



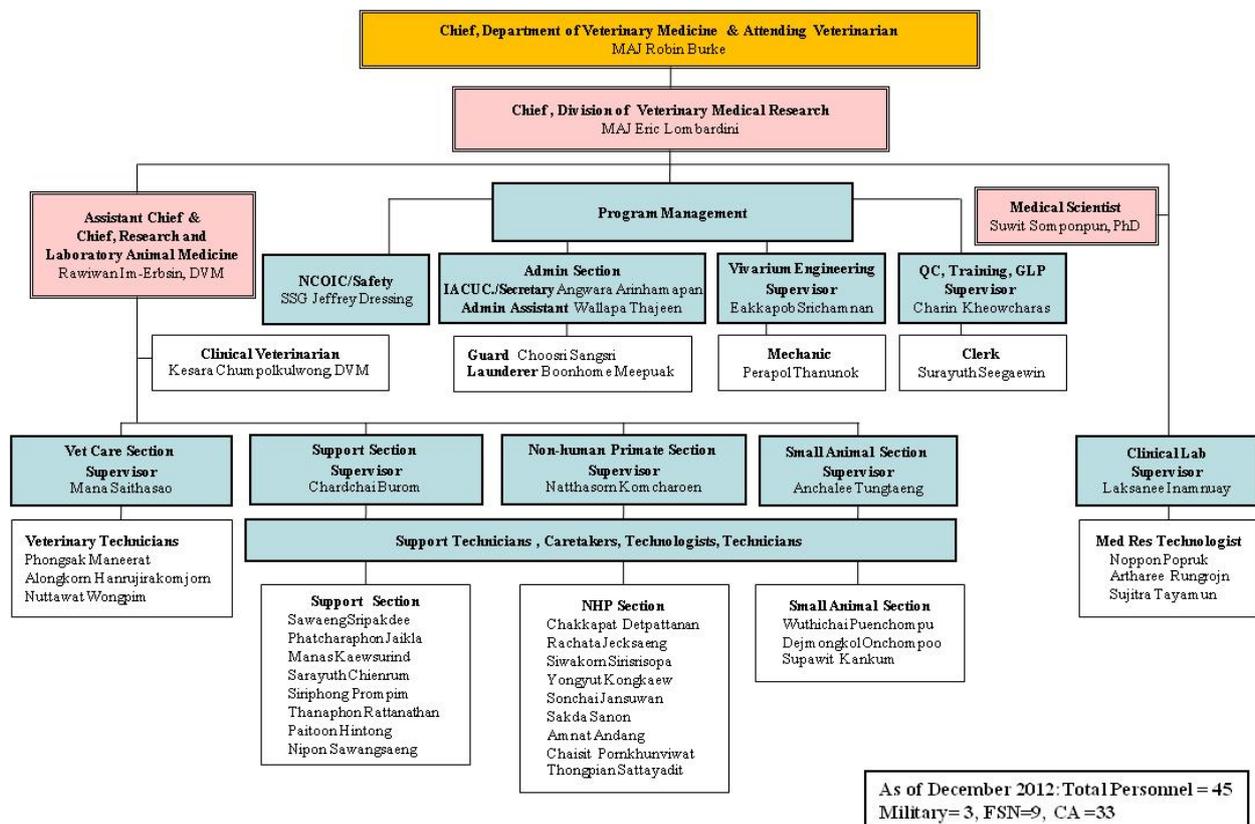
DEPARTMENT OF VETERINARY MEDICINE

DEPARTMENT MISSION

To protect military personnel and their families against tropical disease threats through pre-clinical product development of new prophylactic and therapeutic drugs and new or improved vaccines.

To fulfill this mission, the Department of Veterinary Medicine (DVM) conducts bio-medical research in animal models and zoonotic disease surveillance, provides veterinary expertise and research animals that are free of confounding diseases to intra- and extra-mural collaborators, and ensures that all animals receive humane, proper, and safe care and that the USAMC-AFRIMS' Animal Care and Use Program complies with appropriate laws, regulations and guidelines.

PERSONNEL



IN-HOUSE TRAINING PROGRAMS AND OUTSIDE TRAINING OF PERSONNEL

In-House Training of Personnel

- American Association for Laboratory Animal Science (AALAS) Certification Training and Official Testing Site (weekly training with annual or biannual certification testing as needed)
- Weekly Staff Training on SOPs, Protocols, Departmental Policies and other topics as appropriate
- The Humane Care and Use of Lab Animals

- Animal Euthanasia
- B-Virus Post-Exposure Intervention and Prophylaxis
- Animal Bites and Other Injuries/Reports
- Non-Monkey Animal Related and Non-Animal Origin Injuries
- Animal BSL-3 Hazard & Procedures
- Occupational Health Program & Safety Communication
- Occupational Health Program for Department of Veterinary Medicine
- Emergency and Disaster Management
- Equal Employment Opportunity and Prevention of Sexual Harassment (EEO & POSH)
- Fire Prevention, Protection, Report and Investigation
- Fire Fighting Training
- Accident, Illness and Complaint Reporting, Records and Investigations
- DoD Information Assurance Awareness
- Counter Intelligence, Operations Security, and Anti-Terrorism
- Hazard Communication Program
- Chemical Safety
- Safety Equipment Usage
- Biosafety in Laboratory
- Laboratory Waste Management
- Respiratory Protection Program
- Composite Risk Management
- Routine Prophylaxis and Screening and Post-Exposure Prophylaxis and Intervention
- Radiation Safety for X-Ray Use
- Blood-Borne Pathogens Exposure Control Plan
- GLP Training – Initial and Refresher
- Ethics Training

Outside Training of Personnel

- FSN Supervisory Skills Workshop. Bangkok, Thailand. January and February 2012
- 2nd Thai National Symposium on Animal Care & Use for Scientific Purposes & Laboratory Animal Trade Exhibition 2012. Bangkok, Thailand. 29-31 July 2012
- The 5th Asian Federation of Laboratory Animal Science Association Congress and IACUC Training - International Standard Approaches. Bangkok, Thailand. 9-12 October 2012
- American Association for Laboratory Animal Science (AALAS) National Annual Meeting. Minneapolis, MN. 4-8 November 2012
- University of Texas Medical Branch (UTMB) National Biocontainment Training Center Laboratory Biosafety Training Program, “ABSL-3 Rodent” and “ABSL-3 Nonhuman Primate” Training. 26-30 November 2012
- American College of Veterinary Pathologists (ACVP) Annual Meeting. Seattle, Washington. 1-5 December 2012
- Immunohistochemistry Training, Chulalongkorn University, Department of Pathology, Bangkok. December 2012

AWARDS

Non-applicable

CORE RESEARCH ACCOMPLISHMENTS



Malaria Research

The mouse model for screening new candidate antimalarial compounds for blood stage activity has been used for over 30 years and is very effective for making comparisons between drugs and for choosing promising drugs for advanced development. It is rapid, relatively inexpensive, and makes reliable predictions of how drugs will act in higher mammalian hosts, including humans. However, a new and economical mouse model for screening antimalarial compounds for exoerythrocytic (EE) activity (i.e., liver stage) was developed in 2006. Prior to development of this model, an *in-vivo* test for EE screening was not readily available. In 2007, this established model was validated and optimized. In 2012, 9 compounds were screened in 2 experiments.

The ongoing efficacy model for drug development and therapeutics in the nonhuman primate (*Macaca mulatta*) was also conducted in 2012. This model provides a mechanism to identify effective new drugs for the enhanced prevention and treatment of malaria infections. One radical curative experiment was conducted by using 10 monkeys and a variety of anti-malarial compounds were administered (chloroquine in combination with Imidazolidinedione (IZ) derivative, Deoxo-Imidazolidinedione (DIZ) derivative, Imidazole (IM) derivative and Deoxo-tafenoquine analog).

Zoonotic Surveillance

A previous study collected animal serum samples to perform surveillance for several important zoonotic diseases, including leptospirosis, anthrax, melioidosis and brucellosis. After discussion with Thailand National Institute of Animal Health (NIAH), some of those serum samples were submitted for additional testing. Of the total serum samples, a 15% random group (1,630 samples) from Mae Hong Son, Kanchanaburi, and Tak provinces were additionally submitted for a series of tests for *Coxiella burnetti* (Q fever). Of those tested samples, 2.14%, 1.16%, and 96.7% were found to be positive, suspect, and negative from these samples for Q fever, respectively.

Research Support

The DVM provided research support to five departments at the USAMC-AFRIMS for 19 active animal use protocols studying disease mechanisms of and developing therapeutics and vaccines for tropical disease threats in Southeast Asia.

The DVM maintains breeding colonies of rhesus monkeys and rodents to support the USAMC-AFRIMS research needs. Forty-three (43) baby rhesus macaques were born in the colony. 2,739 ICR mice (*Mus musculus*) were produced and 1,434 mice were used for 5 active protocols. Four hundred seventy (470) ICR mice were used for one protocol to maintain mosquito colonies. The veterinary clinical laboratory is an integral part of the research support for malaria drug development, malaria vaccine testing, drug-drug interaction investigation, dengue anti-viral drug development and diarrhea model development. The clinical laboratory performed nearly 4,092 malaria parasite counts, 484 doses of test compounds, 1,634 complete blood counts, 2,022 serum chemistries and 1,548 tissue sections.

ACCOMPLISHMENTS AND CURRENT STATUS

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

2. Care and Maintenance of Rhesus (*Macaca mulatta*) and Cynomolgus (*Macaca fascicularis*) Monkeys and Management of Breeding Colonies: Forty-three baby rhesus

monkeys were born this year. Since our production of rhesus in the USAMC-AFRIMS colony was higher than the target goal of 50: One experiment using 10 monkeys was performed this year. Four new compounds were tested naïve animals annually, the number of animals utilized for breeding was decreased by 50% and the weaning age of the infants was extended from 6 months to one year of age.

3. Care and Maintenance of Laboratory Rodents and Rabbits, Maintenance of Rodent Breeding Colonies and Quality Assurance/Quality Surveillance Program: 2,739 ICR mice (*Mus musculus*) were produced, and 1,434 mice were used for five active protocols.

4. A *Plasmodium berghei* - Mouse Model for Screening Blood-Stage Antimalarial Drugs: No experiments were conducted in 2012.

5. *Plasmodium berghei* - *Anopheles dirus* Sporozoite - ICR Mice Malaria Model for Screening Exoerythrocytic Antimalarial Drugs: A total of 9 compounds in 2 experiments were tested for their potential anti-malarial efficacy.

6. A *Plasmodium berghei* – Mouse Malaria Model for Studying Hemoglobin Based Oxygen Carrier (HBOC) Treatment of Severe Malaria Anemia: A pilot experiment was performed, and the results are still pending. There was no activity or animal use in 2012. The protocol will be closed.

7. Efficacy Testing of Two Novel Anti-Viral Compounds against Dengue Fever Virus in the Rhesus Monkey (*Macaca mulatta*): There was no activity or animal use in 2012. The protocol will be closed.

8. Mosquito Feeding Using *In Vitro* and *In Vivo* Techniques with Mice (*Mus musculus*) as a Blood Source: 470 culled mice were used to maintain mosquito colony.

9. Evaluation of Scrub Typhus Vaccine Candidates by Natural and Artificial Challenge Models in ICR Mice (*Mus musculus*): 120 mice were used in 2012.

10. Maintenance of the *Leptotrombidium* Larval Mite Colonies: Chigger Feeding on ICR Mice (*Mus musculus*): 600 mice were used to maintain the chigger mite colony.

11. Continuation of "Evaluation of a Tetravalent Dengue Purified Inactivated Virus (TDEN PIV) Vaccine in *Macaca mulatta*, Phase II: Adjuvant Down Selection and Duration of Immunity". This protocol is a follow-up study in rhesus monkeys to test the most effective tetravalent dengue purified inactivated (TDEN PIV) vaccine formulations that confer long-term immunity. Sixty monkeys were used to test the dengue vaccine in the different formulations. One positive control group (Live attenuated vaccine-LAV) that planned to use six monkeys was cancelled. Titer levels of nab were higher in the groups inoculated with the TDEN PIV vaccine in the presence of the AS03 adjuvant (>1:2500) at the two highest concentrations (AS03_A and AS03_B) than in the AS01 and AS03_C groups. Titers in all groups waned after day 56 but remained detectable through day 168. Five randomly selected monkeys within each group were challenged subcutaneously (SC) with either the wild type DENV2 at day 252 or wild type DENV1 at day 308. Viremia in the serum was measured for 15 days post-challenge. While all macaques in control group developed viremia as early as day 1 post-challenge, all TDEN PIV vaccinated groups showed fewer viremia days. The TDEN PIV AS03_A conferred the highest protection to



both virus challenges.

12. Comparison of Room Temperature Preservation to Hypothermic Preservation of Renal Autograft in the Rhesus Macaque (*Macaca mulatta*) Renal Transplantation Model.

In 2012, new the anesthesia machine and patient monitoring equipment have been shipped from the sponsor with new surgeons and anesthesiologist. The experiment was conducted by using 4 monkeys. Belzer and Lifer perfusates were utilized in static storage condition. The result showed 2 monkeys had anuria after surgery and were euthanized since study day 4. The other 2 were maintained until the study endpoint 21 days after surgery.

13. Characterization of the Disease Course and Immune Response of Scrub Typhus in Rhesus Monkey (*Macaca mulatta*) by Intradermal Injection of *Orientia tsutsugamushi*:

7 rhesus monkeys were utilized in this study. The establishment of the scrub typhus in rhesus was successful by challenging the infectious agent via intradermal injection, all experimental monkeys demonstrated eschar at the injection site with lymphadenopathy.

14. Evaluation of the Local Reactogenicity after Intradermal Administration with Different Formulations of Adjuvanted Seasonal Influenza Vaccine in Rhesus Monkeys:

3 monkeys were utilized and tested for 7 adjuvants in the different doses for development of effective malaria vaccines.

15. Evaluate Protective Immunity of the Pre-Erythrocytic Vaccine Antigen CeTOS in a Nonhuman Primate Model against *P. knowlesi* Sporozoite Challenge:

22 monkeys were utilized for 2 experiments developing the *Plasmodium knowlesi* model by using mosquito natural challenge and the efficacy test for malaria vaccines.

The DVM has directly contributed to 122 published scientific articles dating back to 1966. In 2012, six journal articles were published with DVM authors.

The DVM organizes and conducts weekly American Association for Laboratory Animal Science (AALAS) certification training and serves as an official testing site. Training was held for one hour twice weekly (8 times per month) for a total of 56 classes in 2012. All DVM personnel and personnel from outside collaborating institutions who work with laboratory animals are encouraged to participate in the AALAS certification course. The supervisors and qualified technicians conducted the regular classroom training for staff seeking all levels of AALAS certification. The AFRIMS' English teacher set up a course called "Essential English for AALAS ALAT Certificate Seekers," which was held every week on Tuesday from 1500-1700 hrs (total of 39 classes). The 3 levels of certification (in order, from most difficult to least difficult) are Laboratory Animal Technologist (LATG), Laboratory Animal Technician (LAT), and Assistant Laboratory Animal Technician (ALAT). AFRIMS-DVM serves as Thailand's AALAS Certificate official testing site for institutes and agencies outside of AFRIMS; (i.e., Mahidol University, Chulabhorn Institute of Research, etc.). Twenty (20) personnel, both from AFRIMS and outside AFRIMS, were trained. AALAS certification exam was held on 21 May 2012 with 10 personnel testing from AFRIMS, CRI, and Mahidol University. The pathology division has provided direct training to veterinary pathologists, veterinary students and PhD students at Chulalongkorn University as well as at several regional meetings on topics of zoonotic disease pathology, fish and wildlife pathology, histopathology description, macroparasite histopathology and has been involved in the early stages to enable the establishment of a Thai veterinary pathology training center with a regional board examination.

COLLABORATIONS

MOUs

No active MOUs.

CTAs

No active CTAs.

CRADAs

- University of Mississippi, School of Pharmacology and AFRIMS-DVM engaged to perform work on *ex vivo* antimalarial and PK/PD screening of antimalarials and their metabolites in mice and rhesus monkey models.
- Mahidol-Oxford Research Unit, AFRIMS-Entomology and AFRIMS-DVM engaged to perform work on Scrub typhus development in rhesus monkeys.
- GlaxoSmithKline, Belgium, AFRIMS-Virology and AFRIMS-DVM engaged to perform work on novel anti-viral compounds for dengue fever virus using a collaborative research and development agreement.

FUTURE PLANS AND STRATEGIES

- Continue to use and improve animal models of malaria in the mouse and monkey for testing new anti-malarial drugs, especially to develop and refine a new model of humanized mouse in collaboration with the Tropical Medicine Faculty, Mahidol University.
- Continue to use and improve animal models of malaria in nonhuman primates. This effort will include not only the relapsing malaria model for *Plasmodium cynomolgi* but also a model of severe cerebral malaria (*Plasmodium coatneyi*) and the fifth human malaria *Plasmodium knowlesi*.
- Develop new animal models of human disease, including a natural challenge Rhesus scrub typhus model in collaboration with Mahidol-Oxford Research Unit, a Rhesus dengue vaccine model utilizing a mosquito challenge and potential models for norovirus pathogenesis in the Rhesus macaque, *Salmonella/E. coli* vaccine model in ICR mice and humanized mouse models for ongoing malaria research.
- Continue GLP capabilities for preclinical studies, including a projected enteric GLP study.
- Continue to develop partnerships for testing vaccines and therapeutics against dengue fever in the rhesus macaque model.
- Develop new animal models of human disease, including a Rhesus simian-human immunosuppressive virus (SHIV) model in collaboration with the Military HIV Research Program (MHRP).
- Provide expertise and assistance to support development of a regional primate center in Thailand
- Provide expertise, training and assistance to the Chulalongkorn University, Department of Veterinary Pathology through technician exchanges, and direct training of faculty and students.
- Continue to support the USAMC-AFRIMS research mission by providing veterinary expertise and animal resources for product testing.
- Continue to maintain an exceptional animal care and use program and prepare for the 2014 AAALAC site visit.