

b. Objectives:

- 1) To determine the effects of carbon dioxide and mouse excrement on the walking distance of host-seeking chiggers towards a glue board.
- 2) To compare the trapping efficacy of A-Rat glue (Thailand) *versus* Stick-EM glue (USA) using various glue board modifications.
- 3) To evaluate the ability to retrieve mites from glue boards that have caught live rodents.
- 4) To compare the catching rate between the snap trap and live trap.
- 5) To compare the stickiness of double-sided tapes in capturing chigger mites.
- 6) To determine if a self-baiting (rodent) ectoparasite trap will out-compete prototype chigger traps developed in FY 08 at AFRIMS.

c. Methods/Results:

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

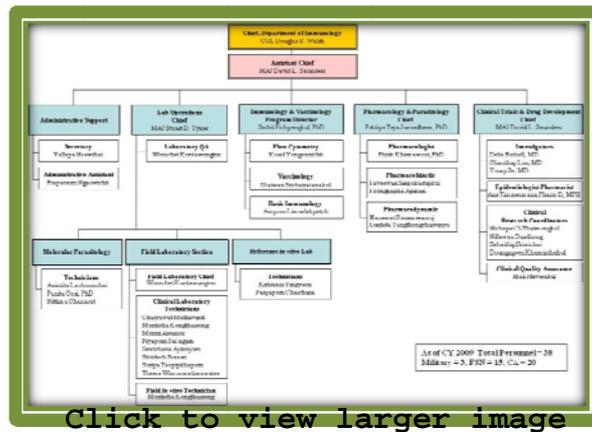
DEPARTMENT OF IMMUNOLOGY AND MEDICINE

DEPARTMENT MISSION

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

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

PERSONNEL



Investigators:

- Dr. Mark Fukuda, MD
- Dr. Douglas Walsh, MD
- Dr. Sathit Pichyangkul, PhD
- Dr. Paktiya Teja-Isavadharm, PhD
- Dr. Pisit Khemawoot, PhD
- Dr. Panita Gosi, PhD
- Dr. Wiriya Rutvisuttinunt, PhD
- Dr. Delia Bethell, MBBS
- Dr. Youry Se, MD
- Dr. David L. Saunders, MD
- Dr. Stuart D. Tyner, PhD
- Dr. Ans Timmermans, PharmD
- Dr. Jessica Lin, MD
- Dr. Lon Chanthap, MD

IN-HOUSE TRAINING PROGRAMS AND OUTSIDE TRAINING OF PERSONNEL

In-House training

Hosted one U.S. military medical student, and one civilian infectious disease fellow pursuing careers in tropical medicine and research.

Two staff members were trained in GIS training organized by Uniformed Services University of the Health Sciences (USUHS), July 28-31, 2009.

Three senior laboratory technicians from the National Malaria Center (CNM) in Cambodia received advanced training of *in vitro* malaria culture techniques and conditions. They also received an intense molecular parasitology course which consisted of nucleic acid extraction, both DNA and RNA, real-time PCR, standard PCR, and fingerprinting for molecular correction. This training occurred over a 6 week period and was intended to provide them with the analytical skill set to function more independently from the AFRIMS laboratory in Bangkok.

Outside Training Provided by Department

One staff member presented on Epidemiology of Drug Resistant Malaria at the PACOM Disease Surveillance Workshop, organized in Vientiane, Laos, 9-12 Jun 2009

CNM/AFRIMS Workshop on Clinical and Laboratory Training for Survey of *in vitro* and Molecular Markers of Anti-malarial Resistance & Human Influenza Surveillance, Battambang, Cambodia, September 14-18, 2009

Two senior investigators attended the *in vitro* consensus meeting for four days, organized by the WARN (World Anti-malarial Resistance Network) in Paris, where they discussed how to develop and implement appropriate QC/QA procedures for *in vitro* malaria culture and how to use a few key *in vitro* culturing techniques for the analysis of cultured parasites.

AWARDS

Non-applicable

ACCOMPLISHMENTS

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, reference microscopy, assessment of rapid diagnostics for various tropical infectious diseases, and a staff well-versed in conduct of clinical trails to GCP and ICH standards. The animal research teams are all trained in laboratory animal research and regulations, current AAALAC requirements, and laboratory animal test and observation methods. State-of-the art methodologies are available for the study of vaccine and drugs to include advanced molecular biology methods such as sequencing, SNP analysis, and 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, a unique malaria bioassay to measure the *in vivo* anti-malarial bioactivity of potential new anti-malarial medications, sustained malaria cell culture and radio-isotopic uptake, and antibody based methods for measuring *in vitro* drug sensitivity patterns of malaria strains against standard malaria drugs.

RESULTS:

Accomplishments during the period of January-December 2009:

1. Malaria Drug Development

Managed the implementation of departmental quality practices for the execution of studies in agreement with MRMC policies and US FDA standards in support of IV AS drug development program. Work involved upkeep of personnel training and qualification records; space utilization for LCMS lab, sample repository, and field clinical lab; establishment of a controlled sample tracking and inventory system; qualification of equipment used for regulated studies; and continued interaction with Medical Maintenance and service contractors. Helped integrate Departmental QA/QC efforts with those of the subsequently established QA units at the AFRIMS, WRAIR and MRMC, participated in the IPT teleconferences, providing metabolism and pharmacokinetics insight.

Parenteral anti-malarial drugs are indicated for the treatment severe malaria and when oral therapy cannot be given. The goals of treatment are prevention of death and reduction of morbidity. Even when treated with appropriate anti-malarial drugs, severe malaria in austere or resource-limited settings in the developing world may be associated with high mortality rates because of complications for which treatment may not be available, such as acute renal failure and acute respiratory distress syndrome. Little has been reported in the peer reviewed literature about the burden of severe malaria in the government referral hospital in Battambang (BRH), Western Cambodia's second largest city. However, data from the Cambodian National Malaria Center (CNM) in 2007 indicates that Battambang Province had the second highest mortality rate for probable and confirmed malaria in Cambodia. We conducted a retrospective epidemiological survey to establish the burden of severe malaria in this hospital and to assess the potential for conducting clinical trials in the future. All cases of malaria admitted to the BRH from January 2006 to December 2008 with a discharge diagnosis of severe malaria were reviewed for demography, mortality, and referral patterns. There were 2,648 reported cases of severe malaria among 59,848 confirmed malaria cases in 2007 nationally, with a case fatality rate of 8.3%. There were 132 cases and 23 deaths (17.4% mortality) from severe malaria in 2007 reported from BRH, out of 4,105 confirmed malaria cases reported in Battambang province that year.

Mirincamycin is a lincosamide antibiotic structurally related to Clindamycin. Prior work in the 70s and 80s led to discovery of anti-malarial properties in primates, but the drug was never tested in humans. Recently, interest in this compound has resurfaced. A formal oral bioavailability study of this drug was conducted in non-human primates in 2008, and was found to be roughly 10-13% compared to intravenous administration. The drug was reasonably well tolerated. In 2009, an abstract was presented at ASTMH on the comparison of the absolute oral bioavailability (F) and ex-vivo anti-malarial activity against *P. falciparum* (as a W2 clone of *P. falciparum*) of cis-mirincamycin (c-MC) and trans-mirincamycin (t-MC) in 4 groups of healthy rhesus monkeys at a dose of 4mg/kg IV or 20mg/kg PO. No significant differences were observed between single dose c-MC and t-MC in PK or PD parameters by the IV or oral route in non-human primates. Higher ratios of ex vivo activity to concentration in the oral dose groups for the first 90 minutes suggests first pass metabolism with formation of an active metabolite. Further PK-PD analysis in infected primates determined that the compound was safe, though ineffective for treatment of relapsing *P. cynomolgi* malaria. This data supported an IPT decision at the WRAIR to suspend further development of the lincosamide class, with consideration for pursuit of analog synthesis in the future. Timely data provided by the Department was instrumental in achieving a 'quick kill' - an important objective of the Army Drug Development Program; thereby conserving resources to pursue other more promising leads.

AFRIMS Immunology and Medicine supported the WRAIR in obtaining a new Investigational New Drug (IND) application from FDA for a safe, effective human *Plasmodium vivax* challenge model. The department implemented *P. vivax* infected blood donor screening processes to FDA standards to ensure challenge subject safety. In collaboration with the Department of Entomology, we supported the first human challenge in healthy volunteers at the WRAIR using *P. vivax* infected mosquitoes produced under regulated IND from donors in Thailand.

Future Plan:

We are currently working on establishing a human challenge model in healthy volunteers at AFRIMS in collaboration with investigators from the Royal Thai Army. We are also actively pursuing site development for anti-malarial chemoprophylaxis in the Cambodian

military (RCAF) with a view to initiating a large Phase 3 clinical trial in FY 2010 or FY2011, depending on sponsor commitments. Once final site selection is completed, a protocol for a pilot study will be developed in concert with other participating sites to gather prospective epidemiology data in a cohort of 250-500 potential study subjects. The sites will be selected based on data from active and passive epidemiology studies that we will conduct with RCAF. The purpose of this pilot study will be to simulate a phase 3 clinical trial in order to assess feasibility, and to determine the malaria attack rate to determine trial sample size and design.

2. Malaria Drug Resistance Surveillance

Artemisinin based combination therapies (ACTs) are the first line treatment for drug resistant *Plasmodium falciparum* malaria. The current major global investment in ACTs is threatened by the possible emergence of resistance to artemisinins, as signaled by a trend of increasing ACT treatment failure on the Thai-Cambodian border, which has historically been an epicentre of drug resistant malaria. Once it develops and spreads, resistance to the artemisinin derivatives, could very well be the most devastating event in the history of malaria control in the 21st century. There are no effective alternatives to artemisinins for the treatment of malaria either on the market or nearing the end of the drug development process.

Strategies for containing artemisinin resistance require the ability to detect it rapidly and accurately, both in humans (*in vivo*) and in collected parasite isolates (*in vitro*). AFRIMS' proven ability to monitor artemisinin resistance with a consistent regionally applied method and standards for its *in vitro* drug sensitivity testing and *in vivo* efficacy trials is critical in this regard.

Artesunate in combination with mefloquine has been the first-line drug for uncomplicated *falciparum* malaria on the Thai side of the border since 1995 and in Cambodia since 2000. Therapeutic efficacy monitoring is regularly conducted by both the Thai and Cambodian malaria control programs. Both progressively increased parasite clearance times and unusually high failure rates with artesunate-mefloquine have been reported recently on both sides of the border.

AFRIMS began working in collaboration with the Thai MOPH in Trat Province, Thailand to try and determine why the treatment failures described by the Thai National Malaria Program (Vijaykadjia, 2006) were occurring. An integrated *in vivo-in vitro* approach was adopted using existing protocols. This approach comprised anti-malarial treatment in accordance with MOPH guidelines (directly observed treatment with AS (6mg/kg daily for 2 days), MQ (25mg/kg split into 2 doses) and PQ (0.5mg/kg single dose on Day 2) with all doses given as DOT), and *in vitro* culture of parasites with drug sensitivity assays at admission to the study and subsequently if treatment failure occurred. Parasite growth inhibition was used as a measure for drug sensitivity of fresh samples in a HRP2 double-site antigen capture ELISA. Follow-up had previously been to Day 28 in accordance with WHO guidelines (WHO 2003) but was extended to 42 days when AFRIMS became involved since this is the preferred duration of follow-up following MQ therapy. We found that the PCR-corrected ACPR (cure rate) at 42 days for Trat in 2005 was 81% (7 out of 42 enrolled patients failed therapy and 5 were reinfected). The second (and currently on-going) Trat study, (WRAIR #1327) started in September 2007. The study also uses an *in vivo/in vitro* approach yet incorporates a more detailed human use (*in vivo*) study, with plasma drug level measurements and a comparison of 2 and 3 days AS treatment. AFRIMS and the Thai MOPH will continue to work in collaboration during the forthcoming study.

The *in vivo* component aims to compare the efficacy and tolerability of artesunate (12mg/kg) and mefloquine (25mg/kg) given over 2 or 3 days for the treatment of uncomplicated *P. falciparum* malaria in Trat Province, Thailand. This has important public health implications



as it may influence future treatment policy. Due to the changed local epidemiology of malaria in Trat and the malaria containment efforts in border districts in Trat, this site will not generate a sufficient number of enrolled volunteers with *Pf* malaria before the end of the trial in 2012. Rather than closing the study, efforts will be made in 2010 to expand the Trat study with an extra site

Data from AFRIMS' earlier ARC1 study conducted in Western-Cambodia in 2006 suggest that along parts of the Cambodian-Thai border there are individual *P. falciparum* isolates, which are highly resistant to artemisinins. Although the prevalence of these isolates was low, the overall sensitivity of the parasite isolates was significantly reduced as compared to western Thailand. In ARC1 some individual isolates were associated with greatly increased parasite clearance times, treatment failures despite 7 days of artesunate monotherapy (4mg/kg), and very high inhibitory concentrations for artemisinins *in vitro*. Reports from the Ministries of Public Health on both sides of the Thai-Cambodian border indicate increasing numbers of treatment failures with artemisinin-based combination therapies.

In 2009, the Department of Immunology and Medicine completed the ARC2-trial, a follow-up study to ARC1. The aim was to determine whether regimens with increased artesunate doses could overcome the problem of reduced drug sensitivity to artemisinins and to determine whether these experimental regimens, particularly the high-dose regimen, were safe and well tolerated. Similarly like in ARC1, the study was conducted in a purpose-built AFRIMS study ward at Tasanh Health Center in Western Cambodia, due south of Pailin and close to the border with Thailand. Tasanh Health Center and its referral health clinics stand in the middle of the crucial area of the growing reports of emergence of artemisinin resistance. The study was conducted in a designated study ward and staffed by a team of Cambodian and Thai nurses, physicians, microscopists and laboratory technicians, in close collaboration with the National Center for Parasitology, Entomology and Malaria Control (CNM) in Cambodia.

The study determined that increasing doses of artesunate monotherapy given for 7 days did not improve clinical or parasitological outcomes in Cambodian patients with uncomplicated *Pf* malaria. Even with high-dose treatment (6mg/kg/day for 7 days) cure rate was 88%, comparable to previous AS monotherapy studies in terms of efficacy. However, when patients receiving AS 4 mg/kg/day in this study were compared to those treated with exactly the same regimen in our previous 2006 study at the same site, the proportion of patients still parasitemic at 72 hours had almost doubled from 29 to 56%. This finding confirms the emergence over the last 3 years of parasite strains that are more resistant to AS *in vivo*, and underscores the importance of current containment strategies.

The pharmacokinetics and pharmacodynamics of oral artesunate monotherapy were also explored as part of the ARC2 trial. Despite weight-based dosing, a wide variability in artesunate concentrations were observed. There were significant reductions in plasma concentrations between day 1 and day 7 of dosing, suggesting auto-induction of metabolic clearance pathways. Dose limiting hematologic toxicity with neutropenia in 5 of 26 subjects occurred at the 6mg/kg dose level.

In vitro drug sensitivity assays have been used as a tool to characterize the drug susceptibility phenotype of clinical *Plasmodium falciparum* isolates and to screen new candidate drugs in development. Variability in *in vitro* drug sensitivity testing throughout the malaria research world makes comparison between different data sets, different labs, and different time periods difficult. In order to develop a testable model system for generating IC50 values with patients' specimens, we finalized the evaluation of dynamics of W2 standard clones as a mechanism to establish a validated control in 2009.

After these stringent method validations, the ARC 2 study has successfully managed to culture malaria parasites and generate IC50 values for a range of anti-malarial drugs (AS, DHA, chloroquine, mefloquine, lumefantrine, quinine) from 136 fresh patient samples, the largest number of fresh parasite isolates from a single clinical study in the region. IC50 values for DHA (major artemisinin metabolite) were higher in isolates of patients with delayed parasite clearance times, indicating that prior exposure to AS and its metabolites may select for development of resistance.

The *in vitro* methodology used in the ARC2 trial was used to initiate a dedicated *in vitro* survey in Cambodia in September 2009 for the purposes of measuring the distribution of resistant phenotypes, as defined in ongoing clinical trials of artemisinins, and obtaining adequate numbers of samples for planned genome-wide association studies.

Isolates of patients with treatment failures will be further screened for molecular markers of artemisinin resistance, in collaboration with the University of Maryland. Once identified and validated as predictors of clinical outcomes, molecular markers for artemisinin resistance will be used in a surveillance network (such as the World Wide Anti-Malarial Resistance Network-WWARN) employing molecular, *in vitro*, and clinical tools to measure the present extent of resistance and then guide rational containment strategies to deter its further spread. AFRIMS also conducted drug sensitivity assays in 2009 of new and unknown anti-malarial drug candidates (on blood samples containing malaria parasites, both from patients that have successfully completed and that have failed artemisinin treatment) developed by the MMV, a non-profit foundation created to discover, develop and deliver new, affordable anti-malarial drugs through effective public-private partnerships.

Funding for anti-malarial drug resistance work is sourced from DoD-GEIS, WHO, MMV and the Bill and Melinda Gates Foundation.

Future Plan:

Through *in vitro/in vivo* studies with a range of currently used anti-malarial therapies, and in collaboration with other key anti-malarial surveillance research institutions in the region and the U.S., we will continue to monitor drug efficacy, measure the distribution of resistant phenotypes, and collect adequate numbers of samples for population genetics studies that aim to identify molecular markers for artemisinin resistance.

3. Vaccinology and Immunology Studies in Support of Malaria Vaccine Program and Highly Pathogenic Avian Influenza Pathogenesis Studies

3.1 Malaria Immunology

Since the re-emergence of *Plasmodium vivax* in and around the demilitarized zone (DMZ) of South Korea in 1994, 10,000 to 20,000 new cases of *P. vivax* malaria have been reported. As high as 10% of the native population in the DMZ has sero-converted against *P. vivax* malaria. *P. vivax* malaria has also been shown to be a potential threat in Afghanistan, Iran, and Iraq, three other areas strategically important to the U.S. military. Currently, no vaccine exists against *Plasmodium vivax*, a potential threat to military operations.

Our department tested safety and immunogenicity of a *Plasmodium vivax* Circumsporozoite Protein (CSP) Vaccine Candidate in Rhesus macaques. Our aim was to measure the monkey antibody and cell mediated immune responses to VMP001/AS01B and CSV-S,S/AS01B.

Results:

We found that VMP001/AS01B and CSV-S,S/AS01B elicited strong antibody and T cell responses. Interestingly, 1-2 monkeys in each group demonstrated CD8 responses against *P. vivax* CSP.

Future Plan:

Manuscript is in preparation. Both VMP001/AS01B and CSV-S,S/AS01B will be clinically evaluated in *P. vivax* human challenge model at WRAIR.

3.2 Malaria vaccinology

RTS,S/AS02A and RTS,S/AS01B were co-developed by GlaxoSmithKline (GSK) and Walter Reed Army Institute of Research (WRAIR). The vaccines targeted the malaria parasite before it gets into the red blood cells. These two vaccine formulations were safe and immunogenic, but was effective in only 30-50% of the population. Recently, scientists from Otsuka have developed a recombinant baculovirus vector expressing *P. falciparum* CSP. Two independent studies in mice showed that the baculovirus vector vaccine was highly immunogenic and elicited strong immune responses against malaria circumsporozoite protein. This, together with the lack of pre-existing immunity to baculoviruses in humans and non-cytotoxic and non-replicative in mammalian cells even at high multiplicity of infection (MOI), makes the baculovirus-vector promising tool for malaria vaccine development.

Results:

Using a rhesus monkey model, we found that all tested vaccines were safe and well tolerated. Baculovirus vector vaccines elicited moderated CSP-specific antibody responses and all of immune sera inhibited sporozoite invasion into HepG2 cells *in vitro* (>98%). We detected negligible CSP-specific T cell responses in all immunized animals. These results suggest that further studies are needed to enhance immunogenicity of these CSP expressing baculovirus vector vaccines.

Future Plan:

New vaccine constructs are being optimized by Otsuka.

3.3 Highly Pathogenic Avian Influenza Pathogenesis Studies

1) *Reactivity of highly purified intravenous immunoglobulin (IVIG) against homosubtypic influenza A viruses (H1N1, H3N2) and their cross-reactivity against avian influenza H5N1.*

Intravenous immunoglobulin (IVIG) is commonly used to treat both bacterial and viral infections in patients with primary immunodeficiency disease as well as a variety of autoimmune and inflammatory disorders. IVIG contains pooled IgG derived from over a thousand blood donors, and contains antibodies are produced as a result of natural infection or vaccination. To gain further insight into influenza cross-reactive antibodies in humans, we investigated the reactivity of different commercial preparations of IVIG (50 mg/ml of highly purified immunoglobulin) against homosubtypic influenza A viruses (H1N1, H3N2) and their cross-reactivity against avian influenza H5N1.

Results:

We found that IVIGs from three commercial sources contain antibodies against human influenza hemagglutinin (HA), neuraminidase (NA) and matrix 2 ectodomain (M2e). In addition, these antibodies were cross-reactive to avian NA (N1) and M2e derived from

H5N1 viruses. All IVIG tested were able to neutralize H5N1 replication *in vitro*. Our findings highlight the heterosubtypic immunity against influenza A virus of IVIG, and this may provide support for the use of IVIG as adjunctive treatment of severe H5N1.

This work has been published in EID 2009 Sep; 15(9): 1537-9.

Future Plan:

We will evaluate IVIG prepared in 2008 and 2009, and sera samples collected from non-exposed U.S. military personnel for cross-reactive antibody against the novel H1N1 2009 and avian influenza H5N1.

2. Evaluation of In Vitro Cross-Reactivity with Avian Influenza H5N1 Virus in Healthy Volunteers Vaccinated with a Prime Boost Regimen of Seasonal Influenza Vaccine

The hypothesis to be tested in this study is that heterologous prime-boost vaccination of healthy adults with a prime boost regimen of trivalent LAIV (FluMist®) and trivalent IIV (Fluzone®) will induce cross-immunity to a variety of H5N1 avian influenza viruses. Adverse event profile of two doses of influenza vaccine given approximately 8 weeks apart in healthy adult volunteers. The *in vivo* phase of the study will take place at the U.S. Embassy Medical Unit, Bangkok, Thailand. Immunology testing will be done at the Department of Immunology and Medicine, AFRIMS and the Faculty of Medicine, Siriraj Hospital, Bangkok. Subjects will be healthy U.S. citizens residing in Bangkok who fulfill the inclusion criteria and do not meet any exclusion criteria. FluMist® vaccine is not yet licensed for use in Thailand; hence Thai subjects will not be recruited into this study.

Results:

The study was initiated in October 2009 and had enrolled 18 out of 26 required subjects by the end of December 2009. Enrollment is expected to be completed in February 2010. Laboratory analyses and adverse event data are pending.

4. Influenza Surveillance (DoD-GEIS)

This GEIS-funded project will allow for ongoing surveillance of Influenza-Like-Illnesses (ILIs) and detection of influenza and highly pathogenic influenza among vulnerable military and civilian populations in South-East Asia who are not included in other surveillance mechanisms.

AFRIMS is part of the US DoD surveillance program and participates in the collection and characterization of influenza viruses circulating within the human population in Asia. Various AFRIMS departments collect respiratory specimens from sites in Thailand, Nepal, the Philippines, Bhutan and U.S. Embassies in Southeast Asia with plans on expansion to Cambodia and Vietnam, and definitive test results are shared with the Ministries of Health and WHO Flu Net. This surveillance data gathered contributes towards the annual re-formulation of the influenza vaccine as well as early detection of novel influenza strains or existing subtypes with pandemic potential which can increase the lead time for implementation of control and prevention measures.

4.1 Kwai River Christian Hospital Surveillance of Influenza like illness

The Department of Immunology and Medicine, in collaboration with AFRIMS' Department of Virology, conducts ILI surveillance along the Thai-Burma border at the Kwai River Christian Hospital in Kanchanaburi Province.

The Department of Virology is responsible for protocol development and provides principal investigator and laboratory support. The Department of Immunology and Medicine provides clinical and administrative support, training and supervision.

Results:

For CY09, 87 samples have been collected (no samples were collected between October 2008-February 2009 and after November 2009 due to institutional halting of the protocol). Eleven (11) out of 87 samples tested positive for influenza A, and 76 out of 87 samples tested negative by rapid antigen testing. Twenty eight (28) out of 87 samples tested positive for influenza A by RT-PCR, broken down into the following sub-types: 21 out of 28 influenza A samples tested positive for influenza A/H1, 2 out of 28 samples tested positive for influenza A/H3 and 5 out of 28 samples tested positive for influenza A/Sw H1. 59 out of 87 samples tested negative for influenza.

4.2 Sentinel Human Surveillance for Influenza in Western Cambodia

This 5-year study aims to characterize influenza types and subtypes and determine genetic heterogeneity and antiviral susceptibility of influenza A viruses circulating in Western Cambodia. The Department of Immunology and Medicine is responsible for protocol development, clinical support, laboratory support for rapid antigen testing and real-time RT-PCR, site supervision, training. Laboratory support for influenza-negative specimens will be provided by the Department of Virology and reference laboratory support will be provided by the Pasteur Institute in Phnom Penh, surveillance data will be collected and analyzed in collaboration with the Cambodian Communicable Disease Control Department and members of the Technical Working Group for influenza in Cambodia (U.S. CDC, Cambodian CDC, NAMRU-2, Institut Pasteur, NIPH).

Approach:

Respiratory samples will be collected from outpatients with influenza-like-illness (ILI) symptoms at various hospitals. Data regarding household risk factors to avian and human influenza infection and weekly number of ILI-cases will be collected as well. After rapid diagnostic testing on-site, samples will be sent to the AFRIMS-CNM laboratory in Battambang for rapid detection and simultaneous subtyping of clinical influenza specimens. Primers include universal, A, A/H1, A/H3, A/H5, B.

Since the Cambodian Ministry of Health does not allow transfer of influenza specimens out of the country, confirmation of type and subtype by multiplex PCR and viral isolation will be contracted to the Pasteur Institute in Phnom Penh, which is also the National Influenza Center in Cambodia. HA, NA, MP complete genes will be routinely amplified and sequenced. Out of a subset of influenza isolates, full genome sequencing and uploading in GenBank will be conducted. In addition, oseltamivir sensitivity assays will be conducted by measurement of IC50 values using NAStar kit. Respiratory samples negative samples will be sent to AFRIMS for further characterization by MasTag PCR. Data will be shared with DoD-GEIS and key stakeholders in Cambodia through the Technical Working Group for Influenza.

Results:

Protocol WRAIR#1630 was approved by WRAIR IRB in October with minor comments to address prior to implementation. The protocol was submitted to the Cambodia IRB (NECHR) and immediately approved (December 2009). A bio-safety risk assessment was submitted to the AFRIMS Bio-safety Committee, equipment and supplies were purchased, SOPs and SSPs were developed, a real-time RT-PCR training plan was developed in collaboration with AFRIMS Department of Virology.

Future Plan:

The influenza surveillance study at KRCH will be resumed as soon as a new study protocol will be approved by the Thai MOPH. We expect to start collecting samples in our Cambodian sentinel sites as soon as our lab technicians are adequately trained in RT-PCR and the contract with the reference laboratory has been awarded by USAMRAA, most likely in April 2010.

OVERVIEW OF RESEARCH PROJECTS:

1. Human Malaria Vivax Challenge. Conducted *P. vivax* infected blood donor screening processes to FDA standards to ensure challenge subject safety

2. Safety and Immunogenicity of a *Plasmodium vivax* Circumsporozoite Protein Vaccine Candidate in Rhesus Macaques. Status: Manuscript is in preparation. Both VMP001/AS01B and CSV-S,S/AS01B will be clinically evaluated in *P. vivax* human challenge model at WRAIR.

3. Preclinical Evaluation of the Safety and Immunogenicity of a Vaccine Consisting of *Plasmodium falciparum* Liver-Stage Antigen 1 with Adjuvant AS01B Administered Alone or Concurrently with the RTS,S/AS01B Vaccine in Rhesus Primates. Status: Study in life completed. New vaccine constructs are being optimized by Otsuka.

4. Efficacy of Artesunate-Mefloquine Combination Therapy for the Treatment of Uncomplicated *Falciparum* Malaria in Trat Province Thailand. Status: Study ongoing in liaison with Thai MoPH. Expansion to other malarious border areas likely in 2010 due to changed malaria epidemiology in Trat.

5. A Phase II, Randomized, Open-label, Dose-ranging Study of GMP Intravenous Artesunate for Optimizing Parasite Clearance in Uncomplicated *P. falciparum* Malaria. Status: Clinical trial completed.

6. Surveillance and Laboratory Characterization of Artemisinin Resistant *Plasmodium falciparum* Strains to Inform Drug Development. Status: Validation of standard clones completed and data presented. MMV compounds tested and new in vitro protocol initiated.

7. Artemisinin Resistance in Cambodia II (ARC II). Status: Clinical Trial completed.

8. Pharmacologic and Pharmacodynamic Animal Studies in Support of Mirincamycin Development. Status: Bio-availability and tolerability study in primates completed. Development further suspended by IPT at WRAIR due to ineffective treatment of relapsing *P. cynomolgi* malaria.

9. Evaluation of Avian Influenza Hemagglutinin Sequences in Wild Birds. Status: Data analyzed and publication submitted.

10. Kwai River Christian Hospital Surveillance of Influenza-like Illness. Status: Surveillance halted since November 2009.

11. Human Influenza Sentinel Surveillance in Cambodia. Status: Study protocol approved. Study initiation expected in February 2010.

12. Evaluation of *In Vitro* Cross-Reactivity with Avian Influenza H5N1 Virus in Healthy Volunteers Vaccinated with a Prime Boost Regimen of Seasonal Influenza Vaccine. Status: Study will complete *in vivo* part in February 2010.

13. Retrospective survey of Severe Malaria in Battambang Referral Hospital, Cambodia, from 2006 to 2008. Status: Study completed.

14. Site Assessment of Phase 3 studies for Anti-malarial Chemoprophylactics in Cambodia/Thailand/Philippines. Assessment trips made and report written.



15. Detection and Quantification of *Plasmodium* spp. by 18S rRNA Gene Subunit-Based and Species-Specific Real-Time PCR Assays. Status: Study protocol is waiting for approval.

16. *Leptospira* (LPS assay) RAPID PCR Validation Using JBAIDS Molecular Assay Transition Package. Status: Study is ongoing in trying condition.

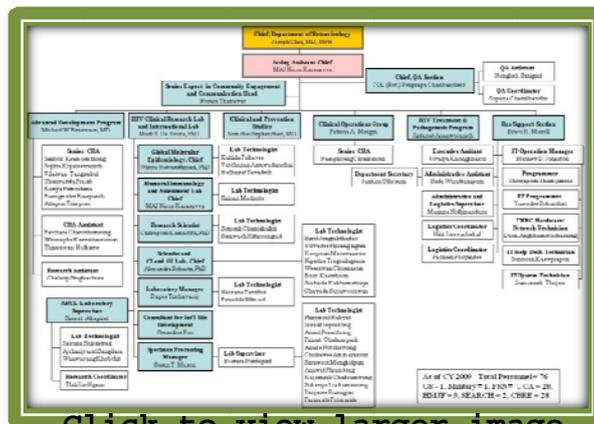
DEPARTMENT OF RETROVIROLOGY

DEPARTMENT MISSION

The mission of the Department of Retrovirology is to prepare for and conduct advanced development of preventive HIV vaccines for soldiers. This mission is achieved collaboratively and supported through i) the performance of preclinical and clinical (phase I-III) trials of candidate vaccines and their evaluations for safety, immunogenicity and efficacy, ii) the identification and characterization of potential cohorts for phase III vaccine trials, iii) the establishment of diagnostic assays which differentiate infection from vaccine-induced immune responses, iv) the characterization of HIV viruses circulating in the region, v) the determination of the natural history of HIV infection and disease.

PERSONNEL

The Department of Retrovirology consists of 76 staff that includes 1 GS and 2 Active-Duty Army Officers (1 Medical Corps, 1 Medical Service Corps). Within the department, there are 21 clinical research (4 MDs) and quality assurance staff, 42 laboratory personnel (5 PhD staff scientists), and 13 logistics/IT support staff. An overview of the organization chart is provided below as well as listing of each departmental staff member. The Department of Retrovirology has added personnel due to initiation of new MIDRP-funded activities and increased laboratory work load resulting from the recently completed Phase III trial that showed modest efficacy in preventing HIV infection.



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