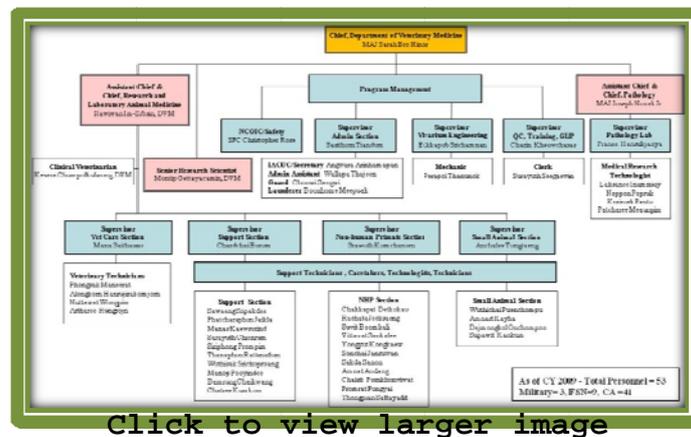


DEPARTMENT OF VETERINARY MEDICINE

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 extramural 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

- 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 (annual)
- Animal Bites and Other Injuries/Reports (annual)
- Animal Euthanasia (annual)
- B-Virus Post-Exposure Intervention and Prophylaxis (annual)
- Animal BSL-3 Hazard & Procedures (annual)
- Occupational Health Program & Communication (annual)
- Equal Employment Opportunity and Prevention of Sexual Harassment (EEO & POSH) (annual)
- Fire Prevention, Protection, Report and Investigation (annual)
- Fire Fighting Training (annual)
- Accident, Illness and Complaint Reporting, Records and Investigations (annual)
- Computer Security Awareness (annual)

- Counter Intelligence, Operations Security, and Anti-Terrorism (annual)
- Hazard Communication Program (annual)
- Chemical Safety (annual)
- Safety Equipment Usage (annual)
- Biosafety in Laboratory (annual)
- Laboratory Waste Management (annual)
- Respiratory Protection Program (annual)
- Composite Risk Management (annual)
- Routine Prophylaxis and Screening and Post-exposure Prophylaxis and Intervention (annual)
- Radiation Safety for X-ray Use (annual)
- Radiation Safety (General) (annual)
- Blood-borne Pathogens Exposure Control Plan (annual)
- GLP Training – Initial and Refresher (annual)
- Ethics Training (annual)

Outside training

- International Symposium on Laboratory Animal Science, 26-29 April 2009, Bangkok, Thailand
- Association of Medical Technologist (AMTT) Annual Meeting, 28 April 2009, Chiang Mai, Thailand
- Association of the United States Army (AUSA) 2009 Army Medical Symposium and Exposition “Army Medicine”, San Antonio, TX, 21-24 July 2009
- American Society for Clinical Pathology (ASCP), Chicago, IL, 27 October- 1 November 2009
- Thailand Association for Laboratory Animal Science (TALAS) and USAMC-AFRIMS Institutional Animal Care and Use Committee (IACUC) and Scientists Training Program, Salaya, Nakhon Pathom, Thailand (15-16 October 2009)
- Association of Primate Veterinarians (APV), Denver, CO, 5-7 November 2009
- American Associate for Laboratory Animal Science (AALAS) National Annual Meeting, Denver, CO, 8-12 November 2009
- American Society of Tropical Medicine and Hygiene (ASTMH) Meeting, Washington, D.C., 18-22 November 2009
- American College of Veterinary Pathologists (ACVP), Monterey, CA, 5-9 December 2009

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. In 2009 a total of 32 compounds in 12 experiments were tested for their potential anti-malarial efficacy.

A new and economic 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 2009, we screened 74 compounds in 39 experiments.

The ongoing efficacy model for drug development and therapeutics in the nonhuman primate (*Macaca mulatta*) was very active in 2009. This model provides a mechanism to identify effective new drugs for the enhanced prevention and treatment of malaria infections. Two experiments were conducted in 2009 using 38 monkeys and a variety of antimalarial compounds were administered (chloroquine combination with cis- or trans- mirincamycin; primaquine and chloroquine combination with cis- or trans- mirincamycin or clindamycin; decoquinate, tinidazole, minocycline, desloratidine, pyrazinamide, trimethoprim, promethazine, imidazolinedione, tafenoquine, malarone[®] (atovaquone and proguanil), and a Rifampin antibiotic combination, among several other combination therapeutics). Results are pending.

A mouse malaria model to test novel hemoglobin based oxygen carriers (HBOC) was initially performed only as a pilot experiment. The pilot Study (Experiment 1) was performed and used a total of 15 mice (n = 3). Mice in experimental groups received artesunate (AS), HBOC-201 and AS, or AS, HBOC-201 and NTG. Results are being analyzed.

Zoonotic surveillance:

In 2009 we continued our protocol for surveillance of zoonotic diseases (brucellosis, leptospirosis, anthrax, melioidosis, and tuberculosis) in provincial and imported livestock in Chiang Rai Province Thailand, funded by GEIS and in collaboration with the Thai National Institute of Animal Health and provincial Thai Department of Livestock Diseases (DLD) veterinarians. In FY09, serologic analysis of these initial samples for anthrax and melioidosis was completed. For melioidosis surveillance, 41 samples were positive (0.62%) by HAI, and for anthrax surveillance, all 6,631 samples were negative (0%).

In 2009 a total of 7,935 serum samples were obtained from cattle, buffalo, goats and sheep in two Northwestern provinces, Maehongson and Tak. In March 2009, 3,315 serum samples were obtained from cattle, buffalo, goats and sheep (from a total of 5,356 animals) in four border districts (Maelanoi, Maesariang, Sobmoei and Muang) of Maehongson province. The 3,315 blood samples included 2,142 cattle, 565 buffalo, 393 goats and 215 sheep. In April 2009, 4,620 serum samples were obtained from cattle and buffalo (from a total of 5,873 animals) in two border districts (Umphang and Maesod) of Tak province. The 4,620 blood samples included 3,887 cattle and 733 buffalo.

In FY09 (November 2008 to April 2009), the Department of Livestock Development also independently sampled 3,062 imported animals in Kanchanaburi (Sangkhlaburi border) and Prajuabkeereekhan provinces, and banked the serum. These samples may be analyzed pending funding availability. The 3,062 blood samples included 1,040 cattle and 1,989 buffalo (Kanchanaburi), and 33 buffalo (Prajuabkeereekhan).

In 2009 samples were also analyzed for leptospirosis and brucella. The sample collection was completed in 2009. The USAMC-AFRIMS animal care and use protocol (PN06-08) expired in December 2009. All serum samples will be donated to the Thai Department of Livestock Development, National Institute of Animal Health (DLD-NIAH). Laboratory assays will be continued in FY2010 as the funding allows.

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-four (44) baby rhesus macaques were born in the colony. 11,147 ICR mice (*Mus musculus*) were produced and 3,036 mice were used for 6 active protocols. 240 ICR mice were used instead 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 laboratory performed over 16,000 malaria parasite counts, 1,400 doses of test compounds, 3,200 Complete Blood Counts, 1,600 serum chemistries and 1,500 tissue sections.

ACCOMPLISHMENTS

1. Antimalarial Drugs Efficacy Testing in the Rhesus Monkey (*Macaca mulatta*)/*Plasmodium cynomolgi* Malaria Models: two experiment using 38 monkeys were performed within this year. More than 30 compounds were tested.

2. Care and Maintenance of Rhesus (*Macaca mulatta*) and *Cynomolgi* (*Macaca fascicularis*) monkeys and Management of Breeding Colonies: Forty-four baby rhesus monkeys were produced and newly imported male monkeys (received 2008) were placed as breeders in gang cages.

3. Care and Maintenance of Laboratory Rodents and Rabbits, Maintenance of Rodent Breeding Colonies, and Quality Assurance/Quality Surveillance Program: 11,147 ICR mice (*Mus musculus*) were produced and 3,036 mice were used for six active protocols.

4. A *Plasmodium berghei*-Mouse Model for Screening Blood-stage Antimalarial Drugs: A total of 32 compounds in 12 experiments were tested for their potential anti-malarial efficacy.

5. *Plasmodium berghei* - *Anopheles dirus* Sporozoite - ICR Mice Malaria Model for Screening Exoerythrocytic Antimalarial Drugs: A total of 74 compounds in 39 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: Pilot experiment was performed and results are still pending.

7. Pharmacokinetic (PK) testing of two novel anti-viral compounds in the Rhesus monkey (*Macaca mulatta*): Six rhesus monkeys were tested with two novel anti-viral compound. PK blood was collected and shipped to the Toyama company for PK analysis.

8. Efficacy testing of two novel anti-viral compounds against Dengue Fever Virus in the Rhesus monkey (*Macaca mulatta*): Step 1 was performed and twenty-five rhesus monkeys were tested the two novel anti-viral compounds. PK blood were collected and shipped to Toyama Company for analysis. The result is still pending.

9. Evaluation of a Tetravalent Purified Inactivated Virus (TPIV) Vaccine for Dengue in *Macaca mulatta*: Forty-eight (48) rhesus monkeys were test the TPIV vaccine. The procedures are complied and being performed.

10. Safety and Immunogenicity of two *Plasmodium vivax* Circumsporozoite Protein Vaccine Candidates in Rhesus monkey (*Macaca mulatta*): Twenty monkeys were tested the malaria vaccine. All procedures were completely performed.

11. Mosquito Feeding Using *in vitro* and *in vivo* Techniques with Mice (*Mus musculus*) as a Blood Source: Two hundred and forty mice were used instead of hamster to maintain mosquito colony.

12. Study on safety and immunogenicity of live-attenuated dengue vaccine candidates in Rhesus macaques (*Macaca mulatta*): Sixteen monkeys were tested with live-attenuated dengue vaccine. The procedures are complied and being performed.

13. Determination and Comparison of Optimum Inoculation Doses of Two *Orientia tsutsugamushi* Strains in ICR Mice: One hundred and thirty three mice were used within this fiscal year.

14. Maintenance of the *Leptotrombidium* Larval Mite Colonies: Chigger Feeding on ICR Mice (*Mus musculus*): Six hundred and twenty five mice were used for maintain chigger mite colony.

15. Evaluation of live, attenuated oral *Shigella dysenteriae* 1 vaccine candidates in rhesus monkeys (*Macaca mulatta*) in an intragastric challenge model: Forty eight rhesus monkeys were use in this Shigella vaccine study. This protocol was the first very Good Laboratory Practices (GLP) study at USAMC-AFRIMS. All animal procedures were completed.

16. *Ex vivo* antimalarial activity and pharmacokinetic/pharmacodynamic (PK/PD) screening of antimalarials and their metabolites in rhesus monkey models: Fourteen monkeys were tested the anti malarial drug and PK blood collection were completely performed.

17. Safety and Immunogenicity of Protein Recombinant Baculovirus Expressing *Plasmodium falciparum* Circumsporozoite (CSP) Vaccines in Rhesus Macaques: Forty two rhesus monkeys were tested with malarial vaccine and all animal procedures were completed.

The DVM organizes and conducts weekly American Association for Laboratory Animal Science (AALAS) certification training and serves as an official testing site. 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 3 levels of certification in order from most to least difficult are Laboratory Animal Technologist (LATG), Laboratory Animal Technician (LAT) and Assistant Laboratory Animal Technician (ALAT). In 2009, 30 personnel attended training; 15 from DVM and 13 from host nation organizations (Thai National Institute of Health [NIH], Mahidol University [MU] and Chulabhorn Research Institute (CRI)). Twenty eight (28) personnel attempted the certification, yielding an overall pass rate of 64%, with 5 AFRIMS' personnel and 13 host nation personnel achieving certification. The breakdown in certification was 6 LATG, 5 LAT and 7 ALAT. The DVM had 34 AALAS certified technicians/technologists (12 LATG, 4 LAT, 18 ALAT) at the close of 2009.

COLLABORATIONS

- Commercial Test Agreement (CTA) between WRAIR/AFRIMS and the National Center for Natural Products Research (NCNPR), University of Mississippi.
- A Memorandum of Understanding (MOU) between WRAIR-ET and the Department of Veterinary Medicine (DVM) was established to allow for primary support to the AFRIMS animal colony in exchange for priority use of our malaria animal models.



- Mahidol University Faculty of Veterinary Science, Sai Yoke Campus, served as site for critical reagent program in sheep to develop antiserum for use in Influenza A assays (CTA) (AFRIMS-Enterics and AFRIMS-DVM)
- Toyama Chemical Company, Toyama Japan, 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 (CRADA)
- Novartis, WRAIR-ET, and AFRIMS-DVM engaged on work with antimalarial compounds in mice (CRADA)
- The National Science and Technology Development Agency, Thailand, engaged on use of the circumsporozoite protein vaccine model for dengue fever virus (AFRIMS-Immunology and AFRIMS-DVM)

FUTURE PLANS AND STRATEGIES

- Continue to use and improve animal models of malaria in the mouse and monkey for testing new antimalarial drugs.
 - Expand staff to complete malaria mission requirements
 - Adapt further the mouse malaria model to test novel hemoglobin based oxygen carriers being developed by the US Navy.
 - Continue GLP capabilities for preclinical studies. A follow on study is expected for advancing a Shigella vaccine.
 - Continue to develop partnerships for testing vaccines and therapeutics against Dengue Fever in the Rhesus Macaque model.
 - Develop partnerships and collaborations for Influenza A surveillance.
 - Expand zoonotic surveillance mission under the Bio Engagement Program
 - Provide expertise and assistance to support development of a regional primate center in Thailand
 - Continue to support the USAMC-AFRIMS research mission by providing veterinary expertise and animal resources for product testing.
 - Continue to maintain and exceptional animal care and use program and prepare for the 2010 AAALAC site visit.

DEPARTMENT OF VIROLOGY

DEPARTMENT MISSION

To develop and evaluate products, and collects epidemic data to protect the Soldier from infectious diseases