specific factors (e.g. pre-existing memory T and B cell responses to DV, HLA genetic polymorphisms, viral burden and replication in the host), virus-specific factors (e.g. DV serotype, serotype infection sequence), and environmental factors (e.g. mosquito population patterns, mosquito viral burden) for asymptomatic and symptomatic secondary DV infections, particularly severe infections (DHF/DSS). Multi-year investigations are crucial to this study due to the year-to-year variations in the incidence and prevalence of circulating serotypes. An improved understanding of the correlations between the host, viral, and environmental factors and dengue disease severity will contribute to DV vaccine development and testing.

c. Study Specific Hypotheses:

1. Subjects with pre-existing neutralizing dengue antibodies above a definable threshold will be protected from DV infection or severe disease on subsequent exposure to virus.
2. The frequency of pre-existing CD4⁺ and CD8⁺ T-cells and their specific cytokine responses to stimulation with DV antigens will correlate with disease severity (protection or enhancement) and the plasma viral RNA levels measured in secondary DV infections.
3. Specific serotype sequence combinations of DV infections will elicit qualitatively and quantitatively distinct immune responses associated with illness of varying severity.
4. Higher viremia levels will be seen in secondary DEN-2 and DEN-4 virus infections in subjects with higher levels of *in vitro* antibody-dependent enhancing capability of pre-illness blood samples.
5. DV infection rates will cluster in households around a DV-infected index case and a correlation will exist between the number of susceptible contacts, and associated mosquito density, and mosquito infectivity (viral RNA levels).
6. DV disease severity will correlate with peak plasma viremia levels and associated mosquito density and mosquito infectivity (viral RNA levels).
7. Genes encoded within the human MHC, the NK killer inhibitory receptor (KIR) gene complex on chromosome 19, and the Fc gamma receptor gene complex on chromosome 1 influence the susceptibility, severity and resistance to primary and secondary DV infections.

d. Methods:

We propose to:

- a) Continue the successful prospective, school-based, study platform to study dengue epidemiology in primary school children in KPP province, and
- b) Add a village-based, cluster surveillance study.

a) This will be a prospective school-based study of 2,000 children, which will begin upon study approval in 2003 and end in January 2008. Students in K2 to grade 6 will be recruited and enrolled into the study in June-July 2003 or upon study approval. Baseline demographics will be recorded and study numbers assigned. Each subsequent year, new K1-Grade 5 students will be newly enrolled. Students will be followed until they are either disenrolled, withdrawn by their parent/guardian, graduate from Grade 6 or when the study ends. Every year, plasma (plasma and PBMCs for Dengue Season 1 only) will be collected from the entire cohort at the beginning of the surveillance period (June). Plasma and PBMC will then be collected from the entire cohort at the end of the surveillance period (January). A one-dilution neutralization assay will be performed on paired sera from the beginning and end of the surveillance period to assess for flavivirus seroconversion. Plasma and PBMCs obtained at the end of the surveillance period in January will serve as pre-illness samples in subjects who have a DV infection that same calendar year.

During the active surveillance period extending between June and the following January, those children who are absent from school (or who report ill to the teacher), will be evaluated either by a VHW or AFRIMS nurse using a questionnaire and oral temperature measurement. Any child who has a documented fever (temperature $\geq 38\text{C}$) or reports illness with subjective fevers during the prior 7 days, will be transported to the PHO where a public health nurse will do an evaluation. An acute blood specimen will be drawn. The child will be referred to hospital at the discretion of the public health nurse. About 14 days later, an AFRIMS nurse will visit the child to administer another questionnaire and to draw a convalescent blood specimen. The acute and convalescent specimens will be evaluated by dengue/JE ELISA and HAI. The acute specimen will be evaluated further by RT-PCR (and virus isolation techniques).

b) Cases 'triggering' a cluster investigation will be identified between Monday and Thursday of each week during the School-Based Component active surveillance period. Most specimens from acutely ill children will arrive at the field station laboratory by 3pm each day. Upon arrival of the specimen, the database will be reviewed to assess whether the child meets all index case inclusion and exclusion criteria. The field teams will be notified of a possible case. The DV RT-PCR result (positive or negative) will normally be available by 11AM the following morning. No more than 30 positive and 30 negative clusters (as defined by the RT-PCR result of the index case) will be initiated in any given year. Once triggered, an Advance Team composed of a nurse and an entomological team supervisor will visit the village and begin the consent form process. The exact location of all houses in each participating village will have been previously determined using a Global Positioning System (GPS) unit. Data points will be used to construct a

digital map which will enable the team to precisely identify houses located within 100-200 meter radius (the exact radius to be pre-determined based on the prevalent average density of homes across all villages) of the index case and rapidly assess the likelihood of enrolling a minimum of 10 contacts. Once at least 10 contacts have been consented, the field teams will be dispatched to the village where the consent form process will continue. A reasonable effort will be made to contact the village leader. If the village leader is available, he or she will be requested to facilitate contact and communication with identified households. A clinical nurse will review the consent form, answer questions, address parental concerns, and obtain informed consent from the parents of susceptible contact children (ages 6 mo-15 yrs) residing within a pre-determined meter radius of the index household. Following the acquisition of parental consent, blood samples will be collected from 10-25 contacts. Those parents (and children) who are unavailable to be consented (and bled) will be visited that same evening or the following morning. The clinical team will return to these homes approximately 5, 10 and 15 days after the initial visit to perform clinical assessments. The children bled on day 0 (initial specimen) will be re-bled on approximately day 15 (follow-up specimen). DV RT-PCR will be performed on all acute specimens. DV IgM/IgG ELISAs will be performed on paired initial and follow-up specimens.

An entomological team will collect mosquitoes, administer questionnaires, and perform insecticide spraying within the pre-determined meter radius of the index household (or an alternative specific pre-determined radius based on pre-study assessments of average household densities within the villages and experience gathered from Dengue Season 1). Another entomological team will collect mosquitoes but not perform insecticide spraying around the classroom and school bathroom areas of the index case.

e. Results (January 2003 - December 2003):

The first meeting of study collaborators and sponsors (10 principal teachers, 10 health teachers, 5 heads of public health office, 24 village health workers and village leaders) occurred on 10 July 2003. Between 19-21 August 2003, five workshops were held for public health office heads, health teachers, and village health workers. Following local, US Army, and collaborating institution IRB approval, study subject enrollment from 10 schools commenced between 17 November to 19 December 2003.

To date 1,977 children (from 2,241 or 88.22 % of the total number of the children) have been enrolled from 10 schools. The goal of 2,000 children is being pursued. Village based cluster investigation logistical dynamics continue to be refined. KAVRU has officially opened (9 JUL 03) and is being supplied and its personnel trained to support the diagnostic requirements of this study.

f. Future plans:

1. Enrollment is designed to reach 2000 children.
2. Logistical and laboratory infrastructure shall be expanded and refined.

3. Serum samples shall be acquired and the surveillance and cluster investigations shall commence.

4. Project Title: *A phase I/II trial of a tetravalent live attenuated dengue vaccine in flavivirus antibody naive children*

a. Background:

The US Army seeks to acquire a licensed vaccine capable of protecting soldiers and their families from disease caused by infection with the dengue viruses. The Kingdom of Thailand shares this goal. For over 50 years the US Army has been active in developing and testing various vaccine candidates. This study represents the first use of the most promising Army dengue vaccine candidate in an overseas (Thailand), non-adult (children) population.

b. Objectives:

1. To demonstrate the WRAIR tetravalent dengue vaccine is safe and well-tolerated in a small number of Thai children between the ages of 5 and 9 years.

c. Methods:

1. Screen and enroll between 5 and 10 healthy, flavivirus naïve, Thai children between the ages of 5 and 9 years
2. Provide 2 doses of the WRAIR tetravalent dengue vaccine as outlined in the study protocol
3. Closely monitor the children following each dose of vaccine for safety and tolerability

d. Principal Investigators:

1. Stephen J. Thomas, MAJ, MC, USAMC-AFRIMS
2. Sriluck Simasathien, MD, Phramongkutklao Hospital (PMK), Bangkok, Thailand

e. Additional Study Personnel:

1. Dr. Rudiwilai, PMK Hospital
2. Dr. Angkool, PMK Hospital
3. Dr. Veerachai, PMK Hospital
4. Dr. Ananda, AFRIMS

5. Drs. Innis and Schuind, GlaxoSmtih Kline
6. COL Sun and LTC Gibbons, WRAIR
7. LTC Mammen, USAMC-AFRIMS
8. Dr. Eckels, WRAIR
9. Dr. Putnak, WRAIR
10. Celia Barberousse, GSK
11. CPT Michael VanHoven, USAMC-AFRIMS

f. Results

1. Seven children were ultimately enrolled and have received 1 dose of the WRAIR dengue vaccine
2. All children tolerated the vaccine well
3. Over 70 children who were screened but not enrolled received 2 doses of the Thai GPO JE vaccine as a benefit to participation

g. Future

1. Seven children will receive the second dose of the WRAIR dengue vaccine
2. Safety and tolerability will be monitored and measured

5. Project Title: *A phase I/II trial of a tetravalent live attenuated dengue vaccine in flavivirus antibody naive infants*

a. Background:

The US Army seeks to acquire a licensed vaccine capable of protecting soldiers and their families from disease caused by infection with the dengue viruses. The Kingdom of Thailand shares this goal. For over 50 years the US Army has been active in developing and testing various vaccine candidates. This study represents the first use of the most promising Army dengue vaccine candidate in an overseas (Thailand), infant population.

b. Objectives:

1. To demonstrate the WRAIR tetravalent dengue vaccine is safe and well-tolerated in a small number of Thai infants between the ages of 12 and 15 months.

2. To assess the immunogenicity of the dengue vaccine in terms of seroconversion 30 days post-dose 2 of dengue vaccine

c. Methods:

1. Screen and enroll 51 healthy, flavivirus naïve, Thai infants between the ages of 12 and 15 months
2. Provide 2 doses of the WRAIR tetravalent dengue vaccine as outlined in the study protocol
3. Closely monitor the infants following each dose of vaccine for safety and tolerability
4. Assess the immunogenicity of the dengue vaccine as outlined in the study protocol

d. Principal Investigators:

1. Mammen P. Mammen, Jr. LTC, MC, USAMC-AFRIMS
2. Sriluck Simasathien, MD, Phramongkutklo Hospital (PMK), Bangkok, Thailand

e. Additional Study Personnel:

1. Dr. Rudiwilai, PMK Hospital
2. Dr. Angkool, PMK Hospital
3. Dr. Veerachai, PMK Hospital
4. Dr. Ananda, AFRIMS
5. Drs. Innis and Schuind, GlaxoSmtih Kline
6. COL Sun and LTC Gibbons, WRAIR
7. MAJ Thomas, USAMC-AFRIMS
8. Dr. Eckels, WRAIR
9. Dr. Putnak, WRAIR
10. Celia Barberousse, GSK
11. CPT Michael VanHoven, USAMC-AFRIMS

f. Results

1. None as of JAN 2004.

g. Future

1. The protocol is currently undergoing local and US Army ethical review. Administration of dose #1 of the dengue vaccine is planned for late FEB 2004.

6. Training and Workshops

a. Background:

The Department of Virology, Armed Forces Research Institute of the Medical Sciences (AFRIMS), Bangkok, Thailand, seeks to expand its diagnostic capabilities in South and Southeast Asia by improving regional laboratory capabilities through the dissemination of diagnostic kits and the training of technical personnel.

b. Goals:

1. To create and improve the laboratory infrastructure of South and Southeast Asian regional laboratories specializing in infectious disease surveillance.
2. To provide the training of laboratory personnel (technicians and supervisors) working in South and Southeast Asia and beyond in infectious disease diagnostic techniques.

c. Activities:

The department conducted numerous on-site and in-house diagnostic training activities.

1. Over 50 student scientists from Srinakharinvirotj University, Chulalongkorn University, Phramongkutklao Medical College, Faculty of Tropical Medicine and Mahidol University received 2 to 4 weeks of training at AFRIMS in diagnostic laboratory modalities.
2. On-site training (Kathmandu, Nepal) in the proper performance of the AFRIMS JE EIA and basic instruction in QA and QC principles was provided to representatives of numerous Nepali health institutions in an exercise facilitated by the the Environmental Health Project (EHP).
3. Training in the proper performance of the AFRIMS JE EIA and basic QA and QC principles was provided to visiting scientists from the National Institute of Hygiene and Epidemiology, Hanoi, Vietnam, and National Institute of Pediatrics, Hanoi, Vietnam.
4. During the past year diagnostic kits and training were provided to the following laboratories:

B.P. Koirala Institute of Health Sciences, Dharan, Nepal

Nepal Public Health Laboratory, Kathmandu, Nepal
Teku Hospital, Kathmandu, Nepal
Bheri Zonal Hospital, Nepalgunj, Nepal
Institute of Medicine, Kathmandu, Nepal
NAMRU-2, Jakarta, Indonesia
ICDDR, Dhaka, Bangladesh
Department of Medical Research, Yangon, Myanmar
Pasteur Institute, Ho Chi Minh City, Vietnam

7. Febrile Disease Surveillance, Kathmandu, Nepal

a. Background:

The Dept. of Virology, AFRIMS, and the Walter Reed Army Research Unit, Nepal (WARUN) field office, have completed several cohort studies on hepatitis E virus (HEV) over the past 16 years. These efforts have established excellent rapport with the health care providers at the Teku and Shree Birendra Hospitals. AFRIMS continues collaborations with the Environmental Health Project (EHP) and Nepal Ministry of Health (MOH) on the study of flavivirus seroprevalence in Terai, the area of tropical lowlands across the southern portion of Nepal bordering India. Terai is a breeding area for vectors, which transmit malaria, kala-azar, and JE. While the incidence of the infectious diseases responsible for these syndromes appears to be increasing, there is no organized national surveillance program in Nepal to monitor for febrile illnesses or emerging infectious diseases.

b. Goals:

1. To determine if the etiologies of fever in travelers and populations indigenous to Nepal are emerging diseases e.g., viral hepatitis, dengue, JE, leptospirosis, and scrub typhus.
2. To establish a surveillance system in selected hospitals and points of health care delivery for the purpose of defining the epidemiologic and clinical characteristics of encephalitis in Nepal.
3. To determine the incidence and etiology of vector-borne febrile illnesses in Nepal.

c. Methods:

A multi-departmental effort was initiated in establishing a fever surveillance system utilizing the Nepali patient base, Nepali physician collaborators, and the diagnostic capabilities of AFRIMS and its collaborators.

d. Progress:

During the past year composition of a human use protocol and supporting documents was initiated. Two visits to Nepal were completed with assessments of the clinical and laboratory resources present at five potential collaborating hospitals. Investigators anticipate

achieving protocol approval and enrollment initiation in March-June 2004. Terrorist activities in and around Kathmandu may pose as an obstacle to achieving our research goals.

8. Hospital-based EID Surveillance, Kamphaeng Phet, Thailand

a. Background:

Kamphaeng Phet Provincial Hospital is located in northwestern Thailand and serves both urban and rural communities with a large population of hilltribe Thais and Burmese. This unique environment offers an opportunity to study and identify emerging diseases with potential impact on regional and national health issues. In Thailand, Leptospirosis is a reportable disease. The annual incidence of Leptospirosis, based on passive surveillance, has markedly increased from 0.3/100,000 in 1982-1995 to 3.3/100,000 in 1997, and 22.6/100,000 in 2000 (Thai MOPH). Attempts to distinguish Leptospirosis from other illnesses has relied on clinical and epidemiological factors. Laboratory confirmation of Leptospirosis is problematic since the gold standard assay and serologic tests are not available at the local level. Rapid serologic tests, including dipsticks, have been developed, and have shown good specificity (89%) and good overall sensitivity (90-92%), though less so in the acute illness setting (60%).

b. Goals:

1. To characterize the etiologies of fever and hepatitis, encephalitis, and hemorrhage in defined geographical regions of northwest Thailand.
2. To investigate the public health impact of the etiologies of fever and hepatitis, encephalitis, and hemorrhage.
3. To expand and improve the scientific and logistical infrastructure of KPP by completing construction, and initiating operations, in a new 2-story building outfitted with clinical and laboratory resources.

c. Methods:

This is a hospital-based study closely related to the study of febrile diseases in Nepal. Focus will be on presentations of febrile illness with icteric, hemorrhagic, or encephalitic manifestations. All the case definitions, surveillance, and laboratory methods, specimen collection, and data processing are similar. Inclusion criteria are also similar, except for the absence of age limitations. Illnesses with an onset more than 7 days previous were excluded. Sera were screened at the field hospital using a rapid dipstick for leptospirosis, scrub typhus, and murine typhus. All those positive by dipstick were confirmed using an IgM ELISA completed at AFRIMS.

d. Progress:

Approximately 1200 cases presented with one of the specified clinical syndromes. The sera from 112 febrile cases screened positive for Leptospirosis by IgM EIA (200 specimens) since EID started at KPP have been sent to Brooke Army Medical Center (BAMC) for confirmatory microscopic agglutination test (MAT). Disease mapping of MAT confirmed Leptospirosis cases identified over the past 5 years may occur.

9. Influenza Surveillance in Southeast Asia

a. Background:

Influenza is an important cause of morbidity and mortality among populations at the extremes of age. Continuous viral surveillance and isolation of influenza viruses provides important information for the creation of annual vaccine formulations based on the identification of new and emerging strains of influenza.

b. Goals:

1. To provide isolates of influenza virus collected in Southeast Asian countries as part of the global surveillance network for influenza, "Project Gargle".
2. To evaluate rapid diagnostic techniques at select sentinel sites in an attempt to validate these tests, increase sample submissions, and improve patient care.
3. To expand the network of participating institutions to include the U.S. Embassies of Southeast Asia.

c. Methods:

Samples were collected from patients with clinically suspected influenza infection (case definition includes fever or history of fever $\geq 38^{\circ}\text{C}$ and two or more of the following symptoms: cough, sore throat, coryza, muscle aches, malaise/fatigue, or headache). Participating physicians and staff identified patients who met the case definition during routine clinic visits. Emphasis was placed on quality samples that may provide genetic data for future influenza vaccines rather than a large number of samples to be tested for incidence and prevalence data. Clinical history forms, including basic demographic and clinical information, were completed by the OPD nurse or AFRIMS research nurses. Throat swabs were collected and placed in viral media and stored at -70°C . All specimens were shipped on dry ice to AFRIMS, which in turn shipped the samples to Armstrong Laboratory, Brooks AFB. Rapid diagnostics for Influenza (FLU OIA) were field tested at the sites in Sankhlaburi, Thailand, the medical facilities of the US Embassy in Bangkok, Thailand, Kamphaeng Phet, Thailand and Kathmandu, Nepal. Laboratory test results were maintained and summarized by Project Gargle and CDC personnel.

d. Progress:

Sample collection for influenza surveillance was suspended during the Asian SARS epidemic to avoid placing health care personnel collecting and processing specimens at unknown risk. As a result, few samples were collected. A resurgence in SARS cases in Asia during the influenzae season will dictate the level of influenzae surveillance activity.

E. Department of Retrovirology, AFRIMS FY03 Research Accomplishments

1. Title of Research Project: Screening and evaluation of potential volunteers for a preventive HIV-1 vaccine trial in Thailand (RV148, HSRRB Log No.).

a. Investigators:

Dr. Supachai Rerks-Ngarm, Dept. of Disease Control, MOPH;
COL Sorachai Nitayaphan, RTA Component, AFRIMS;
Prof. Dwip Kitayaporn, Mahidol University.

b. Objectives:

To evaluate adult Thai volunteers for eligibility and subsequent enrollment in a preventive HIV-1 vaccine trial (Phase III) of a prime-boost vaccine combination for the prevention of HIV infection.

c. Methods:

Volunteers who indicate an interest in participating in HIV vaccine research will receive information and education about the upcoming vaccine trial. They will be evaluated to see that they meet the eligibility criteria. They will receive counseling and education on HIV, aspects of participating in a HIV vaccine trial, and will be tested for HIV-1 infection by a standard ELISA & Western blot algorithm. Those volunteers who test positive for HIV will have CD4 enumeration and HIV viral load testing. Those that are eligible for the Phase III study and pass a test of understanding will be offered enrollment in a vaccine efficacy trial.

d. Results:

The protocol began enrolling volunteers as of 29 September 2003. Thus far over 800 volunteers have been enrolled and screened.

e. Future Plans:

This protocol is projected to last approximately 1 ½ years from time of enrollment in order to screen approximately 25,000 volunteers for the vaccine trial which has a sample size of 16,000.

2. A Phase III Trial of Aventis Pasteur Live Recombinant ALVAC-HIV (vCP1521) Priming With VaxGen gp120 B/E (AIDSVAX[®] B/E) Boosting in HIV-uninfected Thai Adults (RV144, HSRRB Log No. A-11048, BB-IND 8795).

a. Investigators:

Dr. Supachai Rerks-Ngarm, Dr. Supamit Chunsutthiwat- Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand
COL Sorachai Nitayaphan, RTA Component, AFRIMS
Prof. Dwip Kitayaporn, Mahidol University
Assoc. Prof. Punnee Pitisuttithum, Mahidol University

b. Objectives:

Primary: To determine whether immunizations with an integrated combination of ALVAC-HIV (vCP1521) boosted by AIDSVAX[®] gp120 B/E prevent HIV infection in healthy Thai volunteers. Secondary: To determine whether immunization with this vaccine combination results in reduced HIV viral load “set point” among those acquiring HIV-1 infection, comparing vaccine recipients to placebo recipients. To determine whether immunization with this vaccine combination results in an increased CD4 count measured at viral load “set point” among those acquiring HIV-1 infection, comparing vaccine recipients to placebo recipients. To confirm the safety of this vaccine combination in Thai volunteers. To evaluate whether participation in this HIV vaccine trial is associated with behavior change that may increase the risk of HIV infection.

c. Methods:

This will be a community-based, randomized, multicenter, double-blind, placebo-controlled clinical trial (vaccine:placebo = 1:1). Screening of potential volunteers will be carried out under a separate protocol entitled “Screening and evaluation of potential volunteers for a trial in Thailand of a candidate preventive HIV vaccine” (RV148). Eligible volunteers will be enrolled over approximately one year. The statistical assumptions of the study will require that 16,000 persons enroll into the study. Vaccinations for each individual will occur over a 24-week period (0, 4, 12, 24 weeks). Women will be tested for pregnancy and pregnant volunteers will not be vaccinated. The volunteers will be followed with HIV testing every 6 months for 3 years after immunization. Blood will be collected for plasma (for diagnostics and HIV-specific antibodies) at 0, 24 and 26 weeks, and every 6 months during the follow-up phase. The blood collection at 0 and 52 weeks will also be used for cryopreservation and archiving of PBMCs (for HIV-specific cellular immune responses). At week 24 and at each six-month follow-up visit, volunteers will have HIV testing, preceded by pretest counseling and followed (approximately 2-3 weeks later) by post-test counseling. Assessment of HIV risk behavior will be performed at

baseline and at each 6-month follow-up visit. Education on risk behavior reduction will be given at each vaccination visit and at each post-test counseling visit.

d. Results:

Enrollment in this protocol began in October 2003, with the first volunteer injected on 20 October. As of 7 January 2004, 521 volunteers have been enrolled, with 7 serious adverse events (all vaccine unrelated).

e. Future Plans:

This protocol will continue to enroll volunteers until the recruitment goal is met. Volunteers will be followed for 3 ½ years after vaccination.

III. APPENDICES:

A. PERSONNEL ASSIGNED UNDER AGREEMENT

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1. Ms. Bang-on Kesdee
2. Mr. Weerasak Yeephu
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8. Ms. Yinglak Apisitsaowapa
9. Ms. Tippawan Tephassadin na ayuthaya
10. Mr. Somporn Krasaesub
11. Mr. Theerasak Ponepan
12. Mr. Prinya Yoophasook
13. Mrs. Suwanee Thet

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17. Mr. Surapol Ogpai
18. Mr. Sawadi Boonnak
19. Mr. Charan Kajeechitr
20. Mr. Thongchai Duangkaew
21. Mr. Boonthum Jamjang
22. Mr. Komson Boonnak
23. Mr. Somporn Pinpo
24. Mr. Nirutti Boonnak
25. Mr. Chatchai Saeng-ngern
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27. Mr. Yuthana Seemat
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56. Mr. Surind Sisiranond
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156. Mrs. Thanintorn Adeedto
157. Mr. Papangkorn Phaophuak
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159. Mr. Pakornpat Suphanich

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170. Ms. Vilaiwan Tungsakul
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179. Ms. Sopana Chatnibandhu
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182. Ms. Nongluck Sangnoi
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188. Ms. Ajchariyarat Sangdara
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192. Dr. Sanjaya Kumar Shresth

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194. Ms. Ploypailin Klaimanee
195. Mrs. Wiboonrat Jaturonglumert
196. Ms. Wareeporn Wognbowornnan

B. PUBLICATIONS 2003

1. Arakawa T; Tsuboi T; Kishimoto A; Sattabongkot J; Suwanabun N; Rungruang T; Matsumoto Y; Tsuji N; Hisaeda H; Stowers A; Shimabukuro I; Sato Y; Torii M. *Serum antibodies induced by intranasal immunization of mice with Plasmodium vivax Pvs25 co-administered with cholera toxin completely block parasite transmission to mosquitoes.* *Vaccine* 2003; 21(23): 3143-8.
2. Aronoff DM; Watt G. *Prevalence of relative bradycardia in Orientia tsutsugamushi infection.* *Am J Trop Med Hyg* 2003; 68(4): 477-9.
3. Aroonrerk N; Pichyangkul S; Yongvanitchit K; Wisetchang M; Sa-Ard-Iam N; Sirisinha S; Mahanonda R. *Generation of gingival T cell lines/clones specific with Porphyromonas gingivalis pulsed dendritic cells from periodontitis patients.* *J Periodontal Res* 2003; 38(3): 262-8.
4. Chanbancherd P; Paris RM; Torugsa K; de Souza M; Myint KSA; Chitpong A; Brown AE. *High frequency of HIV-1 and hepatitis C co-infection among young Thai men: Evidence for a changing pattern of HIV transmission in Thailand.* *Southeast Asian J Trop Med Public Health* 2003; 34(3): 580-2.
5. Chuenchitra T; Wasi C; Louisirojchanakul S; Nitayaphan S; Sutthent R; Cox JH; De Souza M; Brown AE; Birx DL; Polonis VR. *Longitudinal study of humoral immune responses in HIV type 1 subtype CRF01_AE (E)-infected Thai patients with different rates of disease progression.* *AIDS Res Hum Retroviruses* 2003; 19(4): 293-305.
6. Coleman RE; Monkanna T; Linthicum KJ; Strickman DA; Frances SP; Tanskul P; Kollars TM Jr; Inlao I; Watcharapichat P; Khlaimanee N; Phulsuksombati D; Sangjun N; Lerthusnee K. *Occurrence of Orientia tsutsugamushi in small mammals from Thailand.* *Am J Trop Med Hyg* 2003; 69(5): 519-524.
7. Cui L; Mascorro CN; Fan Q; Rzomp KA; Khuntirat B; Zhou G; Chen H; Yan G; Sattabongkot J. *Genetic diversity and multiple infections of Plasmodium vivax malaria in Western Thailand.* *Am J Trop Med Hyg* 2003; 68(5): 613-9.
8. Currier JR; Dowling WE; Wasunna KM; Alam U; Mason CJ; Robb ML; Carr JK; McCutchan FE; Birx DL; Cox JH. *Detection of high frequencies of HIV-1 cross-subtype reactive CD8 T lymphocytes in the peripheral blood of HIV-1-infected Kenyans.* *AIDS* 2003; 17(15): 2149-57.
9. Edstein MD; Kocisko DA; Walsh DS; Eamsila C; Charles BG; Rieckmann KH. *Plasma concentrations of tafenoquine, a new long-acting antimalarial agent, in thai soldiers receiving monthly prophylaxis.* *Clin Infect Dis* 2003; 37(12): 1654-8.

10. Forney JR; Wongsrichanalai C; Magill AJ; Craig LG; Sirichaisinthop J; Bautista CT; Miller RS; Ockenhouse CF; Kester KE; Aronson NE; Andersen EM; Quino-Ascurra HA; Vidal C; Moran KA; Murray CK; DeWitt CC; Heppner DG; Kain KC; Ballou WR; Gasser RA. *Devices for rapid diagnosis of Malaria: evaluation of prototype assays that detect Plasmodium falciparum histidine-rich protein 2 and a Plasmodium vivax-specific antigen.* J Clin Microbiol 2003; 41(6): 2358-66.
11. Hirunpetcharat C; Wipasa J; Sakkhachornphop S; Nitkumhan T; Zheng YZ; Pichyangkul S; Krieg AM; Walsh DS; Heppner DG; Good MF. *CpG oligodeoxynucleotide enhances immunity against blood-stage malaria infection in mice parenterally immunized with a yeast-expressed 19 kDa carboxyl-terminal fragment of Plasmodium yoelii merozoite surface protein-1 (MSP1(19)) formulated in oil-based Montanides.* Vaccine 2003; 21(21-22): 2923-32.
12. Jones JW; Sithiprasasna R; Schleich S; Coleman RE. *Evaluation of selected traps as tools for conducting surveillance for adult Aedes aegypti in Thailand.* J Am Mosq Control Assoc 2003; 19(2): 148-50.
13. Khuntirat B; Lerdthusnee K; Leepitakrat W; Kengluetcha A; Wongkalasin K; Monkanna T; Mungviriyaya S; Jones JW; Coleman RE. *Characterization of Orientia tsutsugamushi isolated from wild-caught rodents and chiggers in northern Thailand.* Ann N Y Acad Sci 2003; 990: 205-12.
14. Kim JH; Pitisuttithum P; Kamboonruang C; Chuenchitra T; Mascola J; Frankel SS; DeSouza MS; Polonis V; McLinden R; Sambor A; Brown AE; Phonrat B; Rungruengthanakit K; Duliege AM; Robb ML; McNeil J; Birx DL. *Specific antibody responses to vaccination with bivalent CM235/SF2 gp120: detection of homologous and heterologous neutralizing antibody to subtype E (CRF01.AE) HIV type 1.* AIDS Res Hum Retroviruses 2003; 19(9): 807-16.
15. Kollars TM Jr; Bodhidatta D; Phulsuksombati D; Tippayachai B; Coleman RE. *Short report: variation in the 56-kD type-specific antigen gene of Orientia tsutsugamushi isolated from patients in Thailand.* Am J Trop Med Hyg 2003; 68(3): 299-300.
16. Lerdthusnee K; Khlaimanee N; Monkanna T; Mungviriyaya S; Leepitakrat W; Debboun M; Coleman RE. *Development of an in vitro method for the evaluation of candidate repellents against Leptotrombidium (Acari: Trombiculidae) chiggers.* J Med Entomol 2003; 40(1): 64-7.
17. Lerdthusnee K; Khuntirat B; Leepitakrat W; Tanskul P; Monkanna T; Khlaimanee N; Inlao I; Kengluetcha A; Mungviriyaya S; Chandranoi K; Krairojananan P; Bodhidatta D; Rodkwamthook W; Phulsuksombati D; Sangjun N; Watcharapichat P; Jones JW; Coleman RE. *Scrub typhus: vector competence of Leptotrombidium chiangraiensis chiggers and transmission efficacy and isolation of Orientia tsutsugamushi.* Ann N Y Acad Sci 2003; 990: 25-35.

18. Lewis MD; Yousuf AA; Lerdthusnee K; Razee A; Chandranoi K; Jones JW. *Scrub Typhus reemergence in the Maldives*. *Emerg Infect Dis* 2003; 9(12): 1638-41.
19. McKenzie FE; Sirichaisinthop J; Miller RS; Gasser RA; Wongsrichanalai C. *Dependence of malaria detection and species diagnosis by microscopy on parasite density*. *Am J Trop Med Hyg* 2003; 69(4): 372-6.
20. Morgan PA; Chinaworapong S; Excler JL; Wongkamheng S; Triampon A; Buapunth P; Nitayaphan S; Michael RA; Singharaj P; Brown AE. *A joint clinical research center in Thailand: role in HIV vaccine development*. *Southeast Asian J Trop Med Public Health* 2003; 34(1): 126-35.
21. Murray CK; Bell D; Gasser RA; Wongsrichanalai C. *Rapid diagnostic testing for malaria*. *Trop Med Int Health* 2003; 8(10): 876-83.
22. Nasveld P; Russell B; Kotecka B; Rieckmann K. *Lack of in vitro effect of ivermectin on Plasmodium falciparum*. *Southeast Asian J Trop Med Public Health* 2003; 34(3): 552-3.
23. Nisalak A; Endy TP; Nimmannitya S; Kalayanarooj S; Thisyakorn U; Scott RM; Burke DS; Hoke CH; Innis BL; Vaughn DW. *Serotype-specific dengue virus circulation and dengue disease in Bangkok, Thailand from 1973 to 1999*. *Am J Trop Med Hyg* 2003; 68(2): 191-202.
24. Noedl H; Faiz MA; Yunus EB; Rahman MR; Hossain MA; Samad R; Miller RS; Pang LW; Wongsrichanalai C. *Drug-resistant malaria in Bangladesh: an in vitro assessment*. *Am J Trop Med Hyg* 2003; 68(2): 140-2.
25. Noedl H; Wongsrichanalai C; Wernsdorfer WH. *Malaria drug-sensitivity testing: new assays, new perspectives*. *Trends Parasitol* 2003; 19(4): 175-81.
26. Paris R; Sirisopana N; Benenson M; Ampaiphis R; Tuntichaivanich C; Myint KS; Brown AE. *The association between hepatitis C virus and HIV-1 in preparatory cohorts for HIV vaccine trials in Thailand*. *AIDS* 2003; 17(9): 1363-7.
27. Parola P; Cornet JP; Sanogo YO; Miller RS; Thien HV; Gonzalez JP; Raoult D; Telford III SR; Wongsrichanalai C. *Detection of Ehrlichia spp., Anaplasma spp., Rickettsia spp., and other eubacteria in ticks from the Thai-Myanmar border and Vietnam*. *J Clin Microbiol* 2003; 41(4): 1600-8.
28. Parola P; Miller RS; McDaniel P; Telford SR 3rd; Rolain JM; Wongsrichanalai C; Raoult D. *Emerging rickettsioses of the Thai-Myanmar border*. *Emerg Infect Dis* 2003; 9(5): 592-5.
29. Parola P; Sanogo OY; Lerdthusnee K; Zeaiter Z; Chauvancy G; Gonzalez JP; Miller RS; Telford SR 3rd; Wongsrichanalai C; Raoult D. *Identification of Rickettsia*

- spp.* and *Bartonella spp.* in ffrom the Thai-Myanmar border. *Ann N Y Acad Sci* 2003; 990: 173-81.
30. Pattanapanyasat K; Walsh DS; Yongvanitchit K; Piyawatthanasakul N; Wanachiwanawin W; Webster HK. *Robust in vitro replication of Plasmodium falciparum in glycosyl-phosphatidylinositol-anchored membrane glycoprotein-deficient red blood cells.* *Am J Trop Med Hyg* 2003; 69(4): 360-5.
 31. Pichyangkul S; Endy TP; Kalayanarooj S; Nisalak A; Yongvanitchit K; Green S; Rothman AL; Ennis FA; Libraty DH. *A blunted blood plasmacytoid dendritic cell response to an acute systemic viral infection is associated with increased disease severity.* *J Immunol* 2003; 171(10): 5571-8.
 32. Pickard AL; Wongsrichanalai C; Purfield A; Kamwendo D; Emery K; Zalewski C; Kawamoto F; Miller RS; Meshnick SR. *Resistance to antimalarials in Southeast Asia and genetic polymorphisms in pfmdr1.* *Antimicrob Agents Chemother* 2003; 47(8): 2418-23.
 33. Pillai DR; Hajar G; Montoya Y; Marouino W; Ruebush TK 2nd; Wongsrichanalai C; Kain KC. *Lack of prediction of mefloquine and mefloquine-artesunate treatment outcome by mutations in the Plasmodium falciparum multidrug resistance 1 (pfmdr1) gene for P. falciparum malaria in Peru.* *Am J Trop Med Hyg* 2003; 68(1): 107-10.
 34. Pitisuttithum P; Nitayaphan S; Thongcharoen P; Khamboonruang C; Kim J; de Souza Souza; Chuenchitra T; Garner RP; Thapinta D; Polonis V; Ratto-Kim S; Chanbancherd P; Chiu J; Birx DL; Duliege AM; McNeil JG; Brown AE. *Safety and immunogenicity of combinations of recombinant subtype E and B human immunodeficiency virus type 1 envelope glycoprotein 120 vaccines in healthy Thai adults.* *J Infect Dis* 2003; 188(2): 219-27.
 35. Polonis VR; de Souza Souza; Darden JM; Chantakulkij S; Chuenchitra T; Nitayaphan S; Brown AE; Robb ML; Birx DL. *Human immunodeficiency virus type 1 primary isolate neutralization resistance is associated with the syncytium-inducing phenotype and lower CD4 cell counts in subtype CRF01_AE-infected patients.* *J Virol* 2003; 77(15): 8570-6.
 36. Ponsa N; Sattabongkot; Kittayapong P; Eikarat N; Coleman RE. *Transmission-blocking activity of tafenoquine (WR-238605) and artelinic acid against naturally circulating strains of plasmodium vivax in Thailand.* *Am J Trop Med Hyg* 2003; 69(5): 542-7.
 37. Ratto-Kim S; Garner RP; Kim JH; Jagodzinski JL; Michael NL; Paris R; Redfield RR; Birx DL. *Prospective analyses of HIV-1 specific and recall antigen proliferative responses and clinical outcomes in a HIV-1 seropositive cohort.* *Emerg Infect Dis* 2003; in press

38. **Russell BM; Udomsangpetch R; Rieckmann KH; Kotecka BM; Coleman RE; Sattabongkot J.** *Simple in vitro assay for determining the sensitivity of Plasmodium vivax isolates from fresh human blood to antimalarials in areas where P. vivax is endemic.* **Antimicrob Agents Chemother** 2003; 47(1): 170-3.
39. **Sattabongkot J; Maneechai N; Phunkitchar V; Eikarat N; Khuntirat B; Sirichaisinthop J; Burge R; Coleman RE.** *Comparison of artificial membrane feeding with direct skin feeding to estimate the infectiousness of Plasmodium vivax gametocyte carriers to mosquitoes.* **Am J Trop Med Hyg** 2003; 69(5): 529-535.
40. **Sattabongkot ; Tsuboi T; Hisaeda H; Tachibana M; Suwanabun N; Rungruang T; Cao YM; Stowers AW; Sirichaisinthop J; Coleman RE; Torii M.** *Blocking of transmission to mosquitoes by antibody to Plasmodium vivax malaria vaccine candidates Pvs25 and Pvs28 despite antigenic polymorphism in field isolates.* **Am J Trop Med Hyg** 2003; 69(5): 536-541.
41. **Silamut K; Newton PN; Teja-Isavadharm P; Suputtamongkol Y; Siriyanonda D; Rasameesoraj M; Pukrittayakamee S; White NJ.** *Artemether bioavailability after oral or intramuscular administration in uncomplicated falciparum malaria.* **Antimicrob Agents Chemother** 2003; 47(12): 3795-8.
42. **Sithiprasasna R; Linthicum KJ; Liu GJ; Jones JW; Singhasivanon P.** *Some entomological observations on temporal and spatial distribution of malaria vectors in three villages in Northwestern Thailand using a geographic informations system.* **Southeast Asian J Trop Med Public Health** 2003; 34(3): 505-16.
43. **Sithiprasasna R; Linthicum KJ; Liu GJ; Jones JW; Singhasivanon P.** *Use of GIS-based spatial modeling approach to characterize the spatial patterns of malaria mosquito vector breeding habitats in Northwestern Thailand.* **Southeast Asian J Trop Med Public Health** 2003; 34(3): 517-28.
44. **Torugsa K; Anderson S; Thongsen N; Sirisopana N; Jugsudee A; Junlananto P; Nitayaphan S; Sangkharomya S; Brown AE.** *HIV epidemic among young Thai Men, 1991-2000.* **Emerg Infect Dis** 2003; 9(7): 881-3.
45. **Tovanabutra S; Watanaveeradej V; Viputtikul K; De Souza Souza; Razak MH; Suriyanon V; Jittiwutikarn J; Sriplienchan S; Nitayaphan S; Benenson MW; Sirisopana N; Renzullo PO; Brown AE; Robb ML; Beyrer C; Celentano DD; McNeil JG; Birx DL; Carr JK; McCutchan FE.** *A new circulating recombinant form, CRF15_01B, reinforces the linkage between IDU and heterosexual epidemics in Thailand.* **AIDS Res Hum Retroviruses** 2003; 19(7): 561-7.
46. **Tsuboi T; Kaneko O; Eitoku C; Suwanabun N; Sattabongkot J; Vinetz JM; Torii M.** *Gene structure and ookinete expression of the chitinase genes of Plasmodium vivax and Plasmodium yoelii.* **Mol Biochem Parasitol** 2003; 130(1): 51-4.

47. Utaisincharoen P; Kespichayawattana W; Anuntagool N; Chaisuriya P; Pichyangkul S; Krieg AM; Sirisinha S. *CpG ODN enhances uptake of bacteria by mouse macrophages*. *Clin Exp Immunol* 2003; 132(1): 70-5.
48. Watanaveeradej V; DeSouza MS; Benenson MW; Sirisopana N; Nitayaphan S; Chanbancherd P; Brown AE; Sanders-Buell E; Birx DL; McCutchan FE; Carr JK. *Subtype C/CRF01_AE recombinant HIV-1 found in Thailand*. *AIDS* 2003; 17(14): 2138-40.
49. Watanaveeradej V; Endy TP; Samakoses R; Kerdpanich A; Simasathien S; Polprasert N; Aree C; Vaughn DW; Ho C; Nisalak A. *Transplacentally transferred maternal-infant antibodies to dengue virus*. *Am J Trop Med Hyg* 2003; 69(2): 123-8.
50. Watt G; Jongsakul K. *Acute undifferentiated fever caused by infection with Japanese encephalitis virus*. *Am J Trop Med Hyg* 2003; 68(6): 704-6.
51. Watt G; Jongsakul K; Chouriyagune C; Paris R. *Differentiating dengue virus infection from scrub typhus in Thai adults with fever*. *Am J Trop Med Hyg* 2003; 68(5): 536-8.
52. Watt G; Jongsakul K; Suttinont C. *Possible scrub typhus coinfections in Thai agricultural workers hospitalized with leptospirosis*. *Am J Trop Med Hyg* 2003; 68(1): 89-91.
53. Watt G; Kantipong P; Jongsakul K. *Decrease in human immunodeficiency virus type 1 load during acute dengue fever*. *Clin Infect Dis* 2003; 36(8): 1067-9.
54. Watt G; Parola P. *Scrub typhus and tropical rickettsioses*. *Curr Opin Infect Dis* 2003; 16(5): 429-36.
55. Wongsrichanalai C; Arevalo I; Laoboonchai A; Yingyuen K; Miller RS; Magill AJ; Forney JR; Gasser RA Jr. *Rapid diagnostic devices for malaria: field evaluation of a new prototype immunochromatographic assay for the detection of Plasmodium falciparum and non-falciparum Plasmodium*. *Am J Trop Med Hyg* 2003; 69(1): 26-30.
56. Wongsrichanalai C; Murray CK; Gray M; Miller RS; McDaniel P; Liao WJ; Pickard AL; Magill AJ. *Co-infection with malaria and leptospirosis*. *Am J Trop Med Hyg* 2003; 68(5): 583-5.