

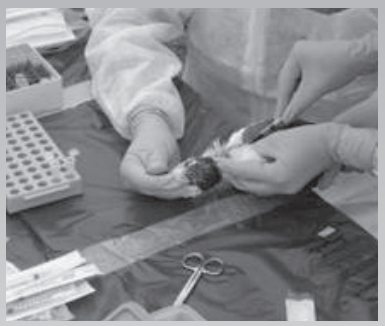





DoD

Global Emerging Infections Surveillance & Response System

A Division of the Armed Forces Health Surveillance Center

Fiscal Year 2008 *Annual Report*

Partnering in the Fight Against Emerging Infectious Diseases

2008: A Year of Transition

DoD Global Emerging Infections Surveillance and Response System

A Division of the Armed Forces Health Surveillance Center

Annual Report
Fiscal Year 2008

Partnering in the Fight Against Emerging Infections

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Welcome Letters

The establishment of the Armed Forces Health Surveillance Center (AFHSC) by the Deputy Secretary of Defense on 26 February 2008 stands as an important milestone in the enhancement of health surveillance capabilities, including emerging infections surveillance, in the DoD. Along with the Defense Medical Surveillance System (formerly operated by the Army Medical Surveillance Activity of the USACHPPM) the Center now manages DoD-GEIS and has assumed responsibility for several health surveillance activities of the office of the Deputy Assistant Secretary of Defense for Force Health Protection and Readiness. AFHSC is supported by the USACHPPM and the Army Surgeon General for the Secretary of the Army, the executive agent for AFHSC designated by the Deputy Secretary of Defense.

Another milestone passed during FY08 was the appointment of Captain Kevin L. Russell as the Director of DoD-GEIS. He assumed the directorship from Colonel Ralph Loren Erickson in a ceremony on 14 July 2008 at the Walter Reed Army Institute of Research presided over by Major General George Weightman, Commanding General of US Army Medical Research and Materiel Command. Kevin is the Deputy Director of the AFHSC and is specifically charged with the integration of GEIS program activities and projects into those of the Center, in light of the broader health surveillance mission of the AFHSC.

The AFHSC was formed to provide relevant, timely, actionable, and comprehensive health surveillance information and support to the armed forces for military and military-associated populations. With the inclusion of DoD-GEIS in this organization, the DoD has aligned its strategic efforts in enhancing emerging infections surveillance with those engaged in analysis of data supporting surveillance efforts across the broad spectrum of exposures, diseases, and injuries of concern for the armed forces. This is a very powerful combination of expertise and capabilities. With the Center's focus on relevant populations at risk, our new organization will build on the accomplishments of the contributing organizations and programs and, by uniting them, improve our ability to support the force, our military and federal partner organizations, and all health surveillance missions of the DoD. I look forward to reporting to you the accomplishments of the AFHSC in our 2009 annual report.

Sincerely,

Robert F. DeFraites, COL, MC, USA
Director, Armed Forces Health Surveillance Center

Colleagues and Friends,

FY08 was a year of transformation for DoD-GEIS. In February, the Deputy Secretary of Defense formally brought together DoD-GEIS, the Defense Medical Surveillance System, the Army Medical Surveillance Activity, and the DoD Serum Repository into the Armed Forces Health Surveillance Center, a visionary forward-leaning public health organization for our men and women in uniform. We have a unique and powerful opportunity to leverage the strengths of each of these legacy organizations to create a better whole for the men and women we serve.

Also this year, I was honored and humbled to assume the leadership of DoD-GEIS from COL Ralph Loren Erickson. It is a privilege to be part of the DoD-GEIS and AFHSC family, where so many incredibly talented and dedicated professionals have found a home.

It was a year of change, a year of opportunities, a year of successes. I think you will agree as you read about the accomplishments of our partners in this 2008 annual report. GEIS is partnership. Through this partnership, we will continue to be an integrating and powerful force for global public health and force health protection!

An Honor,

Kevin L. Russell, CAPT, MC, USN
Director, DoD Global Emerging Infections Surveillance and Response System
Deputy Director, Armed Forces Health Surveillance Center

Armed Forces Health Surveillance Center Staff, 2009

Introduction

On 26 February 2008, the Department of Defense Global Emerging Infections Surveillance and Response System (DoD-GEIS) became a core component of the newly formed Armed Forces Health Surveillance Center (AFHSC). Now known as the GEIS Operations Division of the AFHSC, GEIS joins the Defense Medical Surveillance System and the DoD Serum Repository as part of this larger and more diverse and capable organization serving the DoD. In addition, a new director assumed leadership of DoD-GEIS on 14 July 2008.

Within this new organizational framework, the worldwide partnership that is GEIS continued to promote and facilitate national and international preparedness for emerging infections while maintaining its focus on protecting the health of all DoD health care beneficiaries. GEIS continued to promote, expand, and execute its strategic goals of surveillance and detection, response and readiness, integration and innovation, and cooperation and capacity building. Five categories of infectious diseases and associated clinical states remained the GEIS priority surveillance pillars: respiratory diseases, especially influenza; gastroenteritis syndromes; febrile illness syndromes, especially dengue and malaria; antimicrobial resistance; and sexually transmitted infections.

Efforts by the DoD-GEIS partnership in respiratory infections were many during FY08. The respiratory disease surveillance program at military training centers provided invaluable information on the extent and severity of morbidity, the specific infectious agents responsible, and influenza vaccine effectiveness. In response to the availability of a new generation of meningococcal vaccines and the changing nature of the global epidemiology of meningococcal disease, a military laboratory-based meningococcal surveillance program got underway. When fully operational, this program will monitor the incidence of meningococcal disease, evaluate and describe cases, thoroughly describe etiologic agents, identify vaccine failures, assess the global threat to military populations, and provide data to support DoD policies regarding vaccination, revaccination, and use of prophylactic drugs.

For the third year, DoD-GEIS administrated the DoD influenza surveillance program by coordinating the DoD clinical, syndromic, and laboratory-based surveillance activities for influenza and other acute respiratory infections and influenza-like illnesses, primarily among military and other DoD health care beneficiaries. The position of DoD as a major contributor of current data and information on influenza threats was strengthened by promoting and growing collaborations that improve military health facility and other partner laboratory capacity infrastructure. DoD-GEIS continued to support effective, central communication and coordination strategies among DoD partners and civilian agencies and international organizations. Academic pandemic and avian influenza programs and exercises that enhanced global pandemic preparedness were also supported.

These efforts achieved and strengthened routine seasonal influenza testing capabilities, novel virus and pandemic and avian influenza surveillance, and more timely and efficient case investigations across the DoD. DoD-GEIS was active in expanding the DoD influenza surveillance program to cover all five COCOM areas of responsibility. Expansions included the following:

- PACOM—Bangladesh, Bhutan, Cambodia, Indonesia, Nepal, Palau, Philippines, Republic of Korea, Saipan, Singapore, Thailand, and 7th Fleet;
- SOUTHCOM—four Central American militaries, Panamanian ministry of health, Colombian military;
- CENTCOM—Middle East, former Soviet Union republics, Joint Task Force-Horn of Africa;
- AFRICOM—Cameroon, Ghana, Kenya, Nigeria, Tanzania, Uganda, North Africa;
- EUCOM—Landstuhl Regional Medical Center, USACHPPM-Europe, and 5th Fleet.

Through GEIS-supported programs DoD influenza laboratory diagnostic capabilities expanded both OCONUS and CONUS in FY08. AFRIMS began using its new veterinary BSL-3 laboratory extensively and tested and commissioned its human BSL-3 laboratory that will be operational in FY09; the Walter Reed/AFRIMS Research Unit Nepal continued to operate jointly with the Nepalese Ministry of Health in Kathmandu, Nepal; the Naval Hospital in Yokosuka, Japan, continued serving the 7th Fleet; and NHRC progressed in commissioning its new BSL-3E laboratory. BSL-3 laboratory capacity is planned for the US Army Medical Department Activity in Yongsan, Republic of Korea, and the Landstuhl Regional Medical Center in Germany.

Timely availability of reliable, state-of-the-art diagnostic laboratory capacity is essential to program success. Through GEIS Headquarters, an interagency agreement was signed with the Chemical Biological Medical Systems-Joint Program Executive Office to use the CDC influenza A/H5 assay, recently cleared by the FDA, for use with the DoD JBAIDS laboratory testing platform. USAMRIID is conducting the necessary preclinical assay platform validation studies for FDA clearance. In addition, GEIS has supported USAMRIID's development of a panel of 21 influenza strains that can be accessed to evaluate and validate new influenza diagnostic assays.

Military-military collaborations were forged by GEIS in FY08 to improve consistency among countries in respiratory disease trend analyses and studies about similar at-risk populations. As an added benefit, these collaborations are providing unique insights into cross-country influenza strain circulation and the generation of geographically specific joint threat assessments. These activities strengthen COCOM surgeon discussions and collaborations with host country military, clinical, and public health authorities. GEIS military-military partners include the following.

- Kenyan and Nigerian military and Tanzania People's Defense Forces: surveillance at military health care facilities;
- Polish Military Institute of Hygiene and Epidemiology: surveillance at five basic training sites and preliminary work on future pandemic influenza preparedness and response exercises;
- Israeli Defense Forces: surveillance at two military health care facilities and one basic training site;
- Hungarian Defense Forces: surveillance at five military health care facilities;
- Singaporean Armed Forces: surveillance at seven military health care facilities;
- Colombian Army: establishment of disease surveillance system;
- Peruvian Army/Navy: expansion of Alerta disease surveillance system.

Regarding febrile illness syndromes, GEIS partners made progress toward standardizing malaria drug resistance testing across all DoD facilities and monitored for malaria in all military forces and for the reemergence of *Plasmodium vivax* malaria in Korea. NASA's predictive model for Rift Valley fever outbreaks in East Africa provided reliable advance warning alerts on the pending threats to DoD regional and international partners and encouraged preemptive, targeted action to prevent and/or mitigate human morbidity and mortality for this disease in 2008. GEIS partners are collaborating to develop a similar tool for Japanese encephalitis. These predictive models will provide DoD with data to support risk assessments and policy recommendations on vaccination programs.

A standardized antimicrobial resistance monitoring system for the global MHS came closer to reality in FY08. Several major military medical facilities are striving to reach consensus on the best practices for DoD-wide capability to identify important pathogens associated with wound infections. DoD-GEIS partners are developing a centralized clinical, laboratory, and pharmacy database for antimicrobial resistance monitoring.

The ongoing emphasis by GEIS on determining the incidence of sexually transmitted infections has resulted in a >100% increase over 2001 in annual screenings for *Chlamydia* in at-risk military females. FY08 saw the implementation of a screening program in the US Army in Korea that confirmed its value by identifying of a high prevalence of infected soldiers. A collaboration with the CDC is raising awareness among military organizations for the need to provide drug-resistant *Neisseria gonorrhea* strains to CDC for use in the development of nonculture tests for *N. gonorrhea*.

Throughout FY08, the programs GEIS has put in place continued to generate essential data that bolstered the DoD and global public health efforts. The robust training effort of GEIS continued and grew, notably in a productive new program through the University of Iowa that stretches to Mongolia. Many systems expanded, and additional programs were instituted. Key FY08 global and select accomplishments follow.

Ten FY08 Global Accomplishments of DoD-GEIS

1. Conducted global emerging infection surveillance and response activities and efforts with 39 partners in 111 countries.
2. Expanded the DoD global influenza surveillance program to 72 countries, 20 Navy ships, 1 foreign ship, and 6 clinics along the Mexican border (four in California, two in Mexico); increased laboratory capability system-wide; collected and analyzed more than 21,000 respiratory samples. This represents an expansion of over 230% from levels before the funding for pandemic and avian influenza was available.
3. Served as primary resource for global avian influenza surveillance throughout the world. NAMRU-3 confirmed 15 (3 from Pakistan, 12 from Egypt) of the 57 global human H5 infections in FY08 (26%).
4. Improved laboratory infrastructure at 52 sites in 29 countries, including 16 military and 36 civilian laboratories, with emphasis on influenza and leveraged capability for other emerging infectious disease initiatives.
5. Sponsored and/or conducted 46 training exercises with more than 2,900 representatives from 53 countries.
6. Responded to more than 20 outbreaks globally in military and civilian populations. These outbreaks included influenza, dengue, yellow fever, diarrhea (norovirus and rotavirus), leptospirosis, pneumonia, Rift Valley fever, hemorrhagic fevers, rickettsial illnesses, and poultry die-offs from suspect avian influenza.
7. Discovered emerging and reemerging pathogens:
   - New species of *Anopheles* mosquito in the Republic of Korea revealed by DNA sequencing at WRAIR;
   - The Republic of Korea surveillance program also identified a new Hantavirus, Imjin virus, carried by the insectivore rodent Crocidura laciura.
   - Isolation and description of a new virus, Chapare virus, from a fatal case of hemorrhagic fever in Bolivia published by NMRCD;
   - Reemergence of dengue serotype 4 found by NMRCD;
   - Emerging strains of malaria potentially expressing artesunate resistance described in Cambodia, Sudan, Thailand, and Yemen.
8. Expanded coverage in Africa with projects in Cameroon, Ethiopia, Ghana, Kenya, Libya, Morocco, Nigeria, Sierra Leone, Somalia, Sudan, and Uganda:
   - Improvements in host country diagnostic testing;
   - Emerging infectious disease outbreak detection and control;
   - Surveillance for influenza, rotavirus, and hospital-based bacterial meningitis.
9. Predicted and provided early warning of Rift Valley fever outbreaks and efficient responses in Sudan (June), South Africa (February and May), and Madagascar (February) through collaboration in ecological and climate monitoring and surveillance with NASA, USAID, USDA, WHO, FAO, host countries, and international partners.
10. Standardized laboratory characterization of *Acinetobacter* using uniform laboratory test systems and software at major US military medical facilities treating servicemembers with infected wounds. This accomplishment paves the way for laboratory standardization of other microbes of military interest.

Ten FY08 Select Accomplishments of DoD-GEIS

1. Lassa fever diagnostic laboratory capacity was established for Sierra Leone by USAMRIID, providing earlier diagnosis and more effective care of cases and reducing risk to US forces in area. This activity was a significant FY08 achievement for health diplomacy.
2. A study of archived data from the Australian Army in 1918–1919 conducted by the Australian Army Malaria Institute provided evidence of a protective effect of an early wave of influenza infections that may have critical implications for US pandemic preparedness decisions on prepandemic vaccine development and stockpiling.

3. During FY08 artemisinin resistance in *Plasmodium falciparum* was identified along the Thai/Cambodian border by AFRIMS and in Southern Cambodia by NAMRU-2. This finding raises concern over the antimalarial that is predominately used in the area and emphasizes the existing requirement to develop future control options critical for local public health and troops operating in the area.
4. Timely and actionable information was provided for inclusion of strains in the annual influenza vaccines. The H1N1 Brisbane-like virus seed strain for the 2008–2009 and 2009–2010 live attenuated influenza vaccine was collected by USAFSAM from four dependent brothers at Ellsworth AFB in July 2007 and was shared with the CDC.
5. Recruit training center febrile respiratory illness surveillance by NHRC continued the valuable near-real-time influenza vaccine effectiveness studies and collection of information for the adenovirus vaccination initiative. The resurgence of adenovirus types 3, 7, 14, and 21 was followed among recruit populations.
6. Major steps were taken to obtain FDA clearance for inclusion of the CDC's influenza A (H5) diagnostics in the JBAIDS platform to greatly increase available influenza diagnostic capabilities for deployed forces.
7. Diarrheal pathogen surveillance continued in five sites in Thailand and three sites in Nepal. Because the prevalence of *Campylobacter* was found to be low in Nepal, the use of less expensive quinolones was continued.
8. Important malaria disease variables that facilitate ongoing monitoring were identified and characterized, and the development and targeting of interventions in South Korea have been described in detail. WRAIR used these descriptions to develop MosquitoMap, a web resource to display mosquito occurrences, and designed a Mal-area calculator for estimating future disease risk in South Korea.
9. AFRIMS demonstrated that specimens of chikungunya from a southern Thailand outbreak were caused by a different viral subtype than those that caused outbreaks in the western Indian Ocean islands in 2008.
10. A model sexually transmitted disease surveillance and follow-up treatment protocol was established for arriving soldiers in South Korea. Communication and collaboration with the CDC were enhanced, and a modified CDC instrument for surveying sexually transmitted disease laboratory capabilities was used to assess laboratory tests at Army facilities worldwide. All services exhibited significant increases in annual *Chlamydia* screening rates for at-risk military females.

FY08 Financial Management and Accountability

The total DoD-GEIS partnership budget for FY08 was $51.7 million, which comprised $40 million in supplemental funding for pandemic and avian influenza and $11.7 million for funding GEIS core activities and programs unrelated to pandemic and avian influenza through the Defense Health Program (P8). Of the core funds, GEIS allocated $6,747,600 to the Army, $4,437,000 to the Navy, and $564,400 to USUHS (Figure 1). All financial distributions were made through the Tricare Management Activity after being approved by both internal and external review processes. The pandemic and avian influenza funding was distributed after a formal briefing and concurrence by the Executive Agency Directorate, Office of the Surgeon General, US Army; the Force Health Protection Council; and the Deputy Assistant Secretary of Defense, Force Protection and Readiness.

DoD-GEIS continued to meet its pandemic and avian influenza funding criterion that activities and projects be leveraged by using existing partners' infrastructures. Allocation of the pandemic and avian influenza funding is shown in Figure 2. For the OCONUS laboratories, the Army laboratories (AFRIMS and USAMRU-K) received $7,238,000, and the Navy laboratories (NAMRU-2, NAMRU-3, and NMRCD) received $8,883,500. This support funded emerging infections surveillance activities at 376 sites in 72 countries.

Pandemic and avian influenza distributions to the CONUS-based partners follow: USAFSAM $2,372,000; NHRC $4,336,000; NMCPHC and the NEPMUs $1,395,000. GEIS Headquarters distributed $2,043,165 to CDHAM, $1,318,000 to WRAIR Division of Viral

Diseases, $1,663,000 to the USACHPPM Center for Serosurveillance and Epidemiology for Pandemic Influenza, $390,000 to USAMRIID, $480,000 to Landstuhl Regional Medical Center, and $266,000 to Brooke Army Medical Center. In addition, Headquarters entered a $6,069,188 contract for an administrative infrastructure to provide oversight and management for the pandemic and avian influenza program and to conduct epidemiology training and certification for GEIS partner host country nationals. DoD-GEIS also funded conferences by USACHPPM, NORTHCOM, and EUCOM on pandemic influenza preparedness. DoD-GEIS built these conferences around active-learning tabletop and situational awareness exercises. In general, funding to the CONUS and OCONUS partners was released from Headquarters within one week of availability, resulting in a 99.99% obligation rate.

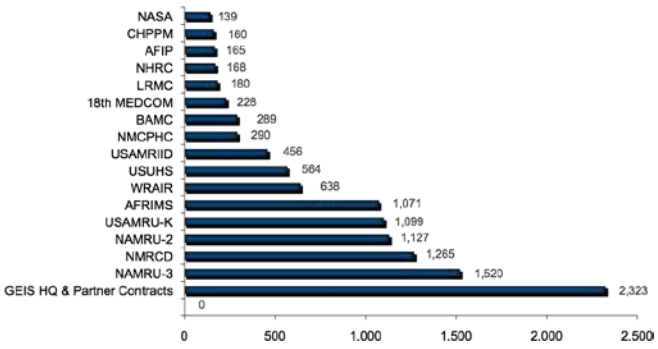


Figure 1. FY08 GEIS core P8 distributions, total $11,749,000.

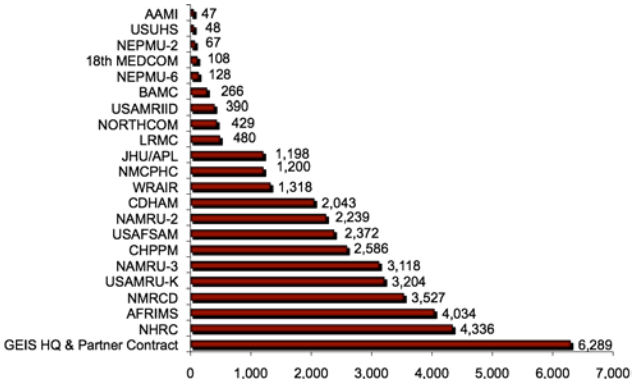


Figure 2. FY08 pandemic and avian influenza surveillance budget distribution, total $40,000,000.

Global Reach of DoD-GEIS Partnerships in FY08

Afghanistan
Albania
Argentina
Australia
Azerbaijan
Azores
Bahrain
Bangladesh
Belgium
Bhutan
Bolivia
Brazil
Burkina Faso
Burma
Cambodia
Cameroon
Canada
Colombia
Cote d'Ivoire
Cuba
Djibouti
Dominican Republic
Ecuador
Egypt
El Salvador
Ethiopia
Gabon
Gambia
Georgia
Germany
Ghana
Guam
Guatemala
Guinea-Bissau
Guyana
Haiti
Honduras
Hungary
India
Indonesia
Iran
Iraq
Israel
Italy
Japan
Jordan
Kazakhstan
Kenya
Kosovo
Kuwait
Kyrgyzstan
Laos
Lebanon
Liberia
Libya
Madagascar
Malawi
Malaysia
Maldives
Mauritania
Mexico
Mongolia
Morocco
Mozambique
Nepal
Nicaragua
Nigeria
Northern Mariana Islands
Oman
Pakistan
Palau
Panama
Paraguay
Peru
Philippines
Poland
Portugal
Puerto Rico
Qatar
Republic of Korea
Romania
Saipan
Saudi Arabia
Senegal
Sierra Leone
Singapore
Somalia
South Africa
Spain
Sri Lanka
Sudan
Suriname
Syria
Tajikistan
Tanzania
Thailand
Togo
Tonga
Trinidad and Tobago
Turkey
Uganda
Ukraine
United Kingdom
United States
Uruguay
Uzbekistan
Vanuatu
Venezuela
Vietnam
Yemen
Zimbabwe

State of DoD-GEIS in FY08

DoD-GEIS partners participated in a workshop entitled "State of the GEIS" on 9–11 January 2008 in Bethesda, Maryland, to review achievements and organizational plans and provide insight and recommendations on strengthening GEIS and maximizing service to its constituents, the men and women of the DoD (Figure 3). All participants acknowledged that commitment to meeting the evolving needs of force health protection, especially in the COCOMs, and effective collaboration with federal agencies in defending the country from emerging infectious disease threats are the core of the DoD-GEIS partnership *raison d'être*. They further agreed that contributions from all partners should synergize so that the whole of GEIS is greater than the sum of its parts.

Partners organized themselves into work groups to discuss the challenges in and opportunities for strengthening the GEIS partnership. Work groups covered the following subjects:

1. Balancing mission priorities;
2. Integrating emerging infections surveillance and best laboratory methods;
3. Setting priorities, goals, and objectives for expansion;
4. Coordinating with other federal agencies;
5. Preparing for pandemic influenza;
6. Evaluating strategic goals and priority surveillance pillars.

Each workgroup reached general consensus on its recommendations. These recommendations, which follow, form the first steps toward a stronger DoD-GEIS partnership committed to continuous quality assessment and improvement.

Figure 3. Participants in State of the GEIS workshop, January 2008.

Work Group 1: Balancing Mission Priorities

Partners need to achieve balance among their GEIS priorities and those of their home organizations to avoid over commitment or unnecessary competition among multiple masters. By starting with the understanding that stronger public health supports force health protection, partners can leverage their GEIS projects with their existing organizational missions. Then both GEIS and host organization assets and activities can mutually reinforce one another.

Recommendations for Headquarters

1. Create a strategic communication plan for inter-partner situational awareness.
2. Develop a product that synthesizes, analyzes, and shares relevant value-added medical information that also identifies gaps in DoD-GEIS surveillance efforts.

Work Group 2: Emerging Infections Surveillance and Best Laboratory Methods

The use of best practice laboratory methods (defined as a gold standard) is critical to achieving reliable and valid disease surveillance data. Meeting this requirement presents a daunting challenge to partners because diagnostic platforms differ greatly among laboratories.

Surveillance tools used by GEIS partners include Alerta (Peru), EWORS (Indonesia), UBS (Thailand), and ESSENCE (United States and within MHS). Appropriately, the epidemiologic methodology of these tools varies to meet the requirements of working in the respective geographic, social, and cultural environments. Strict DoD-GEIS partnership-wide standardization is not a valid approach. Host countries view the GEIS role in providing appropriate surveillance information for their country as essential. The COCOMs support GEIS by providing insights into, and requirements for, priorities in their areas of responsibility as part of their emerging infections surveillance proposal process. Collaboration and coordination with academia may serve as a reasonable method of improving the GEIS partners' capabilities to meet best practice standards.

Recommendations for Headquarters

1. Advocate for best practice compliance methods and measures in all funded projects and activities;

2. Share information on advances in best practices with partners;
3. Support the evaluation of existing surveillance systems using standardized methodologies;
4. Promote the development of standardized laboratory diagnostic panels for multiple pathogens that can be shared with partners;
5. Facilitate the process of moving diagnostic assays from a homebrew to a test eligible for clinical diagnoses.

Work Group 3: Setting Priorities, Goals, and Objectives for Expansion

DoD-GEIS needs a clear and concise mission statement and plan of action for demonstrating the DoD-GEIS partnership's operational uniqueness and growing contribution to global public health and force health protection.

Recommendations for Headquarters

1. Consider asking an outside group of respected authorities to develop recommendations for how DoD-GEIS might enhance its future operational and programmatic effectiveness;
2. Feature partner accomplishments and successes in its communications with DoD leadership and the public;
3. Clarify DoD-GEIS priorities, interests, gaps, requirements, and funding decision processes;
4. Expand existing programs for discovering new and emerging pathogens and for studying the social and environmental factors that may contribute to their emergence.

Work Group 4: Coordinating with Other Federal Agencies

Partners in other federal agencies often wish to collaborate with DoD domestic and overseas laboratories. These proposed collaborations are often location-specific and activity-based. Also, within the DoD, the services have different strategic missions and priorities. For these reasons, routinely administering these interagency collaborations from a centralized GEIS office may adversely impact or inadvertently alter the intended collaborations. Nevertheless, centralized promotion and coordination have a role.

Recommendations for Headquarters

1. Facilitate, plan, and promote interagency collaborations and initiatives with federal agencies, partners, and COCOMs;
2. Provide GEIS partners with key messages and risk communications guidance.

Work Group 5: Preparing for Pandemic Influenza

DoD-GEIS is the primary point of contact for DoD pandemic and avian influenza and emerging infections surveillance. In this capacity, Headquarters serves installation commands by advising preventive medicine/public health emergency officers on pandemic and avian influenza surveillance and preparedness and response issues and activities. DoD-GEIS also supports the services and COCOMs in their force health protection responsibilities to deployed units and regional medical commands. These responsibilities were articulated and are listed under "Roles and Responsibilities of DoD CONUS and OCONUS Laboratories in Pandemic and Avian Influenza Surveillance." Selective role and capacity redundancies found in DoD-GEIS, as an entirety, assure the continued ability of DoD to effect essential response activities during pandemics. They also reflect the geographic and functional uniqueness of GEIS partners.

Recommendations for Headquarters

1. Routinely update the Force Health Protection Council and senior DoD leadership about the status of pandemic and avian influenza surveillance and response capabilities;
2. Actively share DoD pandemic and avian influenza surveillance information, including GEIS-approved project lists, with COCOM surgeons and command public health emergency officers;
3. Incorporate GEIS partner laboratory pandemic and avian influenza roles and responsibilities into the AFHSC DoD directive;
4. Encourage partner laboratories to track consumable supplies, plan for adequate surge capacity, and coordinate with local and regional civilian counterparts;
5. Consider the development of a searchable, web-based database of reports, articles, and other useful pandemic and avian influenza documents and training materials;
6. Promote the use of standardized influenza-like illness surveillance and laboratory data acquisition tools and sample questionnaires, led by USAFSAM;
7. Consider forming a committee to advise partners on common database issues in epidemiology, laboratory, and risk factor data;
8. Represent GEIS partners at Avian Influenza Action Group/Department of State interagency group meetings;

9. Consider leveraging OCONUS capability to further surveillance efforts characterizing influenza infection in migratory birds, poultry, and other animal hosts.

Work Group 6: Evaluating Strategic Goals and Priority Surveillance Pillars

Over time, the strategic goals and priority surveillance pillars of DoD-GEIS have evolved to reflect changes in global public health threats and the necessary GEIS programmatic refinements for overcoming those threats. They provide strategic direction to the DoD-GEIS partnership. A DoD-GEIS mission and vision statement are needed.

Recommendations for Headquarters

1. Periodically assess, clarify, and refine the DoD-GEIS mission statement;
2. Develop a strategy for rapidly and effectively communicating with partners and the DoD community at-large;
3. Periodically perform critical evaluations of all GEIS-supported work to assess alignment with DoD-GEIS goals and priority surveillance pillars.

Way Forward: DoD-GEIS into FY09 and Beyond

As this and previous annual reports describe, the DoD-GEIS network has expanded in 11 years and provides invaluable contributions to force health protection and global public health. In the last few years the expansion has been rapid because GEIS received substantial funding targeting the recently acknowledged threat of pandemic and avian influenza.

This section will review the founding implementing actions for DoD-GEIS, examine progress evident in FY08, and describe priorities going forward. The rapid growth of the last few years required a creative and expanded vision, and the time is ripe for examining that expansion within the guidelines of the founding documents and for ensuring that all elements of GEIS are linked in a cohesive, united system of partners.

Founding Directives

DoD-GEIS was established in 1997 in response to Presidential Decision Directive NSTC-7, which was issued in June 1996. Subsequently in 1998 GEIS published its strategic plan that outlined the goals, objectives, and activities of the new program. These two documents, NSTC-7 and the strategic plan, formed the cornerstone from which DoD-GEIS grew.

The five implementing actions of NSTC-7 that are most relevant to GEIS follow, along with commentary about each to convey how GEIS meets each directive. This awareness is provided to guide current partners going forward in their work with GEIS.

A. "Enhance the surveillance and response components of our domestic and international public health infrastructure."

The GEIS directive is simple and powerful: GEIS is empowered to use its resources for the good of the local public health infrastructure as it supports the needs of uniformed men and women. The wisdom in this freedom was understood by the DoD administration. Support of the host countries, through humanitarian assistance, was a clear theater security benefit. The countries were strengthened; GEIS and DoD relationships were strengthened. These strong benefits for all continue and must remain a priority for DoD-GEIS. Capacity building, infrastructure improvement, and technology transfer are strongly supported in the FY08 work covered in this annual report. GEIS must avoid excessive administrative bureaucracy so that this necessary flexibility is not lost.

Initial funding for DoD-GEIS came at a time of great need for the OCONUS laboratories. For the laboratories to operate as visitors in their respective countries, support was needed through which value could be added to the local public health community as the laboratories supported the DoD. However, such funds were scarce or simply unavailable. For example, to assist in the conduct of a simple outbreak investigation was impossible or, at best, extremely difficult. The expertise existed, but flexible funding did not. DoD-GEIS provided such funding. While assisting the local public health response, the infectious pathogen risks were more fully understood, which was directly in line with GEIS objectives. New collaborations were forged that strengthened the position of the OCONUS laboratories and improved bilateral relationships, ensuring many years of successful coexistence and cooperation.

Future partner funding will be used to encourage assimilation of the rapidly expanding and available techniques for pathogen discovery into GEIS surveillance activities. For example, it is not enough to outline the flow diagram for a diagnosis that will be used to identify the known pathogens causing a febrile illness. GEIS must embrace state-of-the-art capabilities and ask, "What is causing the illness in the remaining undiagnosed cases?" Mindful of the word "emerging" in its name, GEIS will encourage partners to go one or two steps further, dig deeper, ask the hard questions, and analyze with a critical mind.

B. "Enhance biomedical and behavioral research efforts on emerging infectious diseases."

Research was not, and will not in the future, be the first priority of DoD-GEIS. However, by using knowledge gathered in GEIS surveillance activities, limited science initiatives should be supported as a bridge to more comprehensive funding from organizations such as the Military Infectious Disease Research Program or other governmental or civilian entities. Work in this realm should emphasize an eventual product that will enhance force health protection, such as drug or diagnostic development.

C. "Expand formal training and outreach to health care providers."

Since its inception, GEIS has embraced the training mandate of NSTC-7. From formal training programs of the OCONUS laboratories to GEIS-sponsored tabletop exercises conducted by CDHAM, training has been

extensive in places encircling the globe, including Colorado, Germany, Burkina Faso, Cote d'Ivoire, Kyrgyzstan, Ethiopia, Tajikistan, Thailand, Cambodia, Singapore, Hawaii, Australia, Trinidad and Tobago, and Panama. Further training opportunities exist, and training will continue to be a priority of DoD-GEIS.

D. "Encourage other nations and international organizations to assign higher priority to emerging infectious diseases."

GEIS works intimately with host nations by assisting with infrastructure improvement, technology transfer of laboratory capability for emerging infectious diseases, and training of local public health professionals and health care providers. Through these interactions, all GEIS collaborators and colleagues become more aware of, more in tune with, and more capable in the investigations of and responses to emerging infectious diseases. Continued emphasis on this directive, working with host country professionals as pathogen discovery capabilities are expanded, will be part of the GEIS mission moving forward.

E. "Support the World Health Organization and other bodies in playing a stronger role in the surveillance, prevention, and response to emerging infectious diseases."

The ten global and ten select FY08 accomplishments presented in the Introduction demonstrate opportunities Headquarters and the GEIS partners have taken to interface, collaborate, and coordinate with the WHO and the CDC, USAID, Department of State, and other government and civilian organizations. A liaison is supported by DoD-GEIS at WHO in Geneva, and discussions have begun for support of an additional uniformed professional at the WHO. A liaison with the CDC is also leveraged through regular teleconferences and communications.

Coordination and collaboration with these international and governmental organizations are challenging because the organizations are immense, bureaucracy extensive, and turnover constant. Therefore, GEIS must persistently and continually interact with these organizations to ensure that GEIS goals are met without duplicating efforts while maximizing the benefit for all.

Progress Evident in FY08

DoD-GEIS priorities have always remained consistent with those outlined in the strategic plan. Nevertheless, as the new millennium began new priorities arose within the global public health community, particularly concerns about pandemic influenza risks. The US government responded with three documents, and DoD-GEIS was ideally placed to meet some of the actions directed by these documents.

The document with directives specific to GEIS is the *DoD Implementation Plan for Pandemic Influenza* (August 2006), which complements the *National Strategy for Pandemic Influenza Implementation Plan* (May 2006) and the *National Strategy for Pandemic Influenza* (November 2005). The actions of the DoD plan for which DoD-GEIS has direct responsibilities follow:

4.2.2.5. Inpatient and outpatient disease surveillance

4.2.2.7. Assist with influenza surveillance in host nations

4.2.3.8. Develop/enhance DoD network of overseas infrastructure

6.2.2.9. Enhance public health response capabilities

6.3.4.7. Enhance influenza surveillance reporting techniques

To meet these responsibilities, DoD-GEIS received congressional supplemental funding in FY06 that increased the annual GEIS budget from $12 million to $50 million. In managing these increased program and fiscal responsibilities, DoD-GEIS Headquarters closely follows the achievements of the AFHSC and all partners in the above areas and regularly reports progress to DoD-Health Affairs.

Vision

Successfully develop, implement, support, and evaluate an integrated global emerging infectious disease surveillance and response system that supports the Armed Forces Health Surveillance Center and promotes preparedness in US forces, the military health system, and the global public health community.

Mission Statement

To contribute to force health protection and global public health by centrally coordinating a global system of partners that conducts emerging infectious disease surveillance and response; training and capacity building; research, innovation, and integration; and assessment of value added in a timely, efficient, comprehensive, and communicative manner.

Strategic Goals

Surveillance and response
Training and capacity building
Research, innovation, and integration
Assessment and communication of value added

Priority Surveillance Pillars

Respiratory infections (RI)
Gastrointestinal infections (GI)
Febrile and vector-borne infections (FVBI)
Antimicrobial resistance (AR)
Sexually transmitted infections (STI)

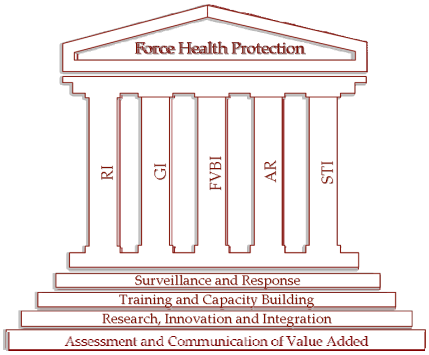


Figure 4. DoD-GEIS vision, mission statement, strategic goals, and priority surveillance pillars.

Into FYO9 and Beyond

The historic documents, increasing requirements, and organizational change have contributed to confusion among some staff and partners regarding the direction of DoD-GEIS. To form a basis for the way forward, the GEIS vision, mission statement, strategic goals, and priority surveillance pillars for FY09 and beyond are presented in Figure 4.

Last, and at least as important, for GEIS to accurately steer the way forward, GEIS needs feedback from its partners and customers. The January 2008 State of the GEIS workshop updated staff of achievements and provided GEIS leadership with valuable input on the entire network. The documents and recommendations developed from this workshop are being carefully considered by GEIS Headquarters. Many recommendations are already being implemented; many are reflected in this section; and the influences of many are seen in the DoD-GEIS priorities articulated in the box below.

In summary, DoD-GEIS is achieving many of its objectives. The ability of GEIS Headquarters to successfully implement the expanded program required by its role in pandemic influenza surveillance is a strong testament to the creative and innovative vision of Headquarters and the GEIS partners during the last few years. The current challenge for the GEIS network is to embrace an expanded global perspective that can be expressed as follows.

Think as partners, not as individuals.
Think as systems, not as institutional programs.
Think globally, not regionally.

This mindset must permeate the thoughts and actions of all GEIS staff and partners, or the products may not benefit the whole. This will require effort by the partners to consider, to act, to become part of the whole system rather than just a program out of their own organization. Communication in the form of workshops and teleconferences is the only way to achieve such unity and coordination. Communication takes time. Only by creating global systems as a unified whole, however, can GEIS influence be more than the sum of its parts.

Priorities for the Way Forward

Unify priority surveillance pillars into coordinated global surveillance systems.

Mature current surveillance network; reduce emphasis on immediate expansion.

Publish requests for proposals that emphasize the following:

- Accomplish the vision and mission of DoD-GEIS and AFHSC;
- Fulfill requirement(s) in the *DoD Implementation Plan for Pandemic Influenza*;
- Coordinate/leverage with other partners;
- Standardize protocols;
- Fit into a global system;
- Process laboratory standard operating procedures that explain pathogen discovery among unknowns;
- Focus on population-based surveillance studies and understanding of denominators;
- Explain how response will be given with surveillance initiatives;
- Evaluate metrics for program effectiveness processes;
- Justify research proposals that explain how work will bridge surveillance gaps and present other potential funding sources.

Dedicate time for Headquarters staff and partners to communicate:

- Support GEIS partner telephone call twice per month;
- Feed good news stories to Headquarters.

Include COCOMs as partners and in priority communications.

Facilitate projects that leverage resources and capabilities from multiple DoD sectors.

Consider funding for non-DoD entities only if partnering with DoD groups and demonstrating lasting capability enhancement with DoD.

DoD Overseas Laboratories

Armed Forces Research Institute of Medical Sciences

Bangkok, Thailand

The Armed Forces Research Institute of Medical Sciences (AFRIMS) is located in the heart of Southeast Asia, a region of the world with a high rate of emerging infections. GEIS-funded programs at AFRIMS have expanded surveillance, response capabilities, laboratory capacity, and training in Thailand and surrounding countries in southern and southeastern Asia. Through these programs, the US military is assisting the region to build its laboratory capacity and surveillance programs, share data within the region and the world, and ultimately contribute to global stability through timely recognition and response to new and reemerging pathogens.

AFRIMS conducts diarrheal pathogen surveillance at five sites in Thailand and three in Nepal. Based on initial data, 74% of the *Campylobacter* isolates in Nepal and 62% of those from Thailand are resistant to ciprofloxacin (Table 1). Given the relatively high cost for azithromycin and the relatively low prevalence of *Campylobacter*, the continued use of the much cheaper quinolones may be a cost-effective strategy in Nepal. The prevalence of enteroaggregative (EAgg) *Escherichia coli* is high in Nepal and northern Thailand. As a chronic infection, EAgg *E. coli* tends to be more antibiotic-resistant, making treatment more difficult and expensive, and 29% and 12% of EAgg *E. coli* isolates in Thailand and Nepal, respectively, are resistant to azithromycin.

Table 1. Preliminary Prevalence of Resistance of Campylobacter,Salmonella, Shigella, ETEC, EPEC, and EAgg E. coli Isolates to Selected Antibiotics in Children in Thailand

Pathogen	*n*	Ampicillin	TMP-SXT	Ciprofloxacin	Azithromycin
Campylobacter	105	11%	39%	62%	4%
Salmonella	158	79%	22%	0%	0.6%
Shigella	5	0%	100%	0%	0%
ETEC	9	67%	33%	0%	11%
EPEC	50	52%	34%	0%	8%
EAgg *E. coli*	31	77%	71%	3%	29%

Surveillance and Detection

AFRIMS continued to provide comprehensive emerging infectious disease surveillance for Southeast Asia with its sister laboratory, NAMRU-2. In addition to significant expansion of influenza surveillance capability, AFRIMS continued to be the vanguard of surveillance for resistant *Plasmodium falciparum* along the borders of Thailand. Ongoing surveillance for multidrug-resistant enteric pathogens, febrile illnesses, zoonotic diseases, and respiratory pathogens was conducted to expand surveillance programs throughout the region.

With evidence of reduced overall sensitivity of *P. falciparum* to artemisinin derivatives along the Thai-Cambodian border, AFRIMS is conducting an in vivo study with artesunate monotherapy to assess and ensure the safety of patients taking high-dose therapy in comparison with two other dosing regimens. A second study is characterizing molecular aspects of malaria infections associated with resistance to drug therapy along the Thai-Cambodian border.

In response to the threat of emerging zoonoses, AFRIMS assisted the ministry of agriculture with zoonotic disease surveillance for brucellosis, leptospirosis, and melioidosis using serologic tests of native livestock; 6,631 blood samples were obtained with positive brucellosis antibody titers in 48 cattle (0.95%) and 8 buffaloes (0.83%), leptospiral antibodies to one or more serovars in 3,262 cattle (64.03%), 651 buffaloes (67.53%), 47 goats (9.38%), and 4 sheep (5.71%), and high titers for melioidosis in 39 cattle (0.77%) and 2 buffaloes (0.21%).

Comprehensive influenza surveillance is critical in Southeast Asia, and AFRIMS has established sentinel influenza surveillance sites throughout the region; 1,166 samples were collected with 39% positive for influenza A or B confirmed by RT-PCR. One H1N1 strain isolated from Kamphaeng Phet, Thailand, was resistant to oseltamivir, and this unique finding was reported to the Thai Ministry of Public Health and the WHO.

In addition to these surveillance sites, the influenza surveillance conducted jointly by AFRIMS and the Royal Thai Army in six remote military hospitals continued. PCR testing at AFRIMS on 583 samples found 449 negative and 134 positive: 61 influenza A/H1, 44 influenza A/H3, 2 influenza A/unsubtype, and 27 influenza B. Further testing by MassTag PCR on 409 samples found 266 negative and 143 infected by virus (influenza A, 20-B, RSV-A, RSV-B, human metapneumovirus, enterovirus, adenovirus, and parainfluenza virus type 4 subtypes) and bacteria (*Mycoplasma pneumoniae*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*). In addition, MassTag PCR was used to identify coinfection of pathogens such as RSV-B with *S. pneumoniae*, adenovirus + coronavirus-OC43 + *H. influenzae*, *H. influenzae* with *S. pneumoniae*, influenza A, or parainfluenza virus type1.

In FY08, AFRIMS started surveillance programs at two new sites in South Asia: Bangladesh and Bhutan. In Bangladesh, a screening program for influenza-like illness was instituted, and patients were evaluated with rapid tests on site. In Bhutan, AFRIMS conducted training in laboratory sampling, testing, and transport and provided rapid tests for initial testing. Beginning in FY09, specimens will be shipped to AFRIMS.

Response and Readiness

AFRIMS assisted the Thai Ministry of Public Health and regional governments in diagnosing emerging infections. In FY08, AFRIMS tested human and animal samples for evidence of infection with the causative agent of tularemia, the first time this agent was potentially isolated in Southeast Asia. Reports of chikungunya cases have been increasing in Southeast Asia, and to prepare for the eventual presence of chikungunya in the region, AFRIMS has purchased equipment and supplies to support vector testing and is collecting mosquitoes from high threat areas.

Kingdom of Bhutan Establishing Human and Animal Influenza Surveillance

At the request of the Royal Government of Bhutan, AFRIMS and DoD-GEIS supported training and surveillance efforts for pandemic and avian influenza in Bhutan in FY08.

AFRIMS organized an influenza rapid response training course for administrative, clinical, and laboratory personnel from the Bhutanese health and agriculture ministries 28–30 July 2008 in Punakha, Bhutan. During this course, experts from AFRIMS, the WHO Regional Office for Southeast Asia, and the WHO Influenza Reference Laboratory at Saint Jude Children's Research Hospital (Memphis, TN) provided current information on the global status of H5N1, guidance on conducting outbreak investigations including sample collection and handling, laboratory diagnosis, and treatment of infected patients.

Participants in rapid response course sponsored by AFRIMS and GEIS in Punakha, Bhutan, July 2008.

AFRIMS is actively working with the Ministry of Health and Education and Ministry of Agriculture to conduct influenza surveillance throughout the country. Seasonal influenza surveillance with the health ministry began in October 2008 with specimen collection in the Bhutanese capital, Thimphu, and two other large cities in

Rapid response course included lectures and hands-on training.

Continued

Kingdom of Bhutan Establishing Human and Animal, *Continued*

Countryside and dzong (castle) at Punakha, Bhutan.

western Bhutan, Punakha and Paro. Future plans include expansion of surveillance to Gelphu and Mongar, the largest towns along a west-to-east trade route, and Phuntsholing, the main overland trade center connecting Bhutan and India. This program will provide valuable information about the seasonal burden of influenza in the region and develop capacity to respond to outbreaks. AFRIMS serves as an influenza reference laboratory for Bhutan while the health ministry establishes laboratory capacity for influenza in Thimphu.

The Ministry of Agriculture has already established a surveillance program to monitor avian influenza in response to outbreaks of H5N1 across the border with India. Although strict poultry import measures and vigilance have allowed Bhutan to prevent H5N1 from entering the country so far, continuing support for these efforts and maintaining surveillance capacity to respond to new outbreaks will be critical to help control H5N1 in the region. AFRIMS is also partnering with the Academy of Education and Development (Washington DC) to assist the Ministry of Agriculture with a survey of knowledge, attitude, and practices regarding influenza in Bhutan.

Bhutan.

Cooperation and Capacity Building

AFRIMS works with multiple agencies throughout Southeast Asia to integrate important public health data with existing surveillance networks. All influenza test results are provided to the respective countries for inclusion in the WHO FluNet program. In addition, several projects use innovative techniques to bring new data to the attention of public health agencies.
The system established by the Royal Thai Army for early warning of medical threats continued to provide meaningful data from areas along the Thai border that are controlled by the military and inaccessible to the Ministry of Public Health network. At the field site in Cebu, Philippines, AFRIMS uses geographic information systems to establish baseline spatial, demographic, and health facility utilization data for households to determine spatial and temporal relationships of disease incidence and transmission correlated to possible causative factors.

In collaboration with Ehime University in Matsuyama, Japan, AFRIMS has developed a new method for hemagglutinin production using a cell-free expression for influenza diagnostics. Recombinant and whole virus antigens produce comparable results in detecting serum antibodies against avian influenza, and these efforts are expected to facilitate future H5N1 surveillance studies.

An important goal at AFRIMS is to strengthen regional capacity, using AFRIMS assets to provide diagnostic support and improve its partners' capabilities. AFRIMS added diagnostic capabilities for West Nile virus, brucellosis, hepatitis A, B, and C, leptospirosis, and malaria as well as blood cultures and antibiotic sensitivity testing to the Walter Reed/AFRIMS Research Unit-Nepal. AFRIMS also improved its capability to perform veterinary influenza A diagnostics and worked with Mahidol University in Bangkok in testing 833 samples from 50 species of wild birds and 210 stored serum samples from birds and mammals collected during the 2004–2006 avian influenza epidemic. Of the retrospective samples, 41 were positive, with one dog positive for both H10N9 and H12N5.

AFRIMS has developed a robust system to support its influenza surveillance and research efforts. The flagship accomplishment for FY08 was the successful testing and commissioning of the AFRIMS BSL-3 laboratory, the first fully certified BSL-3 laboratory in Thailand. This facility will benefit AFRIMS, the US government, Thailand, Southeast Asia, and PACOM by providing capabilities for working with highly pathogenic avian influenza and other potentially dangerous agents in a safe, biosecure environment.

AFRIMS and the Ministry of Public Health developed the Illness Surveillance by Information Technology, an information management tool to facilitate zoonotic, emerging, and reemerging infectious disease detection, response, prevention, and coordination. This year the

Ministry of Public Health established a working group on zoonotic disease cooperation at 10 pilot provinces that facilitated outbreak investigations on avian influenza, tularemia, leptospirosis, and rabies.

Training is an important component of the GEIS mission at AFRIMS. AFRIMS set up a clinical training program on the Thai-Burmese border and conducted classes in malaria diagnostic microscopy. Trainees attending these classes included visiting US and Thai medical students and infectious disease fellows, public health workers, and technicians employed by malaria clinics in the Thai-Cambodian border areas.

United States Army Medical Research Unit-Kenya

Nairobi, Kenya

GEIS-supported programs at the United States Army Medical Research Unit-Kenya (USAMRU-K) cover Kenya and much of sub-Saharan Africa. During FY08, the GEIS program at USAMRU-K grew to comprise 60 scientific, clinical, field, and administrative personnel. The number of active surveillance projects tripled and involved activities in Uganda and Cameroon that included the renovation of three BSL-2 laboratories. USAMRU-K is the African component of the overseas research activity of WRAIR and is hosted by the Kenya Medical Research Institute (KEMRI).

Figure 5. GEIS influenza technician at National Influenza Center, Nairobi, Kenya.

Surveillance and Detection

USAMRU-K has an impressive balance of disease surveillance projects of interest to the US government and the local health officials in Kenya, other sub-Saharan countries, and the WHO Regional Office for Africa.

Human Influenza Sentinel Surveillance

Kenya's National Influenza Center, which was refurbished last year, continued to conduct human influenza sentinel surveillance at eight sentinel sites interspersed throughout the country (Figure 5). There were 3,128 samples collected from patients and screened by real-time RT-PCR: 399 (12 %) were influenza A, and 167 (5 %) were influenza B viruses. Of these, 41 influenza A, 102 influenza B, and 446 noninfluenza respiratory viruses were isolated from culture. The hemagglutinin gene of 119 of these influenza isolates was sequenced, shared with the WHO FluNET, and published in GenBank. In addition, USAMRU-K began efforts in influenza surveillance in Uganda and Cameroon.

Arbovirus Surveillance by Entomologic Collections

USAMRU-K conducted mosquito and tick collections at 10 sites in Kenya, and pools were subjected to molecular screening, virus isolation, and genome sequencing in the laboratory. More than 205,000 mosquitoes were collected and tested, and 77 probable virus isolates were obtained. Nine viruses were identified fully (Babanki and Ndumu viruses), 11 to family level (Alphavirus and Flavivirus), and the rest remain unknown. During FY08, 12,795 ticks have been tested with 45 virus isolates (23 Dugbe, 5 Dhori, 1 Dugbe-like, and 16 unidentified) obtained. Partners are being sought to identify the unknown isolates. This project hones the specialized skills necessary for arbovirus outbreak investigations common in the region.

Enterics Surveillance

In FY08, USAMRU-K researchers obtained 651 stool samples for pathogen identification, antibiotic susceptibility testing, and associated virulence factors. Antibiotic susceptibility was assessed by conventional biochemical methods and PCR, and 115 bacteria were isolated (18%

of total collected): pathogenic *E. coli* (11%), enteroaggregative *E. coli* (9%), enterotoxigenic *E. coli* (1%), enteroinvasive *E. coli* (1%), Shiga toxigenic *E. coli* (0.5%), *Salmonella* (4%), *Shigella* (2%), and *Vibrio cholera* O1 (1%). Significant antibiotic resistance was also documented.

Migratory Bird Surveillance

In collaboration with the National Museums of Kenya, NAMRU-3, CDC-Kenya, and the Kenyan government, USAMRU-K conducted migratory bird surveillance along East African flyways to identify possible highly pathogenic avian influenza infections and characterize avian influenza subtypes in Kenya. In the 2007–2008 migration season, 1,882 birds from 106 species were sampled at nine sites along these flyways. Ornithologists trapped migratory birds using mist nets and collected duplicate cloacal swabs for shipping to NAMRU-3 for full characterization.

Malaria Drug Resistance

USAMRU-K continued to monitor *P. falciparum* drug resistance in FY08. By using its renovated laboratory at Kisumu, USAMRU-K screened 107 *P. falciparum* specimens collected from four sites in western Kenya against six antimalarials using a nonradioisotopic intercalated SYBR-Green dye method. This effort was the first time this method was used in East Africa. PCR techniques detected mutations in the pfMDR1 and other genes, indicating resistance in 80 immediate ex vivo and 27 culture-adapted parasite specimens. Wild-type isolates showed chloroquine (28%) and mefloquine (51%) resistance trends. pfMDR1 mutation rates were unremarkable. This ongoing surveillance helped track the evolution of drug resistance and informed antimalarial formulary selection in Kenya. In vivo studies are warranted to confirm these in vitro findings.

Rickettsia Surveillance in Livestock

Rickettsioses are increasingly being detected in international travelers originating from Africa. USAMRU-K researchers collected whole blood and ticks from 1,661 domestic animals (982 cattle, 300 sheep, and 379 goats) at six major abattoirs that receive animals from Kenya's 42 districts (Figures 6 and 7). PCR testing targeting two rickettsia genes found that 166 cattle (17%), 45 sheep (15%), and 25 goats (7%) were infected with various rickettsial infections. Most were mixed infections, but the spotted fever group predominated (91%) over the typhus group (42%). Rickettsia hot spots tended to be areas occupied by nomadic tribes in the following areas: Keiyo Valley, Mau Narok, Thika-Machakos, and Meru-Isiolo-Wajir. This work provided the first evidence of the potential for human infections in the region. Future work will include human febrile screening targeted to these hot spots.

Figure 6. Technician at Kenya Meat Commission, Athi River, Kenya.

Figure 7. GEIS staff taking blood sample from sheep for livestock *Rickettsia* surveillance study.

Rodent-borne Virus Surveillance

Evidence of rodent-borne viruses remains limited in East Africa. As part of a 3-year study to characterize their presence, USAMRU-K selected four study sites based on a pilot study in 2007. From May to August 2008, 264 rodents representing 21 genera were trapped. Peroxidase enzyme-linked immunosorbent assay (polyacrylamide gel electrophoresis IA ELISA) methods were used to determine seropositivity of serum samples, and these were confirmed by RT-PCR. Six were confirmed positive for Hantavirus group, and these will be further characterized by cloning and sequencing. Specimens will also be tested for Arenaviruses, Rift Valley fever virus, rickettsia, *Bartonella*, pox viruses, and leishmaniasis in collaboration with the National Museums of Kenya, USDA, and CDC.

Acute Febrile Illness

USAMRU-K conducts surveillance to determine the etiology of febrile syndromes in Kenya. Fever is a common presentation in health care facilities in Kenya, and patients are often empirically treated for malaria without laboratory confirmation, which leads to inadequate treat-

ment, inaccurate disease reporting, late recognition of emerging infections and epidemics, and increased drug resistance. USAMRU-K collected 215 specimens (whole blood and nasal swabs) from malaria-negative febrile patients at three western Kenya hospitals and is testing them for a range of pathogens including *Salmonella typhi*, dengue, measles, rickettsiosis, viral hemorrhagic fevers, and leptospirosis. Once fully established, this surveillance system will provide insight on the etiologies of acute febrile illness, identify emerging diseases, and develop evidence-based standards for treatment in resource-constrained settings.

Response and Readiness

Fortunately no response to a regional epidemic like the East Africa Rift Valley fever outbreak of 2006–2007 was required in FY08. Nevertheless, the USAMRU-K GEIS program remained vigilant for epidemic disease and promoted preparedness and capacity strengthening with the Kenya government and regional partners. Through its integrated KEMRI/GEIS arbovirus laboratory in Nairobi, a WHO-designated regional reference laboratory, USAMRU-K staff tested 17 patients for suspected arboviruses from hospitals throughout Kenya and southern Sudan (all negative). The Enterics and Influenza Laboratories and USAMRU-K field staff assisted the Kenya Ministry of Health with cholera outbreaks in Nairobi and Kisumu and an adenovirus outbreak at the International School in Kisumu.

Cooperation and Capacity Building

USAMRU-K continued to be intimately involved in regional cooperation and capacity building. Collaborative efforts for regional public health were initiated by USAMRU-K through the nascent AFRICOM.

In FY08, GEIS and USAMRU-K continued collaborative efforts already established in human and avian influenza and arbovirus surveillance with other US government agencies, and new collaborative projects were implemented. USAMRU-K and CDC-Nairobi independently managed two national human influenza sentinel surveillance collection networks. These two networks are complementary and together cover all of the significant population and epidemiologic zones in Kenya. Both organizations, through frequent collaborative planning and communication, were involved in managing, equipping, and funding Kenya's National Influenza Center in support of KEMRI. Likewise, FY08 collaborative avian influenza surveillance in migratory birds continued. As with the human influenza projects, USAMRU-K took the lead in coordinating efforts with other partners including the CDC-Nairobi, NAMRU-3, the National Museums of Kenya, the Kenya Wildlife Service, and the Ministry of Livestock Development. USAMRU-K also collaborated with the National Wildlife Research Center, USDA-Fort Collins, on Hantavirus and avian influenza surveillance projects this year.

One host country national staff sponsored by USAMRU-K completed a master's degree in FY08, and six other staff members continued their graduate training. USAMRU-K provided numerous other training opportunities for its staff and affiliated scientists including courses in good clinical practices, malaria diagnostic microscopy, and statistics. The Second Annual GEIS Training Conference included 30 hours of epidemiology training led by USUHS faculty for 60 Kenyan attendees.

With support from USAMRU-K and GEIS, the Makerere University Walter Reed Project initiated an influenza surveillance project in FY08 with the intent to strengthen infrastructure and human resource capabilities in Uganda. Construction finished for a newly renovated BSL-2 laboratory for animal studies at the Faculty of Veterinary Medicine at Makerere University (Kampala, Uganda), and it was commissioned by the US Ambassador to the Republic of Uganda in May 2008. Renovation of a BSL-2 human laboratory at the Uganda Virus Research Institute (Entebbe, Uganda) began in September 2008. This work was undertaken through collaboration with the Uganda Ministry of Health and CDC-Uganda, and the laboratory is designated the WHO National Influenza Center with completion anticipated in Spring 2009. USAMRU-K sponsored and conducted an intensive laboratory-based sub-Saharan regional pandemic and avian influenza surveillance training for 18 participants, representing Kenya, Uganda, Cameroon, and Nigeria, to create a regional pool of trained laboratory scientists. Human and animal sample collections will continue into FY09.

In Cameroon, USAMRU-K partnered with the University of Buea (Buea, Cameroon) to focus on infrastructure and human resource development for an emerging infections program. An extensive renovation of the university's animal facility at the Molyko campus produced a state-of-the-art BSL-2 laboratory that will begin work in FY09.

Naval Medical Research Center Detachment

Lima, Peru

The Naval Medical Research Center Detachment (NMRCD) conducts infectious disease surveillance, pathogen identification, outbreak detection and response, and epidemiological training in nearly all countries of South America and continues to be a crucial DoD-GEIS asset in Latin America. Activities are coordinated with SOUTHCOM and relevant US government agencies such as the respective US embassies, USAID, CDC, and USDA.

Surveillance and Detection

NMRCD conducted a broad array of surveillance projects that provided data for most infectious diseases that are relevant to the military and to public health in Latin America. Surveillance efforts extend into 14 countries in Latin America. Only Brazil, French Guiana, and some countries in Central America that are traditionally covered by USACHPPM are not included. The GEIS efforts at NMRCD represent a coordinated and comprehensive emerging infections surveillance system for South America. GEIS-related projects are included in all the NMRCD programs that cover bacteriology, parasitology, entomology, virology, and emerging infections. Assets are leveraged to assure flexibility, efficiency, and a maximum return of investment for time, personnel, and other resources. For example, the same physicians are used to recruit patients for febrile illness, influenza, and bacterial enteric pathogen surveillance projects.

The addition of pandemic influenza funding from GEIS allowed the rapid expansion of a network of laboratory-based strain surveillance that is unparalleled in Latin America. This effort strengthened regional biosecurity and built significant capacity within the networked countries. Most recently NMRCD, in collaboration with the CDC, PAHO, the Johns Hopkins University School of Public Health, Greater National University of San Marcos (Lima), and the Peruvian ministry of health have established several cohorts in various locations in Peru to determine burden of illness, characterize influenza seasonality, and elucidate the transmission dynamics of influenza in the tropics.

NMRCD has conducted respiratory disease surveillance since 2000 and in FY08 vastly increased its capacity for laboratory detection of pathogens such as those in the Bocavirus, Polyomavirus, and Coronavirus genera. Furthermore, NMRCD increased its capacity to detect novel and emerging zoonotic pathogens by enhancing laboratory capacity and epidemiological training at the School of Veterinary Medicine, Greater National University of San Marcos. More than 6,000 respiratory samples were submitted, with an isolation rate of over 35%. Isolates are now collected from 57 sites in 10 countries throughout South and Central America. Most sites were in Peru ($n = 25$), with the others in Argentina, Bolivia, Ecuador, El Salvador, Honduras, Nicaragua, Paraguay, Venezuela, and, most recently, Colombia. These samples were characterized at NMRCD by their hemagglutinin subtype, and a portion was sequenced. NMRCD entered novel isolate results into GenBank. Through collaboration with USAFSAM and the CDC, potential candidate vaccine strains were included for WHO annual vaccine component consideration. Furthermore, the NMRCD virology program has recently begun to characterize the patterns of antiviral resistance among South American influenza strains.

Febrile illness surveillance for novel pathogens continued to leverage the increased number of sites for respiratory disease surveillance. NMRCD isolated several novel viruses and has the most comprehensive collection of dengue isolates in South America. During FY08, 2,789 individuals from Bolivia, Ecuador, Paraguay, and Peru provided specimens, and viruses were isolated from 515. Dengue infection was the most frequent finding, with dengue 3 the predominant serotype. This year NMRCD also saw the reemergence of dengue 4 and low level transmission of dengue 1 and dengue 2. Alerta, the electronic disease surveillance system coordinated by the emerging infections program at NMRCD, also detected an increase in clinically confirmed cases of dengue among Peruvian military personnel in Iquitos (Figure 8). Machupo (or Bolivian) hemorrhagic fever was detected from two fatal cases in a region of Bolivia not previously known for Machupo transmission.

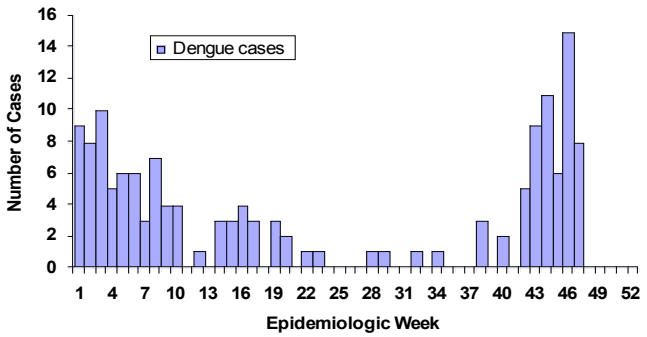


Figure 8. Epidemic curve of dengue fever in Iquitos, Peru, generated by Alerta, that demonstrates increase in clinically confirmed cases among Peruvian military personnel, September–November 2008.

The NMRCD bacteriology program continued to work primarily on enteric pathogen resistance patterns. In addition, NMRCD conducted surveillance for brucellosis and sexually transmitted infections. During FY08, 1,741 participants with acute febrile diseases were enrolled in this protocol. Stool cultures were processed at collaborating sentinel sites in Peru and Paraguay, yielding 640 Gram-negative pathogens, including *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., and enterotoxigenic *E. coli* (ETEC). This year, a continued trend of increasing resistance among many of the enteric bacteria, especially *Campylobacter* spp., was found. Over 92% of *Campylobacter* isolates were found to be resistant to ciprofloxacin, surpassing the same trends found in other regions of the world such as Southeast Asia. More than 50% of *Shigella* isolates were resistant to trimethoprim/sulfamethoxazole. Diarrheal surveillance also continued among Peruvian military populations and among travelers and US embassy personnel, with similar findings of resistance among enteric bacteria. For sexually transmitted infections, molecular technology was used to track fluoroquinolone resistance among *Neisseria gonorrhea* and *Treponema pallidum.*

The NMRCD bacteriology program recently demonstrated that the lysis centrifugation procedure for the detection of *Brucella* was superior to the traditional Ruiz-Castaneda and Bactec methods in terms of shorter mean detection times and lower cost. A pilot study also showed that seminested PCR amplification using primers BRU UP, BRU Low, BRU IN, and B4-B5 allowed detection of *Brucella* spp. in dairy products. Given the increasing importance of brucellosis, these tests could offer rapid and specific screening for the presence of *Brucella* spp. in routine food quality control. Furthermore, in detection of inactivated microorganisms, the test may be used to monitor *Brucella* in the entire cheese production chain. In collaboration with the Johns Hopkins University and Cayetano Heredia University (Lima), recent human *B. melitensis* isolates from Peru were genotyped by multiple-locus variable-number repeat analysis and demonstrated six genomic groups, including nine unique genotypes. Isolates were most closely related to two isolates from Mexico and were clearly distinct from the East and West Mediterranean groups of *B. melitensis* genotypes, suggesting that they may constitute a unique Latin American cluster.

The NMRCD parasitology program was successful in studying antimalarial resistance mechanisms via molecular methods. Active and passive surveillance for *P. falciparum* and *P. vivax* drug resistance was conducted via molecular markers and multilocus sequencing analysis to identify polymorphisms and populations of parasites that carry signatures indicative of resistance. The SYBR-Green dye method pioneered at NMRCD continues to be used and optimized and was shared with GEIS partners throughout the world. As requested by SOUTHCOM, NMRCD studied the molecular etiology of *Leishmania* in Colombia and Peru. This parasite represents a significant potential infection for US and Colombian forces involved in the war on narcotics. Molecular methods to differentiate among New World *Leishmania* species were developed and exported to partners in Colombia and to WRAIR.

Electronic surveillance programs continue to be extremely successful at NMRCD. The outbreak notification system Alerta was consolidated within the Peruvian Army and optimized by the Peruvian Navy. Alerta continued to detect numerous outbreaks, the most significant of which were several influenza outbreaks, two leptospirosis outbreaks, and the beginning of the dengue outbreak in Iquitos, Peru, in late FY08 (Figure 8). These efforts led to timely awareness about the outbreaks within the Peruvian Navy and enhanced cooperation between its health services and NMRCD. Use of Alerta led to the training of more than 1,000 Peruvian military public health and medical personnel in epidemiology, a significant achievement in a developing country. Leveraging support from GEIS and SOUTHCOM, NMRCD is expanding Alerta to three other South and Central American countries: Colombia, Ecuador, and Panama. This ambitious expansion will be implemented per SOUTHCOM priorities and will potentially create the only regional surveillance network utilizing the same system. The Colombia agreement was recently signed by the minister of defense in Colombia, and the agreements with Ecuador and Panama are under review.

NMRCD continues to conduct environmental sampling for avian influenza among migratory birds. In FY08, NMRCD identified nine low pathogenic avian influenza viruses isolated from seven avian species, including subtypes H3N8, H4N5, H10N9, H13N2, and, most recently, H7N3 along the coast of Peru. These findings

provided evidence that influenza viruses are present and circulating in wild birds in South America and thus can be potential sources of influenza transmission among various avian species, including poultry.

Response and Readiness

The ability to conduct comprehensive outbreak investigations remains one of the capacities of NMRCD that is critical to GEIS. The outbreak response team was strengthened in FY08 and now includes a staffmember trained by the CDC's Epidemic Intelligence Service, a Peruvian physician and veterinarian, and field laboratory technicians. Formal and informal advice and laboratory support are provided when requested, and full epidemiological teams are deployed in responses. Response often involves the resources of multiple NMRCD departments and significant collaborations with the ministry of health and subnational health directorates. NMRCD provides any or all of the following depending on the incident: epidemiological consultation, field work, laboratory assistance and training, supplies and media, and relevant scientific expertise.

In FY08, NMRCD responded to several outbreaks, including such simple conditions as diarrhea to more complicated and deadly situations such as urban and sylvatic yellow fever in Asunción, Paraguay. NMRCD staff responded to an outbreak of norovirus among Navy personnel whose ship was visiting the port of Callao, Peru (Figure 9) and investigated severe adverse events, including four deaths, after a yellow fever vaccination campaign in Ica, Peru.

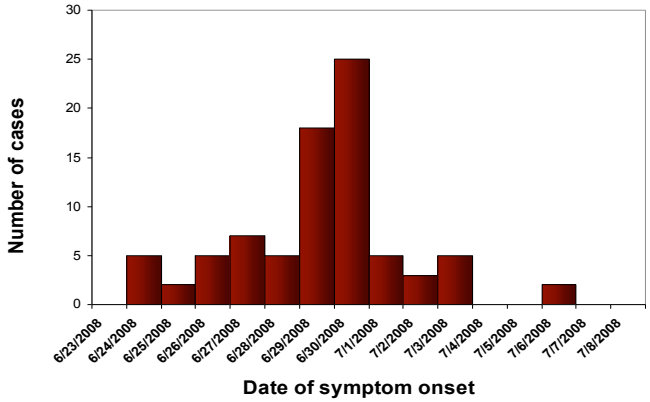


Figure 9. Epidemic curve of norovirus outbreak aboard Navy ship in port of Callao, Peru, June–July 2008.

Cooperation and Capacity Building

By utilizing close relationships with SOUTHCOM, NMRCD continued to actively pursue coordination with the Command Surgeon about disease surveillance priorities within Latin America. To this end, the Navy Bureau of Medicine and the Colombian government signed a bilateral agreement, and NMRCD has initiated several projects in Colombia.

NMRCD has continued to build partnerships with many US government agencies and entities to help create a network of complementary efforts rather than competing or duplicating efforts. In FY08 NMRCD worked intimately with GEIS in the region on the following initiatives:

- Drafting an agreement with PAHO that will serve to formalize the relationship and improve coordination;
- Developing an electronic disease surveillance hub for Latin America in Lima;
- Serving as lead agency for PAHO in initiating the PAHO-CDC generic influenza surveillance protocol and standard operating procedure in Peru, Ecuador, and Bolivia;
- Implementing integrated surveillance methods for detection of emerging and reemerging zoonotic diseases and exploring the effects of changing land use patterns and habitat perturbation on the ecology of infectious diseases in the Americas;
- Drafting an agreement with the Ministry of Agriculture of Peru that will allow NMRCD to provide diagnostic support for the detection of avian influenza in domestic poultry.

With GEIS funding, NMRCD has conducted formal classroom training in outbreak investigation. In FY08, public health officials were trained in Peru, Paraguay, and most recently in Manaus, Brazil. Conducting the training session in Brazil is a significant advancement for the region because the DoD has had little to no activity in Brazil since the 1990s. As a follow-up to this training session, the Brazilian ministry of health has requested that NMRCD conduct a second training session in Rio de Janeiro in 2009. NMRCD continues to collaborate with PAHO and the ministries of health to share costs for these courses and ask them to provide instructors and tutors. The methodology and experience of teaching this course to more than 1,300 Latin American public health officials were published in the 26 October 2007 issue of *Science*.

In addition, NMRCD sponsored several training programs for US and Peruvian military personnel, including GEIS-sponsored USUHS medical students and the military tropical medicine course.

Leveraging its GEIS support, NMRCD also coordinated several efforts with the CDC, specifically in influenza surveillance and outbreak response. USAID has seen the value in the NMRCD training platform, which has resulted in collaboration to replicate the outbreak response training and conduct pandemic influenza preparedness courses throughout the region. Collaborations with PAHO and several ministries of health were strengthened by the training in outbreak management. NMRCD has continued to work with scientists and epidemiologists from the Peruvian ministry of health, Navy, and Army to build collaborations and respond to outbreaks of infectious disease. NMRCD also leveraged the talents and projects of several local and US academicians to enhance surveillance of emerging infectious diseases.

United States Naval Medical Research Unit No. 2

Jakarta, Indonesia

United States Naval Medical Research Unit No. 2 (NAMRU-2) continued to provide disease surveillance, research, and capacity building throughout Southeast Asia, complementing the regional efforts of its sister laboratory AFRIMS in Thailand. NAMRU-2 projects are central to characterizing and mitigating regional infectious disease threats and assisting countries build effective outbreak surveillance and diagnostic infrastructures throughout Southeast Asia. Through the establishment of an extensive network of collaborative relationships, NAMRU-2 continues to assist countries most vulnerable to new or reemerging pathogens and to develop state-of-the-art laboratories and disease surveillance programs. Through the leveraged use of GEIS funds, NAMRU-2 efforts have been essential for the diagnosis, surveillance, and identification of strains of highly pathogenic avian influenza virus H5N1 in the region.

Surveillance and Detection

Although the government of Indonesia suspended the export of all H5N1 influenza samples in January 2007, NAMRU-2 remained an important component of both the Indonesian and regional H5N1 surveillance efforts in FY08. NAMRU-2 continued to work with its Indonesian partners and colleagues on several GEIS-supported projects.

NAMRU-2 continued to monitor the spread of influenza-like illnesses through its extensive network of collaborating hospitals in Laos (Figure 10), Cambodia, and Indonesia. NAMRU-2 findings support the hypothesis that continual intraregional circulation of influenza viruses in Southeast Asia leads to the subsequent seeding of seasonal influenza around the globe. NAMRU-2 work supports an integrated regional influenza surveillance system for identifying circulating influenza strains, providing rapid outbreak responses and laboratory confirmation, and containing possible epidemic/pandemic influenza events in Southeast Asia. This influenza surveillance also provides information that helps guide the WHO annual influenza vaccine formulation process.

Figure 10. Influenza-like illness surveillance sites, Laos, FY08.

With GEIS support, NAMRU-2 continued to upgrade equipment for on-site genetic sequencing to detect potential pandemic mutations and increase the volume of specimens that can be tested with real-time RT-PCR in Laos. Luminex xMAP is fully deployed and this year assisted the Lao National Center for Laboratory and Epidemiology (NCLE) in its surveillance for influenza-like illness, including the ability to detect highly pathogenic avian influenza. During FY08, 296 samples from patients with influenza-like illness were submitted to NCLE for identification. An additional 37 suspected highly pathogenic avian influenza cases were submitted and tested using state-of-the-art equipment placed as part of the capacity building supported by DoD-GEIS. The Luminex system was used to rule out two cases of H5N1 influenza in Laos. Three collaborating hospitals serving the provinces of Champassack, Louang Namtha, and

Savannakhet enrolled in the influenza-like illness study, and personnel received extensive training this year in patient enrollment, specimen collection, and epidemiologic information gathering. These provincial hospitals are providing a network of collaborating clinical centers across the country, from the border with China and Burma south to the border with Cambodia. Additionally, with funding from other sources, Laos has continued to operate the Early Warning Outbreak Recognition System (EWORS), the electronic syndromic surveillance system initiated through GEIS funding. This system has augmented the NAMRU-2 surveillance activities for influenza-like illness at the three provincial hospitals and in the capital.

NAMRU-2 continues to work in Cambodia and has implemented surveillance programs for emerging and reemerging pathogens throughout the country (Figure 11). NAMRU-2 has recently launched a new laboratory and has developed a surveillance program that will identify influenza strains as they emerge. The work has elucidated the epidemiology of influenza viruses recovered during acute febrile illness surveillance in Cambodia from December 2006 through July 2008.

Figure 11. Sites of surveillance conducted by NAMRU-2 in Cambodia.

During FY08, NAMRU-2 implemented a study to examine the incidence of rickettsial agents in humans and the seroprevalence of rickettsial agents in animals. The overall animal seroprevalence of rickettsial agents in rodent reservoirs captured at four sites in Indonesia was high (49%), thus suggesting that the risk for rickettsial infection in Indonesia is high. Seroprevalence varied by island and was highest in Java (83%), followed by Sumatra (72%), Kalimantan (65%), and Sulawesi (26%).

In FY08, using established WHO protocols NAMRU-2 conducted in vivo efficacy studies of malaria drug resistance and surveillance for molecular markers of drug resistance for infections caused by *P. falciparum* and *P. vivax* in Cambodia and Indonesia. In Cambodia NAMRU-2 conducted efficacy studies of mefloquine-artesunate for uncomplicated *P. falciparum* malaria and chloroquine for *P. vivax*. The PCR-corrected treatment failure rates determined by survival analysis at 28 and 42 days were 13.1% and 18.8%, respectively. Treatment failure was associated with increased Pfmdr1 copy number, higher initial parasitemia, higher mefloquine IC50, and longer time to parasite clearance. The results suggest that artesunate-mefloquine combination therapy is beginning to fail in southern Cambodia and that resistance is not confined to the provinces on the Thai-Cambodian border.

In addition to malaria field studies, NAMRU-2 also began using mathematical modeling to identify parasitological and epidemiological factors that favor either the maintenance of a stable equilibrium between resistant and sensitive malaria strains or the fixation of resistance. Two models were created that predicted and partially explained the differing prevalences of chloroquine resistance alleles in Asia and Africa.

During FY08, NAMRU-2 expanded the study of enteric pathogens causing acute diarrhea among children in Indonesia, including two additional referral hospitals. The Indonesian Pediatric Diarrheal Surveillance Program is now located in six cities on five islands of the Indonesian archipelago. This work has identified rotavirus as the cause of diarrheal illness among 52% of the patients enrolled. Bacterial etiologies of disease were identified in 11% of cases and parasites as a cause of 4%. Results of this study have provided valuable surveillance data regarding pathogen distribution and antimicrobial susceptibilities among common enteropathogens stratified by geographic location within Indonesia. These data may be incorporated into the Indonesian health intervention plans of the ministry of health with the aim of reducing morbidity and mortality of childhood diseases, specifically diarrhea.

Sequencing of nontypeable rotavirus strains is critical for diarrheal surveillance programs to identify emerging strains and to determine whether the vaccine will provide adequate coverage. NAMRU-2 found that the predominant rotavirus genotypes circulating in Indonesia are included in the licensed vaccine formulations that have recently become available, a significant finding for public health in Indonesia. The PCR testing algorithms at NAMRU-2, in addition to the sequencing work of nontypeable strains, has enabled identification of >99% of rotavirus strains in positive samples.

Response and Readiness

NAMRU-2 has continued to be a respected regional partner by responding to outbreaks of potential avian influenza and by building capacity for the detection of other pathogens in Laos and Cambodia.

In conjunction with the NCLE in Laos, NAMRU-2 personnel assisted with outbreaks of H5N1 among poultry in northern Laos in February 2008. After the poultry die-off, and while the NAMRU-2 and NCLE team was still in Louang Namtha, NAMRU-2 assisted the hospital in eliminating H5N1 as the cause of death in an infant. Reagents for the Luminex system have now been prepositioned at NCLE to support future outbreak responses.

EWORS is fully operational at 12 provincial hospitals in Laos and ties directly into the outbreak response capabilities. EWORS can trigger a response by NCLE and alarmed twice (standard deviation +2.5) in FY08, which initiated more intensive investigations of influenza-like illness.

Recent outbreaks of hand, foot, and mouth disease among infants and children in China have included the neurovirulent enterovirus strain, enterovirus 71. NAMRU-2 established the capability to detect this virus after the Cambodian National Institute of Public Health in Phnom Penh requested assistance.

Cooperation and Capacity Building

NAMRU-2 shared its results, resources, and expertise with collaborators from regional ministries of health and other government partners. Conferences and publications, as well as close information sharing with Headquarters, allow data to be shared with the scientific community and DoD. NAMRU-2 continued to coordinate its efforts with PACOM, the Department of State, and US embassies in the region.

NAMRU-2 made significant contributions to the understanding of disease threats in this region in FY08. NAMRU-2 facilitated the rapid identification of human influenza A H5N1 infection along with numerous other infectious diseases of importance to the US military and regional public health community. Innovative studies have led to the understanding of emerging and reemerging pathogens, their vectors, and their environment. The use of dried blood spot collection of clinical samples for large-scale screening efforts has simplified shipping of specimens and allowed centralization of laboratory activities for efficiency and cost-effectiveness. Implementation of dried blood spot collection procedures will increase surveillance coverage in areas of the world that are hard to reach.

United States Naval Medical Research Unit No. 3

Cairo, Egypt

The United States Naval Medical Research Unit No. 3 (NAMRU-3) is a forward-positioned laboratory in Cairo with projects in the AFRICOM, CENTCOM, and EUCOM areas of responsibility. NAMRU-3 also serves as an Eastern Mediterranean regional influenza laboratory and a global H5 avian influenza reference laboratory for the WHO. Throughout FY08, NAMRU-3 continued to conduct broad-based infectious disease surveillance in the Middle East, North Africa, and central Asia, regions important in emerging infectious disease surveillance and of strategic value to the DoD.

Surveillance and Detection

During FY08, NAMRU-3 conducted and assisted with influenza surveillance activities and outbreak support in several countries within central Asia, eastern Europe, South Asia, the Middle East, and Africa. In addition to respiratory disease surveillance in human populations, NAMRU-3 conducted avian influenza surveillance in migratory birds in Ukraine, Egypt, and Kenya. NAMRU-3 continued to support satellite laboratories in Afghanistan, Ghana, and Jordan that remain critical for the development of WHO National Influenza Centers. NAMRU-3 also continued to provide regional partners with reagents, supplies, and shipping materials to facilitate influenza specimen collection and transport for NAMRU-3 reference laboratory services.

As a WHO regional influenza reference laboratory, NAMRU-3 continued to proactively support all countries in the region where US forces are deployed. NAMRU-3 received 4,128 samples for seasonal influenza testing and found 232 (9%) positive for influenza, 57 for influenza A H1N1, and 175 for influenza B. In addition, as one of ten WHO global H5 reference laboratories, NAMRU-3 must maintain advanced diagnostic capabilities for influenza H5N1 reference testing; 134 human specimens from Egypt and Pakistan were received for influenza A H5N1 reference testing during FY08, of which 15 were positive.

Surveillance for Influenza Viruses in Middle East, Central Asia, and Eastern Europe

NAMRU-3 conducts influenza surveillance in Egypt, Syria, Oman, Jordan, and Sudan as part of the influenza surveillance network of the WHO Regional Office for the Eastern Mediterranean. As a GEIS-funded laboratory, NAMRU-3 extends this network to the central Asian countries of Afghanistan, Pakistan, Kazakhstan, Kyrgyzstan, Azerbaijan, Uzbekistan, and Ukraine, and the network has been used to respond to reported human and animal H5N1 cases in Eastern Europe.

Conducted under the auspices of the respective ministries of health, the program provides structured active surveillance that supplies data to the WHO on the types of influenza viruses circulating in the region, while the Viral and Zoonotic Diseases Research Program at NAMRU-3 provides training and diagnostic and technical support.

Influenza viruses are isolated and typed and then sent to the WHO Collaborating Centre for the Surveillance, Epidemiology and Control of Influenza, CDC Influenza Branch for further characterization. Results are forwarded to the WHO task force on influenza vaccines for evaluation and potential incorporation into the next season's vaccine.

The procedures used to isolate influenza also identify other respiratory pathogens, such as parainfluenza, adenoviruses, and enteroviruses, that cause morbidity in these regions. These isolates are forwarded for identification to the CDC enterovirus section and the NHRC Respiratory Disease Research Department.

The region covered by this surveillance is of critical strategic value to the military and US government. Knowing the activity of pathogens that can affect deployed forces and civilian populations helps COCOM commanders and local public health efforts.

In addition, NAMRU-3 performed H5 reference testing on 559 animal specimens with 32 positives for H5N1 from Afghanistan, Egypt, Ghana, Pakistan, and Togo. During FY08, NAMRU-3 continued efforts in surveillance for hemorrhagic fever viruses and arboviruses in the CENTCOM area of responsibility. NAMRU-3 also expanded its testing and monitoring for other diseases relevant to the military by collaborating with other DoD groups working on leptospirosis, *Rickettsia*, and Hantavirus diagnostics. This surveillance project provided data on several important, but not well characterized, diseases in regional civilian populations and useful information on the geographical distribution and ecology of these diseases. This information is used by respective ministries of health to develop effective disease control programs and provides baseline data for testing several newly designed molecular diagnostic tests for viral hemorrhagic fever viruses, arthropod-borne encephalitis viruses, and tick-borne diseases in a relevant clinical setting.

The Military Infectious Disease and Operational Health Surveillance Network at NAMRU-3 is a collection of selected clinical sites that conduct surveillance for infectious diseases and other health problems among deployed US military in North Africa, the Middle East, and the CENTCOM area of responsibility. This network utilizes a flexible approach to standardize, coordinate, and centralize study design between populations and settings where US troops are deployed. Specific surveillance activities include a case surveillance study, a sero-survey study, and a self-completed deployment survey. From August 2007 to July 2008, NAMRU-3 successfully obtained more than 19,000 surveys from deployed US personnel. The disease and nonbattle injury data collected from these surveys are undergoing analysis. NAMRU-3 also initiated questionnaires addressing new areas in deployment health, such as diarrhea (Figure 12).

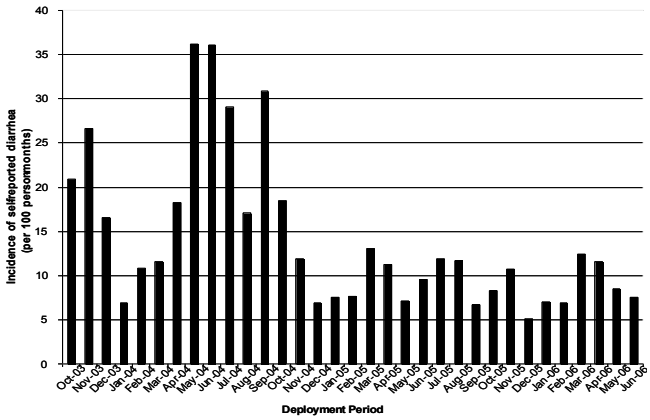


Figure 12. Diarrhea incidence by self-report among troops completing clinic screening.

NAMRU-3 continues to provide scientific (epidemiology and microbiology) and management assistance to a bacterial meningitis laboratory-based surveillance network of more than 25 hospitals in six countries within the Middle East: Sudan, Syria, Pakistan, Yemen, Morocco, and Libya. This opportunity allows NAMRU-3 to establish contact and conduct collaborative studies with personnel from regional ministries of health and universities. The latest additions are scientists from the ministries of health in Oman, Bahrain, and Qatar. With support from DoD-GEIS, NAMRU-3 performed standardized clinical and laboratory procedures and performed cerebrospinal fluid culture to identify more than 13,000 suspected cases from 32 hospitals in the six countries**.** As expected, *N. meningitidis* was the most common bacterial pathogen isolated from suspected Sudanese bacterial meningitis cases. However, *S. pneumoniae* was the most frequent isolate in Pakistan, Syria, and Yemen in children younger than age 5. In Libya, the leading cause of bacterial meningitis was *H. influenzae*. High levels (>34%) of antimicrobial resistance against penicillin, tetracycline, and trimethoprim/sulfamethoxazole were detected in all countries, and resistance levels against ceftriaxone were low (<10%). The use of cerebrospinal fluid PCR as a diagnostic tool improved the yield of cerebrospinal fluid testing (708 positive from 4,042 specimens) and suggested that cerebrospinal fluid PCR may be useful to more accurately define the burden of disease in epidemic and active surveillance settings.

NAMRU-3 has collected more than 1,000 *S. enterica* serovar Typhi, 300 invasive *S. pneumoniae*, and 350 *B. melitensis* isolates from outbreaks, disease surveillance activities, or sporadic cases in the region. All archived isolates have been characterized using general phenotypic (i.e., morphological and serological) characters. In FY08, NAMRU-3 characterized these isolates using molecular techniques (i.e., PFGE, PCR, multilocus sequence typing, and MLVA) to better understand the relationships among strains and to provide essential information about the acquisition, spread, and possible prevention and intervention (i.e., vaccines) strategies for these deadly pathogens. Advanced molecular characterization of 178 *S. pneumoniae* isolates based on multilocus sequence typing showed 84 new sequence types and seven new alleles. Pakistan, Oman, and Egypt had the highest number of new sequence types, demonstrating a great degree of genetic variation among pneumococcus isolates. Potentially, a protein-based vaccine against pneumococcus could provide broader coverage against multiple serotypes. *B. melitensis* isolates from sporadic human cases of brucellosis in Egypt (n = 83), Qatar (n = 17), and Libya (n = 1) were also characterized using molecular techniques.

NAMRU-3 further characterized 1,339 *S. typhi* isolates collected from blood cultures of febrile patients from five countries in FY08: 184 (14%) were found to be resistant to ampicillin, chloramphenicol, and TMP-SMZ. No resistance to ciprofloxacin or ceftriaxone was observed. PFGE was performed on 536 isolates, and 153 unique PFGE patterns were observed, suggesting high genetic diversity among these isolates. Most (91%) of these isolates were at least 76% similar as determined by PFGE. This study suggests that prospective PFGE analysis of *S. typhi* isolates could facilitate early detection and control of outbreaks as well as movement of MDR sequence types across the region.

With support from DoD-GEIS, NAMRU-3 continued to serve as a WHO regional reference laboratory for rotavirus surveillance. NAMRU-3 collected diarrheal stool samples from children older than age 5 for the detection of rotavirus from Afghanistan, Iran, Iraq, Jordan, Morocco, Sudan, and Syria using enzyme immunoassay. They compared this gold standard with several novel molecular methods for rotavirus detection.

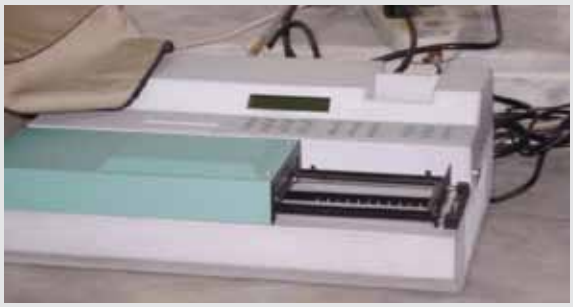
In ongoing support of public health in Egypt, NAMRU-3 continued a survey for antimicrobial resistance among healthcare-related infections. Healthcare-related infections and antimicrobial resistance have emerged as important public health problems in Egypt. Abuse of antibiotics, especially in developing settings, increases the risk of emergence of antibiotic resistance, which contributes to a high morbidity and mortality among patients and adds to treatment costs (Figure 13). This study measured the burden of healthcare-related infections and antimicrobial resistance in hospitals, with an emphasis on catheter-associated urinary tract infections and surgical site infections. The infection rates and antimicrobial resistance levels identified for catheter-associated urinary tract infections and surgical site infections are relatively higher than rates reported from developed countries. NAMRU-3 is working to promote intensive training on

Rotavirus Surveillance in Afghanistan and Iraq

In its capacity as the regional rotavirus surveillance network laboratory for the WHO Regional Office for the Eastern Mediterranean, NAMRU-3 assists the central public health laboratories of participating countries and enables each to become a stand-alone testing facility. At each laboratory, NAMRU-3 trains staff in quality assurance testing and laboratory techniques such as enzyme immunoassay, genotyping, and sequencing. NAMRU-3 also develops standard operating procedures for the laboratories and assists in the assembly and publication of the reference laboratory data.

The rotavirus surveillance program in Baghdad, Iraq, was inspected by NAMRU-3 staff, who also visited the Indira Gandhi Children's Hospital and the Central Public Health Laboratory, both in Kabul, Afghanistan. The Iraqi laboratory equipment, procedures, and reporting forms were assessed, and assistance was given to laboratory personnel in arranging for samples to be shipped to NAMRU-3 for further analysis. Additionally, NAMRU-3 secured the participation of an Iraqi scientist in a rotavirus genotyping training session in Cairo.

During FY08, the Iraqi rotavirus surveillance network collected more than 800 stool samples from six sites throughout Iraq. Analysis of samples indicated that the [P4], G2 genotype, common in Egypt and Sudan, is also dominant in Iraqi children. More than 600 samples collected from the Afghan surveillance efforts were transported to NAMRU-3 for ongoing enzyme immunoassay quality assurance testing and genotyping.



Basic enzyme immunoassay equipment in use in Central Public Health Laboratory, Kabul.

System used to record enzyme immunoassay results in Kabul.

infection control methods for nurses and staff, especially in high-risk wards, to decrease the high infection rates found in Egyptian hospitals.

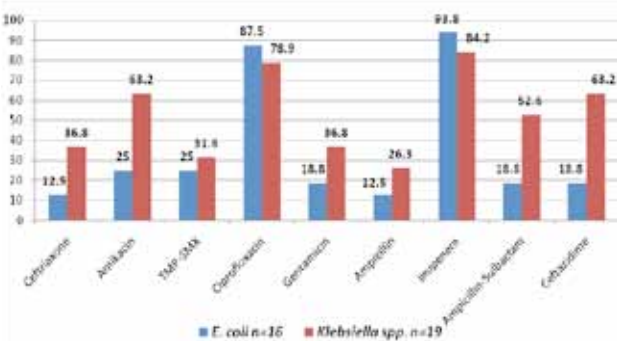


Figure 13. Antimicrobial susceptibility rates in *Klebsiella pneumoniae* and *E. coli* isolates from patients at two university hospitals in Egypt, 2008.

In FY08, NAMRU-3 began a pilot study for antimalarial resistance among active duty populations and surrounding communities in the Horn of Africa. The study will assess the baseline frequency of mutations associated with treatment failure in and around Djibouti City and support WHO efforts to understand, control, and treat malaria. This study will provide a benchmark for further monitoring of antimalarial resistance genes in subsequent years and inform US and regional policymakers of any need to revise malaria drug policies.

Large areas of Afghanistan are endemic for leishmaniasis. The predominant form is cutaneous leishmaniasis, thought to be caused by *Leishmania tropica* and *L. major*. Prevalence as high as 10% for active lesions has been detected in some areas. Despite the scale of the disease, little is known about its transmission, etiological agent(s), or the state of drug resistance. During FY08, NAMRU-3 initiated a project to identify the parasite species, the hosts, and its vectors in Afghanistan. NAMRU-3 provided extensive training to 10 Afghan nationals in

the areas of sandfly collection, surveillance, and identification. These individuals also received training in conventional and real-time PCR including DNA extraction procedures. In FY08, more than 4,300 sandflies were shipped to NAMRU-3 from Afghanistan for species identification and *Leishmania* testing. *Leishmania* parasites (*L. tropica*) were found in both sandflies and human clinical samples, indicating *L. tropica* is a causative agent of cutaneous leishmaniasis in Kabul.

Response and Readiness

NAMRU-3 responds to all requests for outbreak investigations whenever possible, providing CENTCOM, EUCOM, and AFRICOM commanders with increased evidence-based situational awareness regarding disease threats in their respective areas of responsibility. These initiatives are geared toward sustainable surveillance systems utilizing NAMRU-3 as a regional reference laboratory. They should enhance the capabilities of the regional countries for predicting the potential occurrence of an outbreak and responding to it in a timely fashion.

Leishmaniasis is relatively unknown in Ghana. Therefore, an outbreak of cutaneous leishmaniasis in the Volta region during 2005 provided NAMRU-3 with a unique opportunity to characterize the novel epidemiologic aspects of the disease. NAMRU-3 characterized the numbers and seasonal variability of the prevalent sandfly species with extraordinary results. The usual sandfly vectors of leishmaniasis are rare in the Volta region. Observations suggest the disease is seasonal and migratory, affecting different villages in the region during different years. Additionally, PCR analysis of parasites taken from clinical specimens indicated that a previously undescribed species may be responsible for the outbreak. However, attempts to culture the organism for better characterization have been unsuccessful.

NAMRU-3 maintains multidisciplinary teams with advanced field laboratory diagnostics to respond to regional respiratory disease outbreaks in humans and animals. Support is clearly needed, because highly pathogenic avian influenza continues to devastate the poultry industry, posing a health risk to exposed humans and elevating the chances of a pandemic strain developing. By providing outbreak support, NAMRU-3 can identify the etiological agent while providing training, biosafety upgrades, and assessments of preparedness for its regional partners. In the past 2 years, through GEIS support, NAMRU-3 has dispatched field teams to Afghanistan, Azerbaijan, Cameroon, Cote d'Ivoire, Djibouti, Egypt, Iraq, Libya, Pakistan, Palestine, Sudan, Turkey, and Ukraine to provide consultation and/or forward diagnostic capacity (Figure 14).

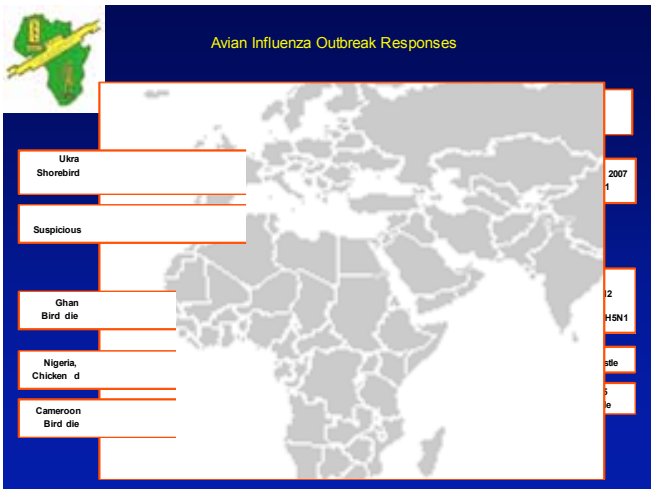


Figure 14. NAMRU-3 avian influenza outbreak responses, 2005–2008.

Capacity Building and Coordination

NAMRU-3 views the enhancement of regional influenza surveillance and US health diplomacy as critical in Africa through the development and support of satellite laboratories with the planned eventual development of WHO National Influenza Centers that will contribute to the WHO Global Influenza Surveillance Network. Little is known of circulating influenza viruses on the continent. With the DoD-GEIS supplemental funding for pandemic and avian influenza, NAMRU-3 is developing a West African regional network, utilizing the previously established satellite laboratory in Ghana to coordinate activities. NAMRU-3 is encouraging new capacity and surveillance efforts for influenza viruses in the Middle East, Central Asia, and Africa through the development of its new satellite sites in Djibouti and eastern Sudan. During FY08, NAMRU-3 completed site assessments and conducted capacity building and training efforts in Burkina Faso, Cote d'Ivoire, Ghana, Sierra Leone, and Sudan. NAMRU-3 also supported GEIS/USAMRU-K efforts in Nigeria, Cameroon, and Uganda by providing oversight, training, and reference laboratory support. Efforts at NAMRU-3 are undertaken in close coordination with the respective combatant commanders in the region.

During FY08, NAMRU-3 used the GEIS funding for pandemic and avian influenza to train 63 persons from 18 countries in influenza surveillance procedures pertaining to specimen collection, avian influenza diagnostics, shipping, and biosafety. In addition, a training workshop for avian influenza molecular diagnostics was conducted for 27 participants from six countries in West Africa: Burkina Faso, Cote d'Ivoire, Ghana, Nigeria, Senegal, and Sierra Leone.

Military Health System

United States Air Force School of Aerospace Medicine

The Air Force influenza surveillance program, now organized within the United States Air Force School of Aerospace Medicine (USAFSAM; formerly Air Force Institute for Operational Health), remains fundamental to the DoD-GEIS influenza surveillance effort. In FY08, USAFSAM continued to improve its ability to rapidly identify and respond to respiratory disease activity by improving case selection, laboratory methods, and procedures for handling specimens and by expanding its geographic presence.

Surveillance

Actively participating sentinel sites and locations for the 2007–2008 influenza seasonal year (30 September 2007–27 September 2008) are shown in Figure 15.

Figure 15. DoD global influenza surveillance sites, 2007–2008 seasonal year.

An additional 40 nonsentinel sites submitted respiratory specimens. Altogether 6,752 respiratory specimens from 82 locations were collected and processed during the 2007–2008 influenza seasonal year. This represented a 16% increase over the 2006–2007 seasonal submissions. Specimens submitted by sentinel sites accounted for 56% (n = 3,806) of total submissions, and nonsentinel sites submitted 44% (n = 2,946) of specimens. Fifty-nine percent (3,967/6,752) were positive for a respiratory virus compared with 42% in the prior season, and 31% (2,094/6,752) were positive for an influenza virus.

Influenza A predominated with 1,521 positives, of which 905 were H3N2 and 513 were H1N1, followed by 573 influenza B. Of all influenza positives, 65% were identified by viral culture and are available as potential seed viruses. Adenovirus accounted for most respiratory viruses other than influenza seen this seasonal year (Figure 16); adenovirus 14 predominated (86%, 1,248/1,450).

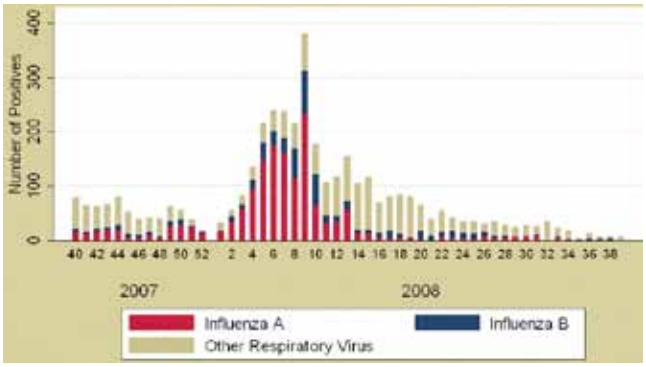


Figure 16. Positive viral results by week and year, influenza seasonal year 2007–2008.

Molecular Sequencing

USAFSAM sequenced and characterized 513 H1N1 strains during the 2007–2008 season and provided more than 350 isolates to the CDC. The H1N1 subtype viruses dominated the first half of the season, beginning in July 2007; although H3N2 viruses became the prevailing subtype at the end of the season, H1N1 strains continued to cocirculate in specific regions. USAFSAM sequenced and characterized 905 H3N2 viruses globally. Asian isolates in particular possessed antigenic changes at all epitopes, yet no dominant strain emerged. The 2007 isolates from USAMRU-K were unique and defined by multiple antigenic changes (one receptor binding site). Conversely, isolates from Europe possessed few changes and, as a group, shared the highest level of genetic homology with A/Brisbane/10/2007. By February 2008, a unique clade of H3N2 viruses emerged with three novel mutations at positions 83, 157, and 173; this clade was most dominant in the United States and dominated to a lesser extent in Asia. The most recent isolates in this group were collected in Guatemala during June 2008. Although influenza A dominated the season, based on overall numbers of isolates, influenza B was the predominant circulating virus in many areas of the world, including regions served by AFRIMS and USAMRU-K.

Vaccine Contributions

The USAFSAM molecular team characterized an influenza A/H1N1 isolate (A/South Dakota/06/2007) collected at Ellsworth AFB (South Dakota) in the summer of 2007 and submitted it to CDC for further antigenic characterization. This virus is an A/Brisbane/59/2007(H1N1)-like virus. Based on the WHO and FDA Vaccine and Related Biologic Products

Committee selection of A/Brisbane/59/2007(H1N1)-like virus as one component of the Northern Hemisphere 2008–2009 vaccine, the manufacturer of the live attenuated influenza vaccine used this Ellsworth virus isolate for the H1N1 vaccine seed strain in the 2008–2009 Northern Hemisphere live attenuated influenza vaccine.

Selected USAFSAM-GEIS accomplishments in FY08 follow:

- The most specimens processed, viruses isolated, and sequences determined during a program year to date;
- Collaboration and expansion of diagnostic capabilities for important respiratory pathogens other than influenza (e.g., adenovirus type 14);
- Year-round influenza-like illness electronic surveillance throughout DoD linked to laboratory surveillance;
- Enhanced detection/molecular characterization of influenza specimens submitted;
- Site support to an increasingly diverse network of surveillance sites with year-round capability;
- Timely characterization of genetic variability through improved molecular subtyping and hemagglutinin sequencing of influenza isolates and communication to global partners and global health entities;
- Preparation and submission of a summary manuscript describing 7 years of the influenza program through 2006;
- Prominent feature in session entitled "International Networks That Work" at 2008 International Conference on Emerging Infectious Diseases, Atlanta, Georgia.

Strain from Ellsworth AFB Used for Influenza Vaccine

The 2008–2009 trivalent influenza vaccine comprised an A/Brisbane/59/2007 (H1N1)-like virus, an A/Brisbane/10/2007 (H3N2)-like virus, and a B/Florida/4/2006-like virus. One of these strains, the A/Brisbane/59-like virus, was identified through the DoD laboratory-based global influenza surveillance program at USAFSAM and served as the seed virus for the H1 component of the 2009 intranasal live attenuated influenza virus vaccine (FluMist).

During 2007, a prior Air Force member and current Army National Guard member and their beneficiaries used Ellsworth AFB (South Dakota) for their primary health care. In July 2007, four of these beneficiaries presented at the base clinic with influenza-like illness (i.e., fever, cough, sore throat). The travel history of the patients was used to explain the presence of influenza-like illness in the Northern Hemisphere during noninfluenza season.

Healthcare providers collected specimens and sent them to the laboratory at USAFSAM. USAFSAM processed the specimens through the standard procedures of viral culture and molecular characterization. Two patients were diagnosed with influenza A. Specimens underwent further molecular testing and were sent to CDC for antigenic characterization.

Like the injectable influenza vaccine, the intranasal influenza vaccine is administered annually and is composed of three strains that are selected each year based on worldwide surveillance and analysis. Strains chosen for the intranasal differ slightly from those in the injectable vaccine because the intranasal vaccine is based on live viruses. Increasingly throughout the military, the intranasal influenza vaccine is being used over the injectable.

Flow of influenza isolate from GEIS network through USAFSAM to CDC for vaccine consideration.

Active duty personnel at Moody AFB (Georgia) receiving intranasal influenza vaccine.

Naval Health Research Center

In 1996, investigators at the Naval Health Research Center (NHRC) in San Diego established a laboratory to survey for respiratory diseases in military populations. Since that time, the NHRC Respiratory Diseases Research Department has become the premier DoD laboratory for diagnosis and characterization of infectious respiratory pathogens, and it has developed an extensive surveillance network spanning eight CONUS recruit training centers, the Mexican border area, forward-deployed shipboard surveillance in three fleets, and several DoD health care facilities in Pacific Rim countries. Specimens and epidemiological data from this network provide a rich array of viral and bacterial isolates that contribute to the global effort to understand and control both adenovirus and influenza infections. During FY08 NHRC continued to address needs of the fleets, other deployments, and recruit populations, by rapidly and accurately identifying respiratory disease threats and conducting basic and applied research to understand and alleviate morbidity from respiratory pathogens (Table 2).

Surveillance

Febrile respiratory illness surveillance is the cornerstone of NHRC efforts, and work conducted in FY08 provided important information on the burden, incidence, and prevalence of respiratory pathogens. NHRC increased the number and frequency of specimens collected and shipped from eight basic training centers. More than 5,000 recruits were sampled in FY08, a 60% increase from levels before the GEIS supplemental funding for pandemic and avian influenza was available.

NHRC identified 400 influenza A and 7 influenza B influenza isolates in specimens submitted from recruits; 262 influenza A cases subtyped as H3. Subtype H1 accounted for the remaining 138 influenza A cases. NHRC's annual estimation of influenza vaccine effectiveness suggested a reduction in vaccine effectiveness against the H1 subtype. Vaccine effectiveness against H1 was 71% in 2007–2008, whereas vaccine effectiveness in the previous four seasons was 86–94% (Figure 17).

Table 2. Scope of Respiratory Pathogens Diagnosed Through NHRC Capabilities

Pathogen	Culture	Molecular	Serology
Adenovirus	□*	□*	□
▪serotyping		□	□
Coronaviruses OC43/229E		□	
Enterovirus	□*	□	
Herpes simplex virus 1/2	□*		
Human metapneumovirus		□	
Influenza A and B	□*	□*	□
▪subtyping	□	□	□
Influenza H5 (laboratory		□	□
Parainfluenza 1/2/3	□*	□	
Respiratory syncytial virus	□*	□	
Rhinovirus		□	
Bordetella pertussis	□*	□	
Chlamydia pneumoniae		□	□
Haemophilus influenzae	□*	□	
▪sensitivity testing	□*		
Legionella pneumophila		□	
Moraxella catarrhalis		□	
Mycoplasma pneumoniae	□*	□	□*
Neisseria meningitidis	□*	□	
▪sensitivity testing	□		
▪serogrouping	□		
Staphylococcus aureus	□*	□	
▪MLST		□	
▪sensitivity testing	□*		
Streptococcus pneumoniae	□*	□	
▪MLST		□	
▪sensitivity testing	□*		
▪serotyping	□*		
Streptococcus pyogenes	□*	□	
▪emm typing	□*	□	
▪sensitivity testing	□*		

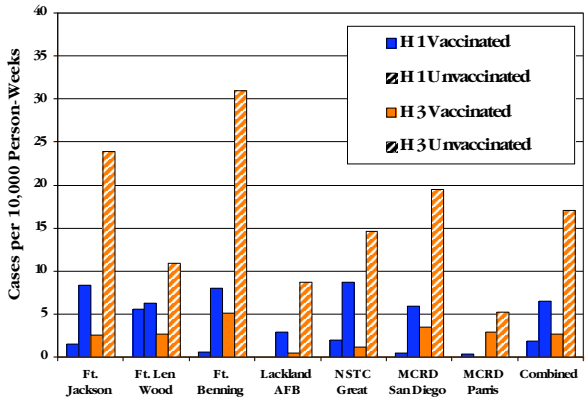


Figure 17. Incidence of laboratory-confirmed influenza among US basic trainees by subtype and vaccination status, 2007–2008

NHRC expanded its shipboard febrile respiratory illness surveillance in FY08 with 20 ships from the 2nd, 3rd, and 7th Fleets submitting a total of 147 specimens, an increase of 26% over FY07; 62 specimens (42%) were positive for influenza A; 59% were H3, and 3% were H1. Many of these cases presented in clusters that occurred immediately after port stops in foreign countries.

Febrile respiratory illness surveillance among non-DoD populations continued along the Mexican border in collaboration with San Diego County, the CDC, and investigators in Mexico. The number of participating clinics increased from three to six. NHRC found that more than 25% (n = 93) of the 368 specimens from presenting cases were positive for influenza: A/H1 (n = 22; 6%) and A/H3 (n = 32; 9%).

NHRC sent influenza isolates to the Influenza Division of the CDC for consideration by the WHO for inclusion in WHO recommendations for the 2008–2009 trivalent influenza vaccine.

Of noninfluenza respiratory pathogens detected, type 4 adenovirus remained predominant at all training sites over 2007 levels. Adenovirus serotypes 3 and 21 were recovered at the Coast Guard Training Center (Cape May, NJ) and Fort Benning (Georgia), and serotype 14 was recovered at Lackland AFB (Texas). Analysis of these samples allowed investigators to track the emergence of adenovirus serotypes 14, 21, and 3. These group B adenovirus serotypes continued to emerge at seven of eight training centers. Additionally, NHRC laboratory analyses of radiologically confirmed pneumonia cases at

Influenza-like Illness Surveillance along Mexican Border

The border crossing at San Ysidro, California, and Tijuana, Mexico, is only 20 miles south of downtown San Diego. In 2004, approximately 50,000 people were documented to cross the border at Tijuana daily. This count, which comprises people who live on one side of the border and work on the other, makes Tijuana the busiest border crossing in the world, according to the Center for Strategic and International Studies (Washington DC). The crossings at Calexico, California, and Mexicali, Mexico, are also busy.

San Diego is home to Naval Station San Diego, North Island Naval Air Station, Naval Base Point Loma, Marine Corps Recruit Depot San Diego, and Marine Corps Air Station Miramar. This large population of active duty members and other DoD beneficiaries often uses regional civilian health care facilities. Because of the proximity to the border, they consequently risk sharing pathogens with civilian populations.

In 2004, the NHRC Respiratory Diseases Research Department collaborated with the CDC Border Infectious Disease Surveillance project and Early Warning Infectious Disease Surveillance program and two county health departments in California to initiate influenza-like illness surveillance at the San Ysidro Health Center (San Ysidro, CA). This collaboration has grown to include four sites in the United States (Brawley, Calexico, San Diego, and San Ysidro) and two sites in Mexico (Mexicali and Tijuana). Mexico's public health department in Baja California entered the program in 2007–2008. Although large numbers of patients with febrile respiratory infections present to these participating community health care clinics, viral testing had not been routinely performed before this collaboration.

Through this collaboration, the CDC hires personnel to collect specimens at the sites, and NHRC provides their training. NHRC tests the specimens using PCR and bacterial and viral culture. NHRC sends weekly laboratory results to all stakeholders. Patients meeting the influenza-like illness case definition (fever of ≥100°F with either a cough or sore throat or pneumonia from any cause) are asked for two nasal and one throat specimen swabs so that rapid antigen influenza tests (Quidel QuickVue Flu A + B) can be performed.

Surveillance for influenza-like illness in 2007–2008 was conducted in Brawley, Calexico, San Ysidro, and San Diego in the United States and in Mexicali and Tijuana in Mexico.

Continued

Influenza-like Illness Surveillance, *Continued*

In 2007–2008, 385 specimens were collected and tested, the highest yearly total up to that time. The most frequent pathogen identified was influenza (25% positive for influenza A or B). Several other viral and bacterial respiratory pathogens were also identified. NHRC has evaluated the performance of the rapid influenza test used during the past 3 years against its gold standard laboratory test system.

Rapid Test Performance from Border Surveillance

	2005–2006	2006–2007	2007–2008
No. specimens (% flu A+B positive)	227 (45%)	209 (21%)	389 (25%)
NPV	76%	94%	85%
PPV	96%	73%	66%
Sensitivity	65%	83%	59%
Specificity	98%	89%	89%

NPV, negative predictive value; PPV, positive predictive value.

NHRC has consistently isolated a high percentage of influenza in specimens from this surveillance. In 2005-6, this program identified the first California isolate similar to the strain chosen to be the H3 component of the 2006-7 vaccine (A/Wisconsin/67/2005 (H3N3)-like). Future plans include expanding the number of sites along the border.

2008 Laboratory Results from FY08 Border Surveillance

	San Diego	Calexico	Mexicali	San Ysidro	Brawley	Tijuana	Total
Influenza-like illness cases	24	82	131	110	27	11	385
Influenza A	2 (8%)	13 (16%)	8 (6%)	25 (23%)	7 (21%)	2 (18%)	57 (15%)
Influenza B	2 (8%)	3 (4%)	15 (11%)	10 (9%)	9 (33%)	0	39 (10%)
Adenovirus	1 (4%)	4 (4%)	2 (2%)	10 (9%)	0	1 (9%)	18 (5%)
Other viral	0	7 (9%)	5 (4%)	0	0	0	12 (3%)
Bacterial	3 (12%)	8 (10%)	15 (11%)	8 (7%)	1 (4%)	0	35 (9%)
Negative	16 (67%)	48 (57%)	90 (69%)	62 (56%)	10 (37%)	8 (73%)	234 (61%)

Some columns sum to more than 100% because of coinfections.

four training centers found that *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* were associated in 10–15% of these cases and that over 50% were coinfected with adenovirus.

With the goal of determining the geographic and temporal trends of antibiotic resistance and distribution, NHRC continued to collect clinical *Streptococcus pyogenes* isolates from basic trainees. Since 1998, NHRC has collected 2,597 isolates, with 303 (11.6%) demonstrating full or partial resistance to erythromycin. Additionally, NHRC started a *Neisseria meningitidis* surveillance program in FY08. Data from this work are under review and will be used to calculate prevalence, incidence, carriage, and serogroup-specific rates.

Response

During FY08, NHRC continued to leverage its expertise in respiratory diagnostics to assist in disease outbreak and fatal case investigations. In July, NHRC provided laboratory support for an investigation of an outbreak of pneumonia among Navy Basic Underwater Demolition/SEAL trainees in San Diego. Within 48 hours of specimen receipt, NHRC identified *C. pneumoniae* as the etiologic agent. NHRC also provided diagnostic support to investigations of 13 severe or fatal respiratory cases at Marine Corp Base Camp Pendleton. In collaboration with the AFIP, NHRC provided laboratory diagnostics for methicillin-resistant *Staphylococcus aureus*, *Streptococcus pneumoniae,* and fatal myocarditis cases occurring at the Naval Medical Center, San Diego. Most of these samples were referred through the AFIP Mortality Surveillance Program.

NHRC provided weekly updates to DoD-GEIS and command partners on influenza and febrile respiratory illness rates throughout the year. NHRC added data-intensive graphics to these febrile respiratory illness updates in FY07 and made further improvements in FY08, including the addition of recruit density data. These reports are published in CDC Epi-X and USACHPPM Health Information Operations Weekly Update.

Integration and Innovation

New and novel diagnostic tests can provide accurate and timely diagnostics in deployment settings. NHRC has conducted clinical evaluations on two of these: LoopAmp and Arbor Vita. LoopAmp, a simple, highly sensitive

Chlamydia pneumoniae Outbreak Hinders BUD/S Training

Respiratory infections account for a high rate of morbidity among military personnel, and moderate disease burden and sporadic epidemics occur most notably in recruit and training populations. Although this phenomenon is incompletely understood, close living conditions, environmental exposures, physical and/or emotional stress, and an influx of immunologically naive persons probably combine as contributing factors. The most likely pathogens associated with respiratory infections in military populations are adenoviruses, influenza, the bacteria group A *Streptococcus pyogenes* (strep throat) and *Bordetella pertussis* (whooping cough), and agents that cause bacterial pneumonia.

BUD/S recruit training is physically harsh and mentally demanding, characteristics that combine to create an ideal scenario for respiratory disease outbreaks

Basic Underwater Demolition/SEAL (BUD/S) training is a 6-month SEAL course conducted at the Naval Special Warfare Training Center in Coronado, California. After an indoctrination and pretraining period that last 5 weeks, a three-phase training cycle starts. Early in the first phase trainees undergo a punishing 5.5-day stage called hell week, which typically eliminates about 75% of the class. Sleep deprivation, extreme heat and cold, mental fatigue, and physical pain are its hallmarks.

Over a hell week in mid-July 2008, several BUD/S students reported to the health clinic with fever, shortness of breath, and cough. Chest radiographs demonstrated moderate consolidation in some lung fields. A mild pneumonia was suspected. On 28 July, the Respiratory Diseases Research Department at NHRC obtained nasal and throat respiratory swabs from five febrile students and conducted molecular laboratory tests to find the primary etiology. Within 24 hours, PCR analysis indicated the presence of *Chlamydia pneumoniae* in all five patients. To determine the extent of a possible *C. pneumoniae* outbreak, 30 BUD/S students from the same class were sampled on 30–31 July 2008. Among these, five (17%) yielded molecular evidence of *C. pneumoniae*, two had rhinovirus, and one had coronavirus infection. Rhinoviruses are the most common viral infective agents in humans and are a causative agent of the common cold. No dual infections were detected.

The unexpectedly high number of *C. pneumoniae* infections among the BUD/S trainees suggests that it was the causative agent responsible for the July hell week outbreak. *C. pneumoniae* infections are responsible for approximately 10% of pneumonia and 5% of bronchitis cases in the United States, and pneumonia and bronchitis are the most common clinical manifestations of *C. pneumoniae* infection. Symptoms can persist for 2–6 weeks. In confined areas, prolonged epidemics can develop among recruit populations. The most likely mode of infection is direct contact from droplets or secretions. A descriptive study initiated in 2001 in Izmir, Turkey, found a high prevalence of military trainees with *C. pneumoniae* infections. Epidemics of respiratory disease caused by *C. pneumoniae* have also been documented in Thai and Swedish military trainees.

Once the diagnosis for the hell week outbreak was determined, infected trainees were treated with a course of antibiotics. In 1998, a randomized, placebo-controlled clinical trial of azithromycin prophylaxis was conducted in BUD/S trainees. Results demonstrated that oral azithromycin (1 g/week) was more successful in preventing respiratory infections in BUD/S populations than benzathine penicillin G prophylaxis. To control the July 2008 outbreak, trainees were given two doses of oral azithromycin (1,000 mg), one during hell week and one the week after. Among the 10 individuals with *C. pneumoniae* infections, four were released from training; three were dropped upon request; and two completed BUD/S training.

Given mission requirements when the United States is at war, the need to reduce the impact of morbidity from infectious pathogens among military personnel is critical. Rapid diagnoses of respiratory infections must guide treatments and limit the emergence of possible epidemics. This is especially pertinent among specialized groups such as BUD/S trainees. Continuous surveillance for respiratory infections among military recruits and trainees is mission critical.

test for H5 influenza, showed promise for application in shipboard and deployed settings. NHRC will test it for seasonal influenza in FY09. NHRC performed an evaluation of the Arbor Vita H5 rapid test utilizing febrile respiratory illness samples from military dependents in San Diego and H5N1 isolates provided by NAMRU-3 in Cairo, Egypt. The FDA is considering NHRC findings in its product approval process.

In collaboration with the La Jolla Bioengineering Institute (La Jolla, CA), NHRC made progress toward the use of the Luminex platform for influenza serological testing by attaching purified whole virus to the Luminex beads. Assay optimization and evaluations using clinical samples are planned for FY09.

The IBIS T-5000 high-throughput diagnostic platform for identifying viral and bacterial pathogens remained a core element of the NHRC respiratory diagnostics capability in FY08. NHRC typed more than 200 *S. pyogenes* isolates using T-5000 in FY08. In addition, NHRC achieved the College of American Pathologists validation for influenza A and B diagnosis and used the T-5000 technology to rapidly identify and subtype influenza clusters occurring at Fort Benning, Great Lakes Naval Training Center (Illinois), and the Marine Corps Recruit Depot (Parris Island, South Carolina).

In collaboration with the University of Iowa, NHRC used novel surveillance methods to predict influenza vaccine performance and demonstrated that a web-based market futures predictive model correctly predicted vaccine performance before laboratory confirmations were available.

Cooperation and Capacity Building

In FY08 NHRC furnished 14 ships with training, protocols, and reagents to perform onboard PCR diagnostics for influenza A and B, adenovirus, *M. pneumoniae*, and *C. pneumoniae*. NHRC also served as the CDC Laboratory Response Network gatekeeper for five ships in the 3rd and 7th Fleets. NHRC stood up and staffed a Pacific Rim surveillance hub in Yokosuka, Japan, for training 7th Fleet ship personnel, gained CDC Laboratory Response Network status for the Yokosuka Navy hospital, and assisted Air Force influenza surveillance in the region. NHRC also provided training and supplies to four US and two Mexican border clinics participating in the border febrile respiratory illness program.

Construction of the new BSL-3E laboratory at NHRC was completed. With this capacity, potential H5N1 infection can be diagnosed and characterized from samples submitted during surveillance or outbreak activities (Figure 18). The laboratory was commissioned in FY08, and USDA certification and operational status are expected in early 2009.

Figure 18. Construction of new BSL-3E laboratory at NHRC was completed in FY08. *Top*, exterior; *bottom*, autoclave and pass-through.

NHRC collaborated with many DoD, US government, and academic partners in FY08, including the CDC, FDA, USAFSAM, NAMRU-2, NAMRU-3, NMCPHC, WRAIR, AFIP, and the Trudeau Institute (Saranac Lake, NY). Increased collaborations are planned as NHRC continues to enhance surveillance activities, develop diagnostics, increase surge capacity, adapt diagnostic platforms, and enhance laboratory capabilities to address the needs of force health protection.

United States Army Medical Research Institute of Infectious Diseases

The United States Army Medical Research Institute of Infectious Diseases (USAMRIID) serves as a national resource for the isolation and identification of infectious disease agents requiring high containment BSL-3 and BSL-4 laboratories. In this capacity USAMRIID serves as a DoD reference center for arthropod-borne and hemorrhagic fever viruses. The institute provides diagnostic support for many CONUS and OCONUS DoD laboratories through its confirmatory testing, reagent supplies, and subject matter expert advice. Development and fielding of new detection assays, technology transfer to government and civilian organizations, production and stockpiling of critical reagents, and a response capacity for outbreaks of emerging and reemerging diseases are components of the USAMRIID program. USAMRIID's important expertise in molecular diagnostics is bringing new influenza diagnostic capabilities to deployed forces through the JBAIDS. DoD-GEIS continues to serve as a primary source of funds to maintain these capabilities within USAMRIID.

High Level Biological Containment: Detecting and Identifying Infectious Diseases

USAMRIID maintains diagnostic capabilities with the production of its repertoire of assay antigens and antibodies and development of new assays. This year BSL-4 biothreat recombinant antigens for use in immunodiagnostic assays were introduced. By leveraging other USAMRIID diagnostic efforts, recombinant Lassa proteins were incorporated into both IgM and IgG ELISAs. In the near future, additional BSL-4 pathogen recombinants will be available. During FY08, 380 assays were conducted for various arthropod-borne and hemorrhagic fever viruses. Of these, 56% were for arthropod-borne viruses, 20% for hemorrhagic fever viruses, and 24% for other viral and bacterial pathogens.

An interesting sample submitted for testing this year was from a hospitalized patient with symptoms consistent with an arthropod-borne virus infection. Because the patient had recently traveled to the southeastern United States, the sample was tested against a panel of arthropod-borne viruses known to occur in that region, but all assays were negative for these viruses. In consultation with the submitting physician, other virus assays were conducted including those for vesicular stomatitis virus, a virus of veterinary importance that rarely causes human disease. The USAMRIID laboratory found the individual had vesicular stomatitis virus-specific IgM antibodies but no detectable IgG antibodies, a condition indicative of a recent infection with vesicular stomatitis virus. Upon further discussion with the physician, it was discovered that the patient reported caring for a sick horse the previous week and was likely exposed to vesicular stomatitis virus at that time.

USAMRIID continued ongoing diagnostic efforts in a Lassa virus hyperendemic area in West Africa spanning the Mano River Union countries of Sierra Leone, Liberia, and Guinea. Within this region, 300,000–500,000 Lassa virus infections occur annually, resulting in 5,000 deaths. Modern diagnostics and training to detect and identify Lassa fever are provided to the Kenema Government Hospital (Sierra Leone) through the collaborative efforts of USAMRIID, WHO, Tulane University (New Orleans, LA), and the Mano River Union-Lassa Fever Network. The collaboration has had tremendous positive impact on the lives of the Sierra Leone people and is an excellent example of effective DoD and US government medical diplomacy. The Lassa fever project comprises the following attributes:

- USAMRIID provides urgently needed diagnostic capabilities for the Sierra Leone people.
- The partnership provides an opportunity to field test assays.
- Hyperendemic areas in Sierra Leone are valuable study areas for diagnosticians.
- This ideal field testing environment furthers the development and refining of the diagnostic Lassa virus immunoassays and real-time PCR assays.

In work with the OCONUS laboratories, USAMRIID personnel continue to advise NAMRU-3 on assay and reagent development and to provide diagnostic reagents for many surveillance activities. USAMRIID personnel served as subject matter experts for a USAMRU-K and KEMRI acute febrile illness protocol conducted at numerous sites throughout Kenya. The team evaluated the collection sites, assays to be used, and the protocol process. They followed up with a comprehensive report outlining strengths and weaknesses and suggested possible improvements. The process strengthened collaborative efforts between USAMRIID and USAMRU-K and helped USAMRIID gain a better understanding of USAMRU-K diagnostic requirements, capabilities, and processes.

Modern Lassa Fever Diagnostics in Sierra Leone

USAMRIID personnel overseeing Kenema technician conducting ELISA technology safely.

Lassa virus, a member of the Arenaviridae family, causes Lassa fever, a severe and often fatal hemorrhagic fever. As many as 300,000 infections and 5,000 deaths occur each year in West Africa, where the virus is endemic. The highest incidence of Lassa fever in the world is in the Mano River Union countries of Sierra Leone, Liberia, and Guinea. USAMRIID represents a unique reach-back support within the DoD for dangerous pathogens such as Lassa virus emerging in Africa. USAMRIID supports work on Lassa fever in Sierra Leone as part of an integrated, multiple-partner endeavor.

From the late 1970s to the early 1990s, the CDC maintained a Lassa fever research station in the Eastern District of Sierra Leone. The station was closed during the brutal conflict that engulfed Sierra Leone in the 1990s. Now, using GEIS funds and in partnership with the WHO, Tulane University, the National Institutes of Health, and others, USAMRIID trains staff and deploys Lassa fever ELISA and real-time RT-PCR assays and reagents to the Kenema Lassa Fever Laboratory for in-country diagnostics. The Kenema Lassa Fever Laboratory is based on the grounds of Kenema Government Hospital in southeastern Sierra Leone. With this capacity, diagnostic test results are now available within a matter of hours, leading to timely and improved patient care and outcomes. In addition, the Kenema laboratory's ability to diagnose Lassa rapidly and accurately has made it the Lassa fever diagnostic center for the entire Mano River Union region. Guinea and Liberia send samples weekly, and Kenema returns test results within 24–36 hours. Having the capability of accurate diagnoses directly where cases occur is allowing bright new light to be shed on the pathogenesis and clinical correlates of this deadly, naturally occurring disease and biodefense threat.

USAMRIID's recent introduction of Lassa-specific recombinant proteins to existing assays has further improved the safety of this diagnostic testing and reduced financial costs. Besides improved patient care, these new assays have demonstrated that Lassa fever survivors develop marked early antibody responses to Lassa glycoproteins. Fatal cases, however, tend to develop strong responses to the nucleoprotein. In addition to improving diagnostics, this finding will direct efforts in vaccine and therapeutics development. USAMRIID has also shown that serum creatinine levels can be used as a patient outcome prognosticator.

Sample processing in Kenema Government Hospital laboratory requires meticulous use of Class II biosafety cabinets and personnel protective equipment.

Pandemic Influenza Surveillance

Development of a DoD influenza virus reference panel and evaluation of molecular-based real-time PCR assays continue to be the emphases of the USAMRIID pandemic and avian influenza efforts. The Influenza Virus Reference Panel consists of 64 influenza virus subtypes collected throughout the world from humans and various animal species. In FY08, 31 subtyped influenza isolates were chosen to comprise the minimum inclusivity/exclusivity reference panel. Each virus was characterized genetically by limited genome sequencing, real-time PCR, pyrosequencing, and T-5006 electrospray ionization time-of-flight mass spectrometer, when available, and antigenically by ELISA. This DoD asset, maintained by the Unified Culture Collection at USAMRIID, will be available to the entire DoD and its collaborators through the Chemical Biological Medical Systems of the Joint Program Executive Office (CBMS-JPEO; Frederick, MD) critical reagent program. Access to this varied collection of influenza virus subtypes will be paramount for any future influenza diagnostic development or test and evaluation.

In unprecedented cooperation, the CDC, CBMS-JPEO, USAMRIID, Idaho Technologies (Salt Lake City, UT), and DoD-GEIS are transitioning the CDC influenza A/H5 two-target assay to the JBAIDS platform for FDA clearance. This diagnostic platform was developed for the rapid detection of biological warfare agents in a deployed setting. USAMRIID, with DoD-GEIS funding, began the process of collecting performance data on the two CDC H5 influenza assays targets, H5a and H5b, to obtain FDA clearance. In FY08, USAMRIID personnel spent significant effort transitioning the CDC assay to the JBAIDS. The initial performance data for the CDC A/H5 two-target assay on the JBAIDS platform using Idaho Technologies reagents represent a critical first step to demonstrate the feasibility of the assay transition. USAMRIID will provide preclinical testing of the assay en route to obtaining FDA clearance for this assay on the JBAIDS platform.

Influenza A/H5 Diagnostic Testing Brought to Forward-deployed Forces

Having the availability of specific and rapid clinical diagnostics for the timely identification of infected patients is critical to pandemic influenza preparedness. Consequently, DoD-GEIS is seeking FDA approval for a specific influenza A/H5 real-time PCR assay to be included on the JBAIDS biodetection platform. Headquarters and USAMRIID are partnering with the JBAIDS program office at CBMS-JPEO, the CDC, and other organizations to achieve FDA approval. Accurate identification of influenza A subtypes is critical for differentiating seasonal influenza varieties from pandemic influenza strains.

The JBAIDS platform is a relatively contained, field-deployable real-time PCR machine that is being placed in more than 300 DoD health care and diagnostic facilities including Army combat support hospitals, veterinary food analysis and diagnostic laboratories, Air Force medical support groups and biological augmentation teams, Navy large deck ships, Marine Corps expeditionary forces, and National Guard civil support teams. These sites are positioned to provide DoD's influenza diagnostic testing to forward-deployed forces. The DoD-GEIS JBAIDS influenza A/H5 effort brings essential preparedness into its laboratory capability for force health protection.

Naval Medical Research Center

DoD-GEIS continued to support the Naval Medical Research Center (NMRC) to conduct surveillance for rickettsial and rickettsial-like diseases. In collaboration with commercial companies, NMRC developed FDA-certified tests for typhus, spotted fever, and scrub typhus and now provides these reagents and training to perform these assays to other DoD laboratory assets and to conduct any required confirmatory testing.

Rickettsial diseases, including epidemic typhus, murine typhus, Rocky Mountain spotted fever, Mediterranean spotted fever, scrub typhus, ehrlichiosis, and trench fever, are endemic, emerging, or reemerging in many parts of the world. Antibiotic resistance has been reported with *Orientia tsutsugamushi*, the agent of scrub typhus. The OCONUS laboratories strive to measure the extent of rickettsial diseases, their threat to military operations, and the emergence of antibiotic resistance; however, rickettsial diseases are difficult to diagnose because symptoms are often nonspecific and share characteristics with many other febrile illnesses. Rapid serologic tests are of limited utility given sensitivity and specificity issues. Complex indirect fluorescence antibody assays can increase accuracy but require preparation by specialized laboratories. Isolation of pathogens is necessary to detect emerging species and strains; however, the hazards intrinsic to pathogen isolation require special training and a BSL-3 laboratory. Accordingly, the NMRC Rickettsial Disease Research Program is ideally suited to fill the DoD-wide need for a reference laboratory to confirm diagnostic results and culture live rickettsiae.

Recent reports utilizing NMRC-developed rickettsiae assays include a case report of the first description of Thai tick typhus in Bangkok, Thailand. The Thai patient presented to a local hospital with fever, rash, and headache. NMRC determined the identity of the causative agent (*Rickettsia honei*) through spotted fever group rickettsiae-specific ELISA serology, genus and group-specific quantitative real-time PCR assays, and species-specific MLST techniques.

A second study using sera from the DoD serum repository determined that in a diverse population of 10,000 servicemembers, 6% were seropositive for previous infection by spotted fever group rickettsiae, but only 0.1% were seropositive for *Anaplasma phagocytophilum* (human anaplasmosis). These results demonstrated that exposure to spotted fever group rickettsiae was common and that *A. phagocytophilum* was rare among this diverse US military population. Moreover, independent risk factors identified for spotted fever group rickettsiae seropositivity in the military include age, home state with Rocky Mountain spotted fever incidence at least as high as the aggregated US incidence, and ground combat occupational specialties.

NMRC determined that 11% (85/756) of adults reporting to a hospital in Kathmandu, Nepal, with fevers of unknown origin had IgM to *R. typhi*. NMRC detected the presence of *R. typhi,* the causative agent of murine typhus, by species-specific quantitative PCR assay in 50 (7%) of 756 blood samples. However, correlation between the presence of IgM antibodies to *R. typhi* and that of *R. typhi* nucleic acid was small because only 11 (13%) of the IgM-positive samples were also quantitative PCR positive. This observation was most likely due to the long life of detectable levels of rickettsia-specific IgM and the relatively short presence of *R. typhi* organisms in the blood before the induction of *R. typhi*-specific IgM.

A relatively new infectious agent with a worldwide distribution, *Rickettsia felis,* causes a febrile illness similar to murine typhus in humans. The genomic variation of *R. felis* isolates throughout the world was unknown until NMRC sequencing of the *R. felis* Cal2 genome demonstrated the agent has two forms of the same plasmid. Plasmids are rare for rickettsiae, and the identification of two plasmid forms was appropriately questioned. After further evaluation of several isolates, the reasons for the discordant findings published in journals and heard in presentations was determined: the two plasmid forms are not found in all isolates, and the plasmid content of an isolate is unstable. The discovery of the unstable presentation of the plasmids is important in determining the identification of isolates from vectors and hosts alike.

NMRC assessed sera from 10,000 servicemembers previously stationed in South Korea for antibodies specific for spotted fever group, typhus group, and scrub typhus group rickettsiae. The seroprevalence of antibodies for these at ≥100 reciprocal titer was 433/8,486 (4.6%), 114/9,249 (1.2%), and 27/9,068 (0.30%), respectively. NMRC then paired seroreactive postdeployment samples with predeployment serum samples and tested for antibodies to spotted fever group, typhus group, and scrub typhus group: 46 (11%), 1 (0.9%), and 8 (30%) individuals showed a four-fold rise in titer for spotted fever group, typhus group, and scrub typhus group, respectively. This study suggests that among rickettsial pathogens, the highest risk for exposure during deployment in Korea is among the scrub typhus group, whereas a lower risk is expected for spotted fever group and typhus group exposure.

Ongoing NMRC projects include the development of new quantitative PCR assays for the detection of *R. conorii* (Mediterranean spotted fever) and *R. raoultii* and *R. slovaca* (tick-borne lymphadenopathy/*Dermacentor*-borne necrosis erythema lymphadenopathy).

Walter Reed Army Institute of Research

FY08 DoD-GEIS activities at the Walter Reed Army Institute of Research (WRAIR) were conducted through the Division of Entomology, Division of Experimental Therapeutics, Division of Bacterial and Rickettsial Diseases, and Division of Viral Diseases.

Division of Entomology

Arthropod-borne infectious diseases are a substantial health threat to troops in combat. Decision makers cannot afford to ignore this health threat nor repeat the mistakes of previous conflicts in which many soldiers were debilitated or killed by preventable infectious diseases. Knowledge of the identity and occurrence of the major vectors is a prime requirement to determine the threat posed by vector-borne diseases. Predicting where the vectors are likely to be found, particularly using ecological niche models, could be a valuable addition to health risk assessment and disease control strategies.

High-resolution maps of mosquito disease vectors would offer important intelligence about where these diseases can and cannot occur given the presence and absence of the vector. In addition, these maps could be used as base layers in geographical information system models that use remotely sensed data to calculate the risk of disease transmission at any point in the world. Global mosquito maps could assist in decisions about where to locate hospitals and bases, the type and extent of vector control and prophylaxis needed for an area, and for quarantine and invasive species management. However, the need for continental and global scale databases of georeferenced insect specimen data is increasing. The paucity of detailed data on the past and present distribution of vectors is a significant limiting factor for global modeling of vector-borne diseases and is the focus of the WRAIR Division of Entomology project.

Mosquito collection records can be obtained from the literature, unpublished reports, survey results, and museum specimens. Higher resolution maps require accurately located and identified mosquito species occurrence records rather than just records of the presence or absence of a species in a country. Perhaps the best resource for distribution modeling is found in survey results that combine global positioning system coordinates with up-to-date mosquito identifications. Such a situation exists in the Republic of Korea (ROK), where members of the *Anopheles* Hyrcanus group are regularly collected and identified by PCR. Malaria control among armed forces personnel is a priority in the ROK and other parts of Southeast Asia. Accordingly, developing high-resolution maps of vector distribution provides valuable intelligence about malaria risk. Past mosquito survey results are vulnerable to loss, which suggests a need for a central data repository that is accessible online.

In FY08, the WRAIR Division of Entomology aimed to build on the progress of its previous modeling work to better understand the distribution of vectors and their spatial relationship to disease. Techniques were improved for applying ecological niche modeling to collection records from the literature and from survey results to derive maps of potential distribution to some major malaria vectors in the ROK and Southeast Asia. These maps will help address many vector problems, including identifying the ecological determinants of distribution and incriminating those species responsible for historical malaria resurgence and current transmission.

The utility of point occurrence records and ecological niche models would be increased if they could be made part of an information management tool for clinicians, epidemiologists, and preventive medicine and public health practitioners to facilitate emerging infectious disease detection, response, prevention, and coordination. Ecological niche models can be used in concert with vector-borne disease models and human population density to investigate the spatial components of disease risk (the Mal-area). A novel web resource (MosquitoMap) was developed for hosting and graphical display of mosquito occurrence records and ecological niche models. Within MosquitoMap a tool called the Mal-area calculator uses ecological niche models and pathogen and human distribution models, obtained from the literature and other sources, to quantify the potential area of co-occurrence of these factors for estimating disease risk. By demonstrating the utility of point occurrence data and ecological niche models, contributions of data from mosquito researchers, preventive medicine officers, and other health professionals will be encouraged. The relationships among some of these outcomes of GEIS FY06–FY08 projects are shown in Figure 19.

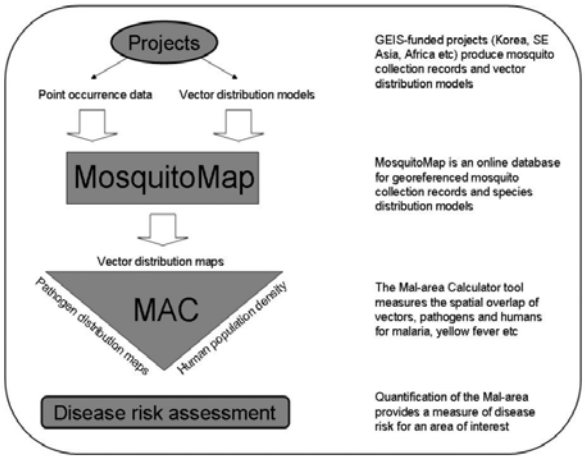


Figure 19. Relationships among elements of GEIS FY06–FY08 proposals awarded to WRAIR resulting in maximum utility of project output for entomologists and health professionals.

How might the Mal-area calculator be put to practical public health use for force health protection? The Mal-area can be calculated using grid files that were rendered from images of the spatial limits of *Plasmodium falciparum* and *P. vivax* published on the Malaria Atlas Project website (map.ox.ac.uk) and by using models of dengue, Japanese encephalitis, yellow fever, and Rift Valley fever distribution obtained from National Center for Medical Intelligence. When performed in this manner, the output of the Mal-area calculator shows an image and associated statistics for the Mal-area, the disease, the vectors, and human population density for the area of interest. Figure 20 shows what the Mal-area might look like for five US bases in the ROK for *P. vivax*. By using such tools, public health practitioners can make decisions about disease monitoring and preventive measures for US forces.

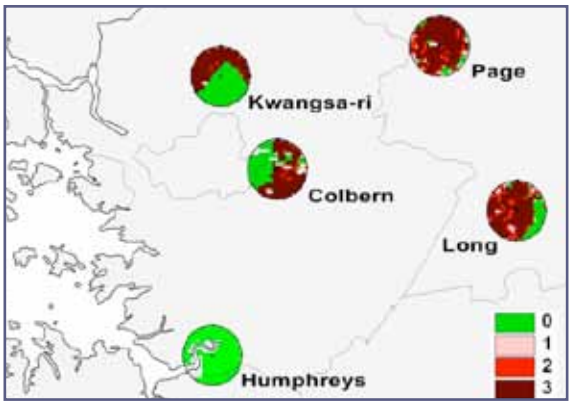


Figure 20. Extent of Mal-area for *P. vivax* within 10 km of five US military installations in the ROK based on vector distribution models and the areas predicted to be suitable for malaria. Mal-area is calculated assuming that any of three species (*An. kleini, An. sinensis,* and *An. pullus*) are equally important as vectors. Higher values indicate greater risk of malaria.

Selected accomplishments for the FY08 GEIS work through the Division of Entomology:

- Ecological niche modeling of mosquito geographic/occurrence records revealed the potential distribution of malaria vectors in the ROK and Southeast Asia.
- Analysis of environmental geospatial data revealed ecological differences among malaria vectors that may assist targeting of vector control measures.
- DNA sequencing revealed a possible new species of *Anopheles* in the ROK.
- Ecological niche modeling has assisted understanding of malaria resurgence in the ROK by incrimination of some of the primary malaria vectors in Asia, specifically the anopheline species responsible in the ROK.
- A website (mosquitomap.org) has been developed to host georeferenced mosquito occurrence data and mosquito distribution models in a geographical information system setting. More than 70,000 mosquito collection records for more than 220,000 specimens from 1,264 species are now available at mosquitomap.org
- A prototype online tool for vector-borne disease risk assessment called the Mal-area calculator is operational for the mosquitomap.org. This novel tool quantifies where humans, pathogens, and vectors are predicted to coincide for a user-defined area anywhere in the world. The Mal-area calculator is configured for malaria, dengue, Japanese encephalitis, yellow fever, and Rift Valley fever, and the funtionality of this tool will expand as new ecological niche models are developed for the vectors of these diseases.
- Four articles (three published, one in review) were produced from FY08 results.

Division of Experimental Therapeutics

Malaria Diagnostic Center of Excellence, Kisumu, Kenya

Worldwide, malaria kills more than 1 million people every year and makes more than 500 million ill. However, because of poor diagnosis, these numbers are only estimates. Historically, malaria has been a serious problem for military forces operating in malaria-endemic areas. Today's forces are not immune from this ancient killer, as evidenced by the 80 of 200 Marines who contracted malaria in Liberia in 2003. Five required treatment in an intensive care unit; fortunately none died. Other isolated cases of severe malaria have occurred in the last 5 years in other US forces deployed to Africa and Afghanistan.

Tools to control and eliminate malaria are available, and more are in development. Several initiatives, including the Global Malaria Partnership and the President's Malaria Initiative, are funding the delivery of effective products and interventions to people who need them. The focus is insecticide-treated bed nets and treatment of infected individuals. As a result of these efforts, malaria transmission has dropped significantly in the general population in mainland Africa. Malaria cases have declined remarkably on the island of Zanzibar with two rounds of residual insecticide spraying in houses and use of artemisinin combination therapies. Malaria eradication has been attempted previously, with some dramatic successes but eventual failure given insecticide or drug resistance and unsustained funding and infrastructure.

Microscopic examination of blood films remains the definitive laboratory test for malaria diagnosis in clinical (drug and vaccine) trials, test and evaluation of new diagnostic assays, and clinical care. The accuracy of microscopy depends on innate ability, training, experience, motivation, and laboratory resources. False-positive and false-negative results (specificity and sensitivity) have serious negative effects on clinical trial results and patient care.

To address the above identified needs, the WRAIR Division of Experimental Therapeutics established the Malaria Diagnostic Center of Excellence (MDCoE) at USAMRU-Kenya in 2002 with the primary objective of training research microscopists supporting clinical trials conducted in Kenya and East Africa. In addition, this facility leveraged its resources and capabilities to build partner nation health system capability by training clinical microscopists from many countries throughout Africa. The MDCoE is available to provide information

and training to DoD about malaria diagnosis and control during military deployments and for protection of US security interests in Africa.

The GEIS-sponsored objectives of the MDCoE in FY08 included continued support of valid malaria diagnosis, assisting host countries in malaria diagnosis capability for malaria control and elimination, and developing a standardized course in entomologic methods. These objectives would then result in technology transfer to host countries, promote international recognition of the center, and establish and encourage other partnerships and funding lines.

The effect of training on microscopy performance was assessed by testing before and after training. The testing comprised parasite detection, species identification, parasite density counting, and picture and written tests. The results of this assessment demonstrate considerable improvements (Figure 21).

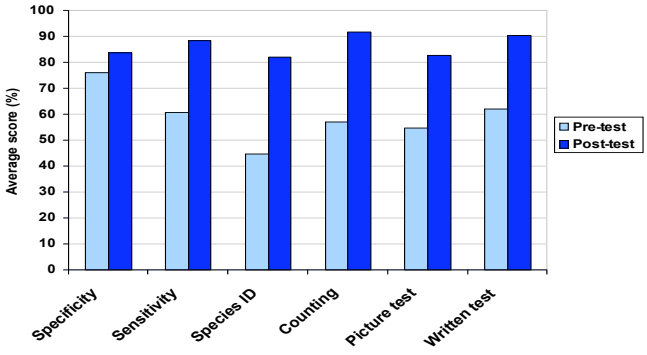


Figure 21. Average test performance before and after microscopy training.

Selected FY08 accomplishments of the MDCoE, achieved with funding from GEIS, WRAIR, and other organizations, follow:

- Training of 117 students from 11 African countries in 2- and 3-week courses;
- Under human use protocols, creation of a standardized set of ~300 human blood films for training and quality diagnosis maintenance;
- Under human use protocols, establishment of an archive of wild-type *Plasmodium* parasite positive blood specimens for testing and evaluation of rapid diagnostic tests;
- Assessment of microscopy training;
- Management of an external quality assurance program that provided a service of malaria slide reading quality assurance with 14 sites throughout Africa involved in malaria clinical trials (Malaria Clinical Trials Alliance);
- Collaboration with the WHO in the revision of the *Bench Aids for the Diagnosis of Malaria*;
- Development of training and diagnosis manuals for the Kenyan ministry of health, Division of Malaria Control, based on MDCoE standard operating procedures;
- Publication of article in 2008 volume of online *Malaria Journal*;
- Progress in establishment of a MDCoE in Kintampo Health Research Center, Kintampo, Ghana;
- Development of curriculum for standardized course in entomologic and vector control to be conducted in FY09.

The MDCoE has gained international renown, thereby attracting funding from and providing services for various organizations such as the Malaria Clinical Trials Alliance, President's Malaria Initiative, and USAID. Training has been shown to significantly improve the performance of microscopists, and the abilities have been leveraged to evaluate various malaria diagnostic techniques that could ultimately improve diagnosis for malaria throughout sub-Saharan Africa.

Malarial Drug Resistance

GEIS work through the WRAIR Division of Experimental Therapeutics comprises two components: surveillance for *P. falciparum* and *P. vivax* drug resistance and quality control and assurance coordination with the OCONUS laboratories. The Division of Experimental Therapeutics supports military operations through identification and characterization of malaria specimens retrieved from infected servicemembers. Serum samples from infected servicemembers are shipped to contract laboratories for antimalarial drug level determinations to address compliance and potential drug resistance questions.

The division also fosters global collaborations with health experts (e.g., university, hospital, and ministries of health) in regions endemic for malaria. These collaborations involve training and technology transfer; malarial species identification, genotyping, and drug resistance marker analysis; and clinical expertise. The Division of Experimental Therapeutics and GEIS assumed the lead in developing a quality control and assurance program involving all GEIS-funded malaria surveillance. In coordination and collaboration with the OCONUS laboratories, the division began shipping reference parasite strains (D6, W2, TM91c235) and standard assayed compounds (e.g., chloroquine, mefloquine, lumefantrine, and pyrimethamine) to overseas laboratories. The quality control and assurance program is in its initial stage,

and the next step is development of a website for placement of quality control and assurance documents (e.g., standard operating procedures); in vitro drug susceptibility determination, genotyping and molecular marker analysis of parasites endemic to the region; and results from quarterly quality control testing. Clinical outcomes, when available, would also be documented on the malaria website.

In addition to these DoD-wide standardization efforts, malaria surveillance efforts were conducted in Honduras and Nigeria and in the South Pacific.

Honduras. Studies were initiated with the Honduran ministry of health in collaboration with CHPPM-West at Madigan Army Medical Center (Tacoma, WA). From Honduras, reference centers throughout Honduras shipped malaria samples to the WRAIR Division of Experimental Therapeutics for genetic diversity and drug resistance marker analysis. Species-specific PCR confirmed 62 *P. falciparum*, 59 *P. vivax*, and 5 *P. vivax -P. falciparum* mixed species infections. *P. falciparum* infections were separated into eight genotypic patterns as determined by *pfmsp1* and *Hinf*I digestion of *pfmsp2*. *P. vivax* infections were separated into six genotypic patterns as determined by *Alu*I digestion of *pvmsp3α*. PCR-RFLP analysis of malaria drug resistance markers, *pfcrt, pfdhfr*, and *pvdhfr*, indicated no drug-resistant alleles were present in the *P. falciparum* and *P. vivax* samples. These findings suggest that genetic diversity of malaria parasites in Honduras has not significantly changed in the last decade.

Nigeria. Studies were conducted in collaboration with the University of Ibadan (Ibadan, Nigeria), Harvard University (Cambridge, MA), and the University of South Florida (Tampa, FL). From 90 Nigerian children presenting with uncomplicated falciparum malaria and enrolled in an artemether-lumefantrine efficacy study, *P. falciparum mdr1* (*pfmdr1*) gene polymorphisms and copy numbers as well as *P. falciparum* Ca2+ATPase (*pfATPase6*) gene polymorphisms were assessed.

The *pfmdr1* haplotype 86N-184F-1246D was significantly associated ($p = 0.000$) with treatment failures and was also strongly selected among post-treatment samples obtained from patients with newly acquired or recrudescing infections ($p = 0.000$, $\chi^2 = 36.5$) and in gametocytes (log rank statistic = 5, $p = 0.0253$) after treatment with artemether-lumefantrine. All pre- and post-treatment samples and gametocytes harbored a single copy of the *pfmdr1* gene and the wild-type allele (L89) *pfATPase6* at codon 89 of this gene. These findings suggest that polymorphisms in *P. falciparum pfmdr1* gene are under directional artemether-lumefantrine selection. *Pfmdr1* polymorphisms may result in reducing the therapeutic efficacy of this newly adopted combination for uncomplicated falciparum malaria in sub-Saharan Africa.

South Pacific. In collaboration with the Australian Army Malaria Institute, the WRAIR Division of Experimental Therapeutics performed surveillance and antimalarial resistance analysis within the South Pacific, an area undergoing a rapid and possibly unique pattern of drug resistance that has not been observed in Africa or Southeast Asia. A 40% increase in chloroquine resistance since 1997, a 20% failure rate to mefloquine, and an increase in drug resistance by *P. vivax* have been observed. These findings are particularly troubling to US forces because mefloquine is the drug of choice for prophylaxis, and no drugs are available to clear *P. vivax* infections that are dormant within the liver hepatocytes.

In 5,239 samples collected from Tanna Island, Vanuatu, a region of the South Pacific that was thought to have a low prevalence of malaria, significantly higher rates of malaria infection were found (1.9%). Preliminary data suggest a two-fold increase in malaria infection than that which was previously reported. This finding is alarming because it signifies that conditions have changed, which supports an increase in malaria infection. Whether environmental conditions and/or drug resistance are involved in the increase is unclear. The DNA extracted from thousands of malaria samples is being analyzed for drug resistance markers. Specifically, markers associated with sulfadoxine, pyrimethamine, chloroquine, and mefloquine resistance will be examined. These markers will be identified in both *P. falciparum* and *P. vivax*. Differences in the genotypic drug markers between malaria species will be interesting because drug treatment policies are not designed for one particular species of malaria. Such practices emphasize the importance of evaluating the drug resistance profiles from mixed infections.

Selected accomplishments in malaria drug resistance surveillance through the Division of Experimental Therapeutics follow:

- Filled gaps in malaria disease surveillance by conducting malaria surveillance, drug susceptibility testing, and infrastructure building in Honduras, Nigeria, and the South Pacific.
- Standardized resistance testing across GEIS network; provided standardized reference strains and assay components.

- Initiated DoD website for quality control and assurance documents, protocols, drug susceptibility data, genotyping data, and clinical treatment/failure data.
- Identified continued lack of malaria drug resistance to chloroquine within Honduras.
- Generated evidence that a new malaria treatment modality may result in polymorphisms that would reduce therapeutic efficacy.

Division of Bacterial and Rickettsial Diseases

Tracking Origin of Multidrug-resistant Organisms at Forward Operating Base Delta, Iraq

Serious infections with multidrug-resistant organisms have increased sharply in military hospitals in recent years and represent a significant challenge for infection control. Multidrug resistance is defined as resistance to three or more antibiotic classes. Nosocomial sources and rates of colonization remain poorly characterized, and infections caused by resistant Gram-negative bacteria, especially multidrug-resistant *Acinetobacter* and extended-spectrum beta lactamase (ESBL)-producing Enterobacteriaceae, have complicated the care of the wounded from Operation Iraqi Freedom and Operation Enduring Freedom. At the time of injury, these organisms are not found in wound flora. However, multidrug-resistant *Acinetobacter* have been isolated from the nosocomial environment of deployed combat support hospitals, and these strains have been linked to clinical isolates. Accordingly, patient colonization and infection with multidrug-resistant organisms are most likely acquired in the nosocomial environment of the evacuation chain, starting with in-theater medical facilities, such as field hospitals. Because the natural history of environmental colonization with multidrug-resistant organisms in field hospitals was unknown, GEIS funded a study conducted by the WRAIR Division of Bacterial and Rickettsial Diseases to describe the evolution of contamination of the forward-deployed treatment environment.

On 6 December 2007, a new operating room and trauma suite were constructed at a deployed field hospital at Forward Operating Base Delta, Iraq. This capacity provided a unique opportunity to observe early events in the development of nosocomial flora and to engage in targeted infection control measures. Twenty-two patient flow areas were comprehensively sampled before patient care ensued, and sampling continued serially for the next 6 months. A total of 795 environmental samples were collected, yielding 366 isolates, including 126 high-risk organisms. The most common organisms were *Pseudomonas putida* (14.2%), *Enterobacter cloacae* (10.9%), *Stenotrophomonas maltophilia* (9.0%), and *Klebsiella pneumoniae* (7.1%). Other high risk organisms isolated included *Acinetobacter* sp. (5.5%), *K. oxytoca* (5.5%), *P. aeruginosa* (2.5%), and *Escherichia coli* (1.9%).

In the new trauma/surgical suite, five of the high-risk organisms were derived from 53 samples (9.4%) taken before opening the rooms for patient use. None of these demonstrated multidrug resistance. After the initiation of patient care, 96 isolates of high-risk organisms were derived from 556 samples (17.3%) from the new suite. At this point, after patient care began, nine multidrug-resistant organisms were recovered from the trauma bay, recovery/intensive care unit, and latrines. In these rooms, multidrug-resistant organisms were isolated from beds, wall vents, a patient monitor, a sink, floors, and showers. One additional multidrug-resistant organism was cultured from the dental clinic, which was not part of the new suite.

The antimicrobial resistance results are summarized in Figure 22 for five organisms of interest. The environmental samples did not yield multidrug-resistant *Acinetobacter*, *Enterobacter*, or *P. aeruginosa* isolates. The 10 multidrug-resistant organisms isolated were five ESBL-producing *K. pneumonia*, one ESBL-producing *K. oxytoca*, and four ESBL-producing *E. coli*—all isolated after the new suite opened for patient care and more than 40 trauma/surgical patients were treated.

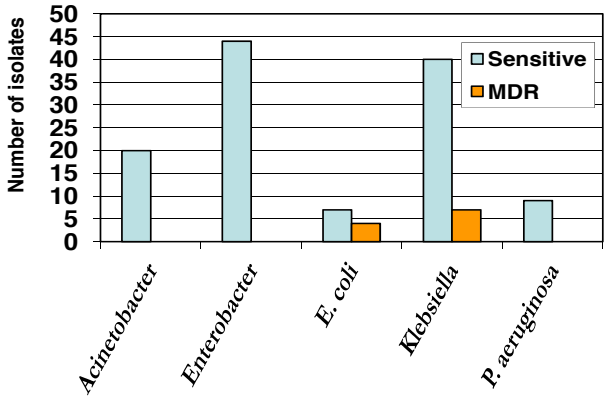


Figure 22. Distribution of susceptibility or multidrug resistance (MDR) isolated from new surgical suite at Forward Operating Base Delta.

Wound infections caused by multidrug-resistant organisms have been known to be seasonal in nature. To address this possibility, the number of isolates during various months during the study was plotted (Figure 23).

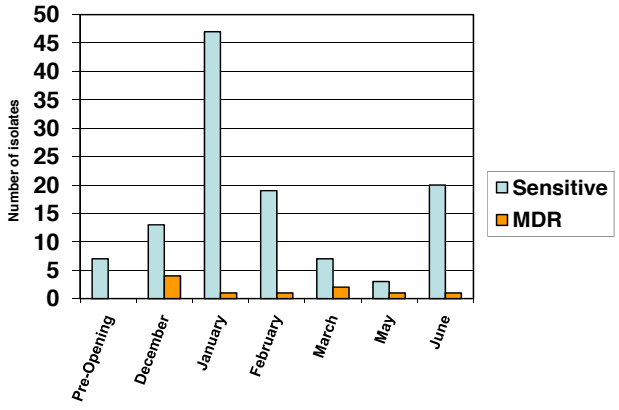


Figure 23. Temporal distribution of multidrug-resistant (MDR) strains isolated from new surgical suite at Forward Operating Base Delta.

The results of this study clearly show that multidrug-resistant Gram-negative pathogens were not isolated from the treatment environment before exposure to patients. However, high-risk pathogens with the potential for resistance acquisition existed in the facility before patient treatment began. Highly resistant environmental flora developed rapidly after patients were brought into the new facility, demonstrating that the challenge of multidrug-resistant Gram-negative bacteria begins early in the evacuation chain. These findings emphasize the importance of early aggressive infection control practices at all levels of treatment in current and future theaters of conflict.

Molecular Epidemiology of Multidrug-resistant Acinetobacter Isolates from National Naval Medical Center

Acinetobacter baumannii complex wound infections have become a troubling issue in MTFs and are associated with wounded servicemembers returning from Operation Iraqi Freedom. DoD-GEIS funded a systematic *A. baumannii* molecular surveillance investigation that comprises the efforts and coordination of WRAIR, NNMC, WRAMC, and BAMC. This project focuses on the genotyping retrospective and prospective military *A. baumannii* complex isolates by PFGE. Goals of this effort follow:

- Establish standardized molecular surveillance procedures for rapid identification of clonal outbreaks;
- Determine transmission maps using genotypic analysis combined with phenotypic traits collected to help develop more sound infection control intervention strategies;
- Examine relationship of molecular fingerprints with organism virulence and treatment success.

The molecular fingerprints of all *A. baumannii* complex isolates were examined and entered into a PFGE database that contains more than 300 genetic fingerprints for comparative analysis and associated antibiotic susceptibility data. Newly obtained *A. baumannii* complex isolates include 116 isolates from NNMC, isolates from LRMC, and isolates obtained from a Canadian civilian hospital that are related to Operation Enduring Freedom.

Each isolate was objectively identified, compared, matched, and analyzed using BioNumerics software (Applied Maths, Kortrijk, Belgium) to analyze the PFGE banding patterns. WRAIR processed 116 *A. baumannii* isolates from NNMC; 112 of the 116 showed clear fingerprint patterns, and 4 were unreadable and could not be compared. Five clusters of similar isolates were defined for 87 isolates. Clusters I–V contained 38, 21, 17, 5, and 6 isolates, respectively. The remaining isolates were less similar to other strains. About 45% of clustered isolates shared 100% similarity. Several WRAMC isolates shared a high degree of similarity with NNMC isolates. All NNMC *A. baumannii* complex strains were isolated between June and December 2007. This relatively short period may explain the high degree of similarity within PFGE clusters.

In October 2007, WRAIR analyzed seven multidrug-resistant *A. baumannii* complex isolates (BC1–7) from a Canadian civilian hospital and identified three groups. Canadian strains BC3 and BC4 were 100% similar, whereas strains BC5, 6, and 7 also shared 100% similarity. BC1 shared >90% similarity with both groups, and BC2 branched apart from these two groups in the dendrogram that shared over 81% similarity. When Canadian PFGE patterns were compared with isolate fingerprints in the database, a 97% similarity was found between the first group (BC3 and BC4) and a WRAIR isolate. The WRAIR isolate was received in early 2005 from LRMC. Accordingly, military *A. baumannii* complex isolates are comparable with multinational force isolates and show high degrees of similarity even when isolated years apart.

Seventeen *A. baumannii* complex isolates from LRMC formed four groups of which three showed 100% similarity. Compared Operation Iraqi Freedom isolates from the same Iraqi location showed 100% similarity, whereas one isolate from another Iraqi location shared low similarity (~70%) between locations. In contrast, a respiratory isolate from the same Operation Iraqi Freedom location shared low similarity with isolates from wound infections.

For a global comparison, all 136 *A. baumannii* complex isolates studied were compared with 337 historic isolates in the database. By using a 90% similarity cut-off level, 18 PFGE pattern groups were observed. The groups ranged from 3 to 88 isolates; 34 of these 88 isolates are from the NNMC archives, and the remaining isolates are from previous studies. Comparisons of BAMC PFGE patterns were not significantly different from PFGE fingerprints obtained at WRAMC.

Additionally, the unified standard operating procedure developed for *A. baumannii* complex PFGE is already being used in partner laboratories and provides standardization of *A. baumannii* molecular methodology. The analysis of genetic fingerprint data related to organisms causing wound infections in servicemembers will lead to better understanding of the infectious process and to better infection control processes. More complete information on the isolates including antibiotic susceptibilities will improve the quality and utility of the data compiled thus far.

The accomplishments of this project for FY08 follow:

- Established unified standard operating procedures among all performing sites, conducted quality control, and established quality assurance procedures;
- Identified a common pattern of genetic fingerprints among MTFs, suggesting that multidrug-resistant *Acinetobacter* is a systemic problem in the MHS;
- Determined that most isolates from MTFs fall into a single genotype, suggesting widespread infection from a common source;
- Showed a clear difference between strains isolated from wounds and those isolated from respiratory infection.

Division of Viral Diseases

Influenza and other emerging respiratory infections continue to threaten the United States. Respiratory infectious diseases account for 25–30% of military hospitalizations, and disease rates in trainees exceed those in similarly aged civilian populations. The agents of respiratory infections include long established viral pathogens such as adenovirus and interpandemic influenza as well as newly emerging threats such as agents of SARS and avian influenza. Influenza, in particular, continues to be an important viral etiology for respiratory infections in the military. As of 20 February 2008, for the seasonal year 2007–2008, 665 influenza isolates (representing 22.6% of all isolates from patients with influenza-like illness) were reported by USAFSAM. Adenovirus is responsible for as much as 20% of hospitalized influenza-like illness among recruits in basic training across all services. Therefore, the burden of respiratory viral pathogens is significant in routine force health protection conditions.

Emergence of Adenovirus 14 and Other Serotypes at Recruit Training Centers

Recruits awaiting vaccinations at Marine Corps Recruit Depot, San Diego. Vaccination in first days of training helps prevent disease outbreaks and improves class graduation rate.

Military recruits are highly susceptible to outbreaks of respiratory pathogens. Adenoviruses have long been known to be the dominant cause of these respiratory outbreaks because of the close quarters and high stress levels in which recruits operate. Adenoviruses were first discovered as agents of respiratory disease during the 1950s by military preventive medicine physicians studying outbreaks in recruit training centers. From 1971 through 1998, the military controlled adenoviruses by vaccination against the two most prevalent serotypes, adenovirus 4 and adenovirus 7. When the vaccine was no longer available (1996), in part because the sole manufacturer discontinued production, the respiratory disease laboratory at NHRC was tasked to document the impact and epidemiology of adenoviruses (and other respiratory pathogens) among recruits and the impact of vaccine discontinuation. NHRC continues this work today.

Continued

Emergence of Adenovirus 14 and Other Serotypes, *Continued*

Since 1998 adenoviral illness rates among recruits have returned to prevaccine levels, causing an estimated 15,000 cases of febrile respiratory illness each year and costing the DoD an estimated $40 million per year for medical treatment and lost training time. From 1998 through 2005, these impacts came almost entirely from a single serotype, adenovirus 4 (species E), with species B1 serotypes such as adenovirus 3 being occasionally detected.

Suddenly in 2006 the situation changed with a rapid return of several species of B1 serotypes (including three common types from the prevaccine era) at several sites, and a simultaneous emergence of a previously rare species B2 serotype, adenovirus 14. At the time, this sudden increase in diversity was not associated with any increase in active-duty member morbidity or mortality. Adenovirus 14 had never been recognized in respiratory disease outbreaks in North America until 2007, when it hit both recruit training facilities and civilian communities hard. Observations suggested a change in disease behavior in comparison with previous adenovirus outbreaks including several severe pneumonias, one fatal pneumonia at Lackland AFB (home of Air Force recruit training), and several fatal pneumonias among civilians in Oregon and Washington.

The work of NHRC, in collaboration with DoD-GEIS, USAFSAM, WRAIR, CDC, and local health departments, was critical in the initial recognition of the emerging (and reemerging) species B adenoviruses. NHRC tracked the viruses, associated them with different epidemiological and clinical disease outcomes, and led surveillance and research into the causal factors of these events. Surveillance at NHRC has shown that the impact of adenovirus 14 was highly variable among sites even though identical viruses were involved in all adenovirus 14 cases. This observation led to two current lines of research: 1) following preventive medicine protocols at multiple sites elucidating how interventions influence outcomes and 2) examining how shifts in preexisting immunity in the general population serve as a predictor of outbreak and symptom severity. The adenovirus vaccine will be resumed, and NHRC intends to monitor its effect among recruits in the near future.

Impact of Adenovirus 14 at Recruit Training Centers

Site	FRI rate in March*	January–March 2008		April–March 2008	
		No. adeno positive†	% Ad14	No. adeno positive†	% Ad14
Fort Benning	0.31	27	0	508	0
Fort Jackson	1.08	182	1	613	4
Fort Leonard Wood	0.63	60	23	393	5
Great Lakes	1.33	163	1	634	1
Lackland AFB	0.54	97	99	290	93
MCRD Parris Island	0.63	91	4	430	37
MCRD San Diego	1.91	93	1	589	6
CGTC Cape May	1.27	74	0	257	0
Total	0.88	787	15	3,714	14

*Cases/100 trainees/week.
†Among sampled FRI cases, actual number of cases is higher.

New and emerging pathogens are causing influenza-like illness with significant morbidity throughout the DoD. An outbreak of adenovirus 14 at a military training center in 2007 interrupted training at a time when the matriculation of trainees into the active duty pool was critical. In addition, respiratory diseases of unknown etiology (for example, eosinophilic pneumonia) have resulted in some deaths and serious morbidity among deployed troops in southwestern Asia. Although the correlation with new onset of smoking or an increase in smoking in the case of eosinophilic pneumonia is strong, the cause of these severe respiratory diseases remains unknown. Without knowing the cause, preventive measures cannot be instituted, and current treatment with steroids, although of short-term benefit, may not be optimal.

Respiratory disease burden threatens the military and the nation's strategic interests when it presents in epidemic and pandemic forms. The grave threat of avian influenza spreading among deployed troops in Iraq, where there have been three laboratory-confirmed cases (two deaths) in the civilian population, and subsequently spreading to the United States by returning troops warrants dramatic improvements in the ability to detect and respond to this pathogen both domestically and abroad. Agents such as human metapneumovirus and SARS-associated coronavirus are emerging pathogens that can cause severe influenza-like illness epidemics. Because of this need, the Defense Health Board Select Subcommittee for DoD Pandemic Influenza Preparedness recommended that the DoD should develop and sustain a pandemic and avian influenza research and development focus.

The CONUS DoD respiratory disease surveillance and laboratory network is based out of two locations: USAFSAM and the NHRC Respiratory Disease Research Department. USAFSAM conducts surveillance for influenza-like illness among military personnel through reporting MTFs throughout the world. NHRC primarily monitors febrile respiratory illness at basic recruit training sites, among Navy shipboard personnel, and in Mexican border populations. In addition, the five OCONUS laboratories have extensive respiratory disease surveillance networks in their areas of responsibility.

Despite the comprehensive surveillance conducted by these organizations, US citizens still live in locations that have limited surveillance for influenza and other respiratory diseases. Many individuals working outside the United States, who have unusual and high-risk exposures, periodically return but are not captured in the current DoD surveillance network. Two examples are foreign service workers on sparsely populated Pacific Islands with a high density of wild fowl who conduct scientific studies with live and dead fowl and US embassy personnel and deployed forces who come in frequent contact with local populations, including people from areas where influenza H5N1 and SARS have been identified. No systematic rigorous surveillance of the causes of respiratory disease exists in many of these populations.

To address these gaps in respiratory disease surveillance in the DoD, the WRAIR Division of Viral Diseases has established a diagnostic laboratory using GEIS funding. This developing capability is called the Emerging Infectious Diseases Research Unit. FY08 efforts have focused on the acquisition of the best technologies, hiring the appropriate personnel, and seeking the necessary training to establish a successful respiratory disease diagnostic laboratory. The unit has acquired three technology platforms: Roche 454 FLX Genome Sequencer, Luminex, and Affymetrix Genechip Array. These technologies along with real-time PCR and traditional Sanger sequencing will be used to augment current capabilities in the DoD for the detection and characterization of viral respiratory pathogens of military importance with an emphasis on influenza. The Roche 454 FLX Genome Sequencer uses pyrosequencing technology to sequence an entire genome in a few hours. Luminex makes an FDA-approved multiplex kit for respiratory pathogens, and TessArae makes a chip with several different respiratory pathogens to be run on the Affymetrix. A testing algorithm has been established for the determination of the pathogens, starting with the Luminex and ending with select pyrosequencing for unknown pathogens.

The Emerging Infectious Diseases Research Unit is a state-of-the-art, molecular-based viral respiratory disease diagnostic laboratory. The equipment, laboratory space, reagents, and personnel needed to perform the functions of the laboratory have been obtained. Collaborations with the Department of State have been established, and a project with several embassies will commence in FY09 once the protocol has been approved.

Establishment and expansion of respiratory surveillance capacity to include foreign service worker and embassy personnel will enhance the capability of DoD to detect and confirm cases of respiratory diseases of pandemic potential. In concert with the other CONUS and OCONUS respiratory surveillance programs and laboratories, emphasis on identification of unknowns and extensive characterization of identified pathogens with cutting-edge platforms will provide a valuable contribution to the GEIS network. This capability will facilitate DoD response planning and execution, leading to disease prevention and/or mitigation.

Australian Army Malaria Institute

In addition to its work with the WRAIR Division of Experimental Therapeutics in tracking antimalarial resistance in the South Pacific, the Australian Army Malaria Institute receives funding from GEIS for an innovative project regarding the 1918 influenza pandemic. Records of World War I Australian Army personnel are being examined to track the movement of the influenza virus. These data are expected to further current understanding of pandemic influenza and its behavior in deployed military forces. GEIS also funded a study, through the Army Medical Surveillance Agency, in which a subset of the 1918 Australian pandemic influenza data was used to evaluate cause of death among Australian Army members.

Knowledge of the mortality and morbidity risk factors in play during the 1918–1919 influenza pandemic can inform pandemic response planning today. Because the 1918–1919 pandemic predated relevant scientific advances, data about its effects on military populations

are limited. Fortunately, extensive records from the Australian Army during World War I have been preserved. With partial support from GEIS, the Australian War Memorial and the Australian National Archives have converted the medical and administrative records of the first Australian Imperial Force into electronic format, and they are accessible for study.

An FY07 analysis of the Australian mortality data demonstrated that hospitalization for an influenza-like illness earlier in 1918 significantly reduced mortality during the subsequent pandemic. A wide range of mortality occurred among infantry battalions during the influenza pandemic. The 49th and 50th Battalions of the 13th Brigade had particularly different mortality rates despite having moved together for the entire war and having received similar numbers of reinforcements (Tables 3 and 4).

Further study in FY08 of casualty records on hospitalizations caused by respiratory infections suggested that respiratory infections requiring hospitalization in early 1918 were caused by an H1N1-related influenza virus that protected against further infections between 1 September 1918 and 31 March 1919. The study also demonstrated that severe respiratory infections during the first wave of the pandemic (April–July 1918) protected soldiers against death but did not protect against infection during the subsequent pandemic. This counters a widely held view that the influenza-like illness in April–July 1918 was a relatively benign first wave of the pandemic. One plausible explanation for the findings in this study is that the earlier wave may have induced a neuraminidase antigen immunity that prevented death but did not result in a hemagglutinin-specific immunity to prevent subsequent infection. This type of protection has been postulated based on animal studies but is difficult to demonstrate in human populations.

Understanding how the 1918–1919 influenza pandemic evolved has practical public health implications. If a highly lethal but poorly infectious H1N1 virus adapts over months or years, then the strategy of rapidly producing a pandemic-specific influenza vaccine may be correct. If neuraminidase-induced immunity is important in preventing mortality, then current seasonal influenza vaccines should protect against any new pandemic virus with a similar neuraminidase, even if the hemagglutinin is distinctly different. Both situations may exist in the H5N1 virus now circulating in Asia.

Reconstructing Pandemic Influenza with Australian Army Records

War and disease often coexist in many places. This is particularly true regarding the 1918–1919 influenza pandemic that coincided with the end of World War I. Whether this coincidence was actually a causal relationship is unclear. Certainly no better system to generate a new respiratory virus can be imagined than millions of soldiers battling from filthy trenches, tens of thousands of wounded crammed into field hospitals, and unprecedented global human mobility and mixing. Because no one could anticipate the arrival of this greatest of recorded human mortality events, finding carefully collected relevant data that expands the DoD's understanding of the evolution of pandemic influenza is valuable.

Armies routinely collect repetitive administrative and medical information about their members. In the case of the Australian Army, records from 1918–1919 have been carefully stored and maintained and now are available digitally on-line. In an heroic data entry effort, GEIS-funded staff at the Centre for Military and Veteran's Health at the University of Queensland (Australia) are gradually piecing together the epidemiologic puzzle of the 1918–1919 pandemic. Their work is not unlike how the fragmented nucleic acid of the virus itself was eventually reconstructed from archived autopsy samples. Unlike the current understanding of the progression of the virus, the Australian Army records indicate that a highly lethal, but poorly infectious, virus circulated for years before acquiring the ability to efficiently spread among people. If this understanding is correct, then World War I would have had a nearly causal role in the evolution of the worst influenza pandemic recorded.

Courtesy of Australian War Memorial (awm.gov.au).

At cemetery near Maubegue, France, Australian Army soldiers carry coffin of comrade who embarked from Melbourne, Victoria, on 22 December 1917 and died of bronchopneumonia on 27 February 1919, aged 31.

Table 3. Incidence of Respiratory Illness or Influenza in 49th Infantry Battalion

Incidence	No.	Percent	Odds ratio	95% CI	*P*
Respiratory illness 1 Nov 1916–30 Apr 1917	217/ 343	16.2	—	—	—
Respiratory illness 1 Mar 1918–31 Jul 1918	200/1,343	14.9	—	—	—
Influenza 1 Sep 1918–31 Mar 1919	252/1,343	18.8	—	—	—
Influenza 1 Sep 1918–31 Mar 1919 Unprotected referent cohort*	170/967	17.6	1	—	—
Received medical care for respiratory illness winter 1917	47/217	21.7	1.28	0.89, 1.86	0.18
Received medical care for respiratory illness spring–summer 1918	45/200	22.5	1.16	0.80, 1.68	0.45

*Unprotected referent cohort, did not get medical care for respiratory illness in winter 1917 or spring–summer 1918.

Table 4. Incidence of Respiratory Illness or Influenza in 50th Infantry Battalion

Incidence	No.	Percent	Odds ratio	95% CI	*P*
Respiratory illness 1 Nov 1916–30 Apr 1917	186/1,235	15.1	—	—	—
Respiratory illness 1 Mar 1918–31 Jul 1918	222/1,235	18.0	—	—	—
Influenza 1 Sep 1918–31 Mar 1919	160/1,235	13.0	—	—	—
Influenza 1 Sep 1918–31 Mar 1919 Unprotected referent cohort*	101/865	11.7	1	—	—
Received medical care for respiratory illness winter 1917	21/186	11.3	0.93	0.56, 1.55	0.79
Received medical care for respiratory illness spring–summer 1918	42/222	18.9	1.38	0.92, 2.08	0.12

*Unprotected referent cohort, did not get medical care for respiratory illness in winter 1917 or spring–summer 1918.

United States Army Center for Health Promotion and Preventive Medicine

To better prepare military personnel to maintain operational readiness and mitigate the adverse impacts of a pandemic, the US Army Center for Health Promotion and Preventive Medicine (USACHPPM) and DoD-GEIS cohosted a pandemic influenza preconference workshop on 11 August 2008 at the 11th Annual Force Health Protection Conference in Albuquerque, New Mexico. The morning workshop was attended by 226 representatives from the Army, Navy, Air Force, Coast Guard, Public Health Service, Veterans Administration, educational institutions, and civilian public health organizations, and 90 attendees participated in an afternoon tabletop exercise.

Workshop presentations covered current pandemic response plans at the national and service-specific levels, the military/civilian interface at the national and local level, and pharmaceutical and nonpharmaceutical interventions during the public health response. Facilitators from CHPPM and DoD-GEIS designed the tabletop exercise to prepare military preventive medicine and public-health personnel to support, develop, and conduct installation-level tabletop exercises. Workshop participants identified several strengths and gaps in military installation pandemic response plans and discussed interventions and strategies, including coordination efforts between military installations and their surrounding civilian communities.

The following areas of progress were noted:

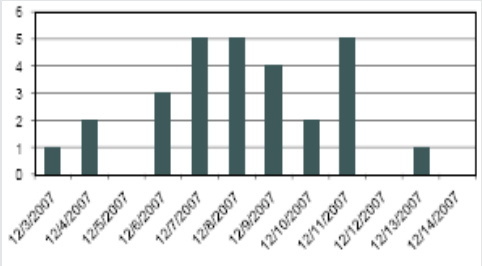
- Coordination with the installation community, local communities, and others;
- Development of plans in coordination with local health departments;
- Tabletop exercises;
- Planning on nonmedical and medical issues;
- Information sharing with the installations' surrounding communities.

Similar and follow-on exercises are planned for the future.

On 4–5 September 2008 DoD-GEIS and USACHPPM partnered to sponsor a deployment health surveillance workshop in Baltimore, Maryland, to examine and discuss difficulties experienced in performing timely and accurate health surveillance in a combat environments. This workshop was a follow-on to the July 2005 deployment health surveillance workshop in Landstuhl, Germany. Forty-eight representatives from the Army, Navy, Air Force, DoD, and CENTCOM attended the 2-day workshop consisting of structured presentations and group discussions. Discussion topics include the following:

Influenza A Outbreak at Fort Gordon Tests Surveillance System

In December 2007 an influenza outbreak occurred among active duty soldiers (n = 28) and dependents (n = 2) stationed at US Army Garrison-Fort Gordon (Georgia). Fort Gordon includes training facilities for advanced individual training in Signal Corps occupational specialties, and approximately 30,000 military and civilian employees are on post, making Fort Gordon one of the largest Army training facilities.



Influenza cases at Fort Gordon, 3–14 December 2007.

Specimens were collected from the patients and sent to USAFSAM. In January 2008, the USAFSAM laboratory received 30 influenza A isolates for molecular characterization, which revealed that the Fort Gordon strains were antigenically unique compared with other US strains previously sequenced by USAFSAM. These strains possessed a marker for adamantine resistance (S46N) and more closely resembled other strains isolated in the Pacific Rim region (e.g., South Korea and Guam). This appearance indicated possible importation. USAFSAM matched these results with Standard Ambulatory Data Record information, and results were forwarded to USACHPPM for a comprehensive follow-up epidemiologic investigation.

Because the isolate that caused this outbreak was different from others found in the United States and because the virus had a recognized drug resistance marker, all 30 cases were contacted for interview via email and telephone between April and May 2008 to obtain demographic, exposure, and impact information. The age of the active duty members averaged 19 years, and 67% (n = 20) were males and 33% (n = 10) females. The predominant symptoms among interviewed cases were fever (>90%), cough (88%), fatigue (88%), and congestion (71%). Most cases were hospitalized, and 88% self-reported an average of two bed days upon admission to Eisenhower Army Medical Center.

The laboratory characterization successfully identified an isolate that had antiviral resistance properties and was unique to the United States. This effort tested the system in the face of an outbreak caused by an isolate that could easily have been similar to that which could cause an influenza epidemic. The Fort Gordon outbreak demonstrates the impact of influenza on readiness and the benefit of a robust surveillance and response program.

- Evolution of theater medical information systems;
- Deployed medical records processing;
- Deployed occupational and environmental health surveillance systems;
- In-theater laboratory surveillance;
- Strategic mapping of disease and injury diagnoses;
- Ongoing DoD/service deployment health surveillance activities;
- Medical surveillance lessons learned.

Attendees at the 2008 workshop found that progress had occurred since the 2005 symposium and that many limitations and obstacles that hamper accurate and timely reporting and analyses of health outcomes continue and are not well understood. However, consistent with the findings of the 2005 symposium, participants suggested that a lack of standardization and predeployment training in deployed surveillance systems and reporting tools used in theater could be a contributing factor for the identified problems. The workshop facilitated much-needed dialogue, provided opportunities to build collaborative thinking, identified in-theater limitations, and formulated actionable recommendations. The most notable success included the naming of individuals from each service to champion the development and fielding of predeployment training programs.

USACHPPM-Europe

The US Army Center for Health Promotion and Preventive Medicine-Europe (USACHPPM-Europe) in conjunction with the Landstuhl Regional Medical Center (LRMC) continued to expand and enhance the GEIS-sponsored influenza awareness, surveillance, and reporting program begun in 2006–2007 in the EUCOM

area of responsibility. During the 2007–2008 influenza seasonal year USACHPPM-Europe worked closely with the LRMC Infectious Disease Laboratory on laboratory surveillance. This enhanced surveillance program increased respiratory virus sample submissions and reporting by three-fold (>2,000 specimens) over previous seasons.

Reporting of influenza virus activity to host country and US officials was streamlined and included periodic influenza reports reaching a wide audience of military, host country, and civilian personnel. Eight countries (37 medical treatment facilities) within the EUCOM area of responsibility participated in the surveillance and submitted specimens to LRMC for reference testing. Health care providers throughout Europe were asked to collect nasopharyngeal swabs from patients presenting with fever of >100.5°F/38°C plus cough and/or sore throat (DoD influenza-like illness case definition). Rapid antigen testing (BinaxNOW) was performed at the specimen collection location, and the samples were sent to LRMC for reference testing. The LRMC Microbiology Laboratory conducted more definitive testing including viral culture, real-time RT-PCR, strain subtyping, and genetic characterization. USACHPPM-Europe provided epidemiologic support for the project by compiling data and coordinating actions with USAFSAM to contribute to the DoD Global Influenza Surveillance Program. The 37 bases/installations within the area of responsibility submitted 2,029 specimens. Of the 2,015 specimens tested, 725 (36%) tested positive for any respiratory viruses, and 470 (23.3%) tested positive for influenza viruses. Influenza A was dominant among the viruses found (64.9%), which was consistent with reported results in Europe and the United States, and influenza A/H1N1 was found in most of the specimens tested. The development and updating of the EUCOM Pandemic Influenza Watchboard managed by USACHPPM-Europe continued in FY08 and provided pertinent information in response to users. This watchboard is a one-stop website for all pandemic, seasonal, and avian influenza information pertinent to the EUCOM area of responsibility. Information on this site addresses health education and risk communication, surveillance, plans and preparation, and the most current influenza-related information.

The Pandemic Influenza Surveillance and Education program, developed by USACHPPM-Europe, continued to provide pandemic influenza outreach, consultation, and training regarding plans and preparations, risk communication and health education, surveillance, and up-to-date information to all stakeholders. Training also included orientation to ESSENCE and the processing of influenza specimens.

The USACHPPM-Europe Epidemiology Division hosted the 4th EUCOM Public Health Emergency Officer Conference on 9–11 September 2008 with 65 attendees from the EUCOM area of responsibility. The EUCOM Joint Force Health Protection Working Group and lead public health emergency officers from each component developed the agenda. Content was geared toward educating installation public health emergency officers, installation medical emergency officers, and others regarding current pandemic influenza plans as well as their specific roles and responsibilities in executing installation pandemic influenza plans. Host country representatives attended the conference, and a breakout session was held to integrate regional planning considerations. As a capstone, GEIS Headquarters personnel led a tabletop exercise on the final day that gave attendees the opportunity to implement their new knowledge and build actionable planning efforts at their home stations in Germany, Italy, Spain, England, Turkey, and Portugal.

Significant accomplishments of USACHPPM-Europe in FY08 include the following:

- Increased influenza surveillance, analysis, and reporting by roughly three-fold with the processing of more than 2,000 clinical specimens;
- Increased coordination with host nation governments and militaries;
- Formed partnerships with LRMC and USAFSAM to ensure long-term program sustainment;
- Improved surveillance reporting to and from EUCOM, USAFSAM, and host nations;
- Provided pandemic influenza preparedness and response training to public health emergency officers

USACHPPM-Pacific

The US Army Center for Health Promotion and Preventive Medicine-Pacific (USACHPPM-Pacific), located at Camp Zama near Tokyo, Japan, is centrally positioned between Yokosuka Navy Base and Yokota Air Base. USACHPPM-Pacific maintains a BSL-2 disease surveillance laboratory and performs ELISA, real-time RT-PCR, electrochemiluminescence gels, and conventional PCR. This spacious, limited-access, climate-controlled laboratory is fully equipped with a −80ºC freezer, several HEPA filter biological safety hoods, 4 × 96 well gradient thermocycler, M1M Analyzer, RAPIDS, and a new ISO shelter. The laboratory is fully operational and actively engaged in processing infectious disease samples from the Pacific Theater including malaria, Japanese encephalitis virus, dengue virus, and ten leading biological warfare agents.

Camp Zama has been designated an MHS sentinel site for influenza surveillance. Historically, collected influenza-like illness specimens were not processed locally and were instead stored in an −80 ºC freezer for periodic shipment to USAFSAM for testing. The newly implemented influenza detection capability at CHPPM-Pacific now permits timely processing of samples locally and could augment Naval Hospital Yokosuka, the nearest DoD laboratory facility, with sample analysis during an influenza pandemic. CHPPM-Pacific is listed as a back-up capability to Naval Hospital Yokosuka during a surge response in the current US Forces Japan Pandemic Influenza Response Plan. CHPPM-Pacific furnished the laboratory with state-of-the-art equipment including QIAcube and Roche LightCycler 1.5 and 480 instruments and an automatic nucleic acid purification device (Figure 24).

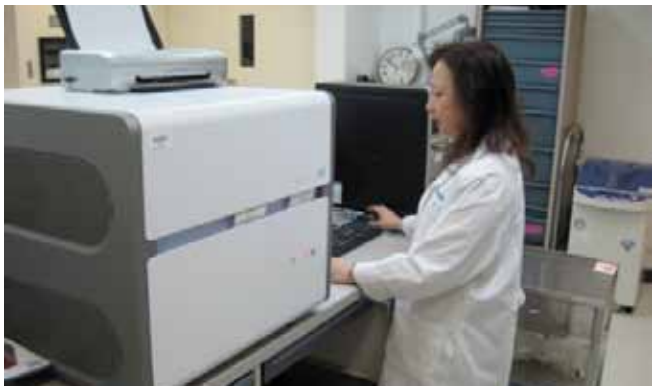


Figure 24. One-step universal influenza A/B detection and subtyping by Roche LightCycler 480.

With assistance of the AFRIMS Virology Department, laboratory personnel are trained in influenza detection, equipment operation, and maintenance. The laboratory adopted the CDC influenza protocol to assure streamlined and comparable detection and subtyping performance. Additionally, Naval Hospital Yokosuka trained laboratory personnel to use a shipboard respiratory surveillance protocol. The technicians obtained transportation biohazardous agent certification training for the international shipping of positive samples.

The newly implemented, nearly real-time influenza identification capability at CHPPM-Pacific serviced local medical treatment facilities and contributed to the development of seasonal influenza vaccines by submitting original specimens and isolates to CDC and WHO through the DoD influenza reference laboratories.

USACHPPM-West

The United States Army Center for Health Promotion and Preventive Medicine-West (USACHPPM-West) continued to expand joint disease surveillance programs in Central America during FY08. Priorities focused on improving the capability of local health agencies to identify and characterize pathogens that cause influenza, leishmaniasis, malaria, and rickettsial and enteric illnesses. Enhanced surveillance provided situational awareness to DoD regarding circulating strains of infectious diseases of interest and assisted healthcare personnel in partner countries make informed decisions concerning public health policy.

Surveillance and Detection

USACHPPM-West collaborated with the ministries of health in El Salvador, Honduras, Guatemala, and Nicaragua to expand existing disease surveillance programs in Central America. Other DoD laboratories at WRAIR and USAFSAM supported these efforts by performing specialized analytical testing. USACHPPM-West trained personnel in Central America on procedures for collection of samples from civilian patients with communicable diseases and performance of laboratory tests to identify pathogens of public health importance. Most collected specimens were submitted to either the USACHPPM-West or USAFSAM laboratory. A portion went to ministry of health laboratories. Highlights from the joint surveillance projects follow.

Respiratory illness surveillance. Routine respiratory illness submissions were received each month from Central America. A total of 727 respiratory specimens were submitted by Honduras (n = 375) and Guatemala (n = 352). This represents a significant increase over the 222 specimens submitted the previous season. Of the 727 specimens collected, 340 (46.7%) were received at USAFSAM for culture and further molecular characterization, and CHPPM-West received 387 (53.2%) (Table 5); 36% (n = 256) were identified as positive for a respiratory virus through culture and/or PCR. Specifically, 14.0% (102/721) were positive for an influenza virus. Ninety (88.2%) of the influenza isolates were type A: 49 were type A/H3, 39 were type A/H1. Most influenza A/H1 isolates (86.2%, n = 28) were collected from Honduras, whereas 76.1% (n = 35) of the influenza A/H3 isolates were collected from Guatemala. Of the 12 influenza B isolates identified, 33.3% (n = 4) were identified as influenza B/Shanghai; 9 of the 12 influenza B isolates were collected from Guatemala. USAFSAM characterized 45 influenza specimens (25 A/H1, 16 A/H3, and 4 B) and determined that the viral sequences were similar to those strains circulating globally and to those that had been included in the 2007–2008 Northern Hemisphere influenza vaccine.

Leishmaniasis surveillance. A total of 124 specimens were submitted from Guatemala, a significant increase in submissions over the 21 specimens submitted the previous season. Of the 124 specimens collected, 61.2%

($n = 76$) were positive for leishmaniasis using primers that targeted kinetoplast DNA. Of those positive, 39.5% ($n = 30$) were identified as *L. braziliensis*, and 25.0% ($n = 19$) were *L. mexicana*.

Febrile illness surveillance. Eighty-nine febrile illness specimens were submitted for dengue evaluation by sites in two countries: Guatemala ($n = 39$) and Honduras ($n = 50$). Five (5.6%) of the samples were positive for dengue viruses: dengue 1, one positive specimen; dengue 2, two positive specimens; and dengue 4, two positive specimens. USACHPPM-West submitted all five positive specimens to WRAIR for genotyping. WRAIR successfully genotyped three of the five specimens and determined that the dengue 2 specimens clustered in the American/Asian dengue 2 genotype (III) group and the dengue 4 specimen clustered in the dengue 4 genotype (II) group. Results of these genetic evaluations are similar to submissions from previous years and suggest that these viruses most likely possess the same antigenic characteristics as those previously detected in the region.

Response and Readiness

Although thousands of US military troops are deployed to Central America each year, the DoD has difficulty acquiring current disease surveillance data for the region given public health limitations in Central America. Regional public health agencies lack the resources to monitor communicable diseases, and the DoD overseas laboratories do not have a permanent presence in the region. The joint surveillance program managed at USACHPPM-West provides situational awareness of disease activity while maintaining close coordination with the ministries of health. In addition to reporting routinely through GEIS and DoD partner mechanisms, USACHPPM-West provides routine consultation to the Joint Task Force-Bravo team concerning readiness issues.

CHPPM-West achievements in FY08 include the following:

- Findings from the malaria surveillance conducted by CHPPM-West and WRAIR are similar to results published in 1999; in both studies the investigators successfully amplified the target chloroquine-sensitive gene in the *Plasmodium falciparum* parasites evaluated, suggesting that the current regimen used by the DoD for malaria prophylaxis is effective.
- USAFSAM detected adenovirus type 14 in four (30.7%) nasal swab specimens submitted by the Honduran ministry of health, through the coordination of CHPPM-West, and forwarded the specimens to NHRC for further genetic characterization. Adenovirus type 14 had not been identified in previous specimens from Central America.

Integration and Innovation

USACHPPM-West collaborated with CombiMatrix (Mukilteo, WA) to design and evaluate a field-deployable microarray system that genotypes influenza A viruses. The microarray chip contains multiple probes that correspond to each hemagglutinin and neuraminidase antigen subtype. The probe data compares the influenza A specimens with known reference strains and allows investigators to estimate the genetic similarity of unknown influenza A viruses with those used in influenza vaccines. The system is flexible, allows easy modification and synthesis of new microarray chips, and provides results within 8 hours.

Cooperation and Capacity Building

USACHPPM Subordinate Commands initiated efforts to develop an operational Public Health Surveillance Laboratory network. Participating USACHPPM Subordinate Command laboratories developed new testing capabilities to support DoD disease surveillance activities, respond to disease outbreaks, and perform epidemiological investigations. The participating laboratories form a network in which each provides unique analytical services. USACHPPM-South developed a surveillance laboratory to assume management of the Central American projects previously located at USACHPPM-West and undertook the molecular component for the Public Health Surveillance Laboratory network.

The new USACHPPM-South surveillance laboratory at Fort Sam Houston (Texas) will allow expansion of the Central American joint disease surveillance programs. The new facility will allow evaluation of new diagnostic technologies, maintenance of high throughput capabilities with rapid screening of respiratory viruses using real-time RT-PCR, and provision of more robust situational awareness reports to DoD and its partners including the Central American ministries of health and military governments.

USACHPPM-West routinely provided laboratory supplies and technical assistance to Central American ministries of health to increase surveillance and laboratory capacity. The CDC Regional Office for Central America and Panama standardized and guided training. These efforts resulted in a more accurate detection of respiratory viruses, as evidenced in the quality of specimen collection and shipments, as well as routine participation from sentinel sites.

Table 5. Laboratory-confirmed Data by Country

Result (PCR and/or culture)	Guatemala	Honduras	Total
Adenovirus, NOS	8	9	17
Adenovirus type 14	0	3	3
Adenovirus and parainfluenza	0	2	2
Adenovirus type 14 and RSV	0	1	1
Coronavirus	0	1	1
Enterovirus	0	1	1
Influenza A unsubtyped	0	2	2
Influenza A/H1	11	26	37
Influenza A/H1 and adenovirus	0	1	1
Influenza A/H1 and rhinovirus	0	1	1
Influenza A/H3	35	14	49
Influenza B unsubtyped	6	2	8
Influenza B /Shanghai	3	1	4
Parainfluenza	15	14	29
Parainfluenza and rhinovirus	0	1	1
Rhinovirus	0	22	22
RSV	21	42	63
RSV and rhinovirus	0	14	14
Quantity insufficient	4	2	6
No virus isolated	249	216	465
Total	**352**	**375**	**727**

Army Medical Surveillance Agency

The Army Medical Surveillance Agency became the Data Analysis Division in 2008 within the newly created Armed Forces Health Surveillance Center. The Data Analysis Division conducts medical surveillance of military members through oversight and management of the Defense Medical Surveillance System (DMSS) and the DoD Serum Repository. DMSS, DoD's premier epidemiological database, maintains a record of each servicemember's health-related information collected from accession through discharge. The DoD Serum Repository, the world's largest serum repository, contains serial specimens from servicemembers since 1985. Linkage of the data in the DMSS with the specimens in the repository provides the Data Analysis Division with an unrivaled ability to perform seroepidemiological studies. FY08 goals for the Data Analysis Division included improved serosurveillance systems for novel and emerging diseases, development and validation of seroepidemiologic studies, and employment of DMSS to evaluate pharmaceutical and vaccine efficacy.

DMSS provides customized, militarily relevant reports and allows drill-down queries to increase functional specificity in outbreak detection and investigations. DMSS enhancements improve surveillance for infectious diseases across DoD, with the deployment setting being a priority. These efforts required the acquisition of rapid access disk storage, servers, and the necessary supporting software for the archiving of additional data, transformation of the data into usable tables, and the creation of customized applications. Additionally, these refinements allow more authorized personnel to query the data and receive reports.

The Data Analysis Division's focus on pandemic and avian influenza uniquely leverages these specialized surveillance and seroepidemiology resources. The DMSS enhancements provide assessments of rates and patterns of influenza infection along with automated and ad hoc demographic and location reports.

The Data Analysis Division reprioritized the data sources integrated into DMSS. Deployed health encounter data have the highest priority: Standard Inpatient Data Record-III and Patient Accounting and Reporting Real-Time Tracking System data are received from the Patient Administration System and Biostatistics Activity, and US Transportation Command Regulating and Command and Control Evacuation System data are received from Transportation Command. The Data Analysis Division focuses on deployed data. The Armed Forces Health Surveillance Center also receives Health Level Seven laboratory and pharmacy files and has considered inclusion of additional data fields for personnel data and deployments within the data files provided by the Defense Manpower Data Center.

The Data Analysis Division specializes in the design and conduct of serological studies, surveillance of respiratory pathogens, and conduct of special studies. The Center of Excellence for Serosurveillance achieved full operating capability as a center of excellence during FY08 and has already made contributions to various special interest study areas including serology proof of concept, vaccine efficacy and adverse events, and avian influenza.

GEIS-supported achievements at the Data Analysis Division in FY08 follow:

H5N1 antibody seroprevalence among deployed servicemembers. No serologic evidence was found for H5N1 exposure among servicemembers in Indonesia or Vietnam, and little to no evidence of exposure to the virus was found in Thailand. These results indicate that mild subclinical H5N1 infections do not occur among servicemembers deployed to these areas.

Evidence of prior immunity against influenza among recruits. Investigation of prevaccination seasonal influenza seroprevalence among recruits found that whereas a third of recruits enter service with antibodies to both H1 and H3 influenza A viruses, nearly a quarter enter without preexisting antibody. This nonimmune recruit population could serve as a source for influenza outbreaks in the recruit setting.

Hepatitis E virus antibody seroprevalence among servicemembers deployed to Afghanistan. A low antibody seroprevalence was found before deployment (1.07%) and very low seroconversion during deployment (0.13%). This exposure study indicated that the risk for hepatitis E virus infection during Afghanistan deployments is low.

Evaluation of cause of death among Australian servicemembers during 1918 influenza pandemic. A cohort review of data from the Australian Army 1918 pandemic influenza study determined that most deaths occurred from secondary bacterial pneumonia. This GEIS-sponsored study supports increased stockpiling of antibiotics and indicates that more effective bacterial vaccines should be developed.

Evaluation of comparative efficacy of live attenuated influenza vaccine and trivalent inactivated vaccine. The study demonstrates that during periods of relatively good match between circulating influenza strains and vaccines, trivalent inactivated vaccine is at least as protective as or better than the live attenuated influenza vaccine at prevention of respiratory pathogens in non-recruits.

Navy and Marine Corps Public Health Center

Public Health Disease Surveillance

The Navy and Marine Corps Public Health Center (NMCPHC, formerly Navy Environmental Health Center) provides public health services for Navy activities and is the medical and deployment health surveillance hub of the Navy. NMCPHC contributes to surveillance through its work with Health Level Seven (HL7) electronic medical data, a national standard messaging format used by health care systems to exchange data. HL7 data from the Composite Health Care System includes patient encounter information such as clinic appointments, pharmacy orders and fills, radiology examinations, laboratory test orders and results, and inpatient admissions and discharges. NMCPHC developed tools to abstract and categorize the information into a searchable database.

During 2008, NMCPHC focused on influenza case identification using laboratory and pharmacy data, tracking of antibiotic-resistant microorganisms, and an estimate of the burden of sexually transmitted diseases. Other activities included the following:

- Case identification for malaria, West Nile virus, tuberculosis, syphilis, shigellosis, salmonellosis, mumps, meningitis, measles, Lyme disease, leishmaniasis, lead poisoning, hepatitis B, heat injuries, cold injuries, and dengue;
- Integration of medical event reports with confirmed laboratory diagnoses;
- Cancer case validation through pathology reports;
- Case finding for ongoing research projects at Eastern Virginia Medical School (Norfolk, VA) and USUHS.

DoD Influenza Surveillance

NMCPHC created reports for seasonal influenza surveillance linking laboratory-confirmed cases with outpatient pharmacy HL7 databases. The linkage of laboratory, pharmacy, and both inpatient and outpatient encounters created a more comprehensive record of all clinical transactions. Data analysis demonstrated that the number of influenza tests performed during the

2007–2008 season increased and that a large proportion of the positive results was found in children. The rate of infection for active duty Navy personnel was lower than civilian rates, probably a reflection of the DoD mandatory vaccination program. These results were conveyed in weekly influenza reports and distributed to Navy public health professionals.

Antibiotic-resistant Organisms

NMCPHC goals are to develop data delivery, display, and analysis tools designed to reduce the burden of reporting for the MTF and to provide pertinent data on antibiotic-resistant organism trends for public health surveillance. The activities utilizing the HL7 microbiology database are examples of this product development in which the results of the antibiotic resistance testing are included. NMCPHC compared the information found in the HL7 database with MTF-generated antibiograms and found good agreement between the two processes. As such, the database has become a primary source for public health surveillance. Another significant advance in antibiotic-resistant organism surveillance was an improved code that organized the HL7 data for use in the WHO software program, WHONET, to identify and track antibiotic-resistant organisms.

NMCPHC used deployment data from the Defense Manpower Data Center contingency file to compare antibiograms for infections of deployed and nondeployed personnel. Deployment- and postdeployment-related infections, especially respiratory or wound and abscess infections, were more likely to be associated with *Acinetobacter baumannii* complex, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, whereas infections unrelated to deployment were more likely to be associated with *Staphylococcus aureus*.

Estimation of Burden for Sexually Transmitted Diseases

NMCPHC completed an estimate of the number of cases of chlamydia, gonorrhea, and syphilis in the DoD using a capture-recapture method to determine the risk of sexually transmitted disease in the military and its possible impact on readiness. By using data from HL7 laboratory databases, DoD Medical Event Reports from the Defense Medical Surveillance System, and medical encounter records from Composite Health Care System, the capture-recapture methodology established case definitions in all data sources, extracted cases, and then aligned the sources. The capture-recapture technique estimated that approximately 154 (95% confidence interval 147.6, 160.4) cases of syphilis were not identified by any source. Chlamydia and gonorrhea are poor candidates for disease burden estimations using this methodology because these cases are overwhelmingly represented in the HL7 laboratory database but not in the clinical encounter reports. The model proved unstable for these diseases because of low reporting rates through the medical event reporting systems or improperly coded or undocumented medical encounters rather than because of a problem with HL7.

Investigations of Tick-borne Disease

NMCPHC conducted surveillance of arthropod-transmitted diseases. Lyme disease and Rocky Mountain spotted fever are reportable medical events for Navy and Marine Corps personnel. Rates for reported cases of both diseases at Camp Lejeune (North Carolina) and Marine Corps Recruit Depot (Parris Island, SC) are much higher than rates in the general population in North Carolina. To ascertain the significance of this finding, a project was initiated to identify the species of ticks present and the human pathogens infecting each species at these locations. This information will be used to improve clinical recognition and treatment of tick-borne diseases and to improve disease surveillance reports.

Navy Environmental Preventive Medicine Unit Two

Navy Environmental Preventive Medicine Unit 2 (NEPMU-2) in Norfolk, Virginia, performed operations in CENTCOM and new initiatives in AFRICOM during FY08. The primary objectives of the NEPMU-2 influenza work were to support USAFSAM in increasing the number of influenza sentinel sites and to increase febrile respiratory illness surveillance specimen submissions from deployed locations and overseas hospitals within AFRICOM, EUCOM, and CENTCOM. NEPMU-2 also partnered with the NHRC shipboard respiratory illness program to serve as its operational liaison for amphibious ships and aircraft carriers stationed on the East Coast.

NEPMU-2 visited EUCOM Navy medical hospitals in Sigonella and Naples, Italy, and Rota, Spain, to conduct follow-up training in influenza surveillance and performance of protocols for specimen submissions by sentinel site personnel. NEPMU-2 also established or maintained influenza training and surveillance sites in the Middle East during two 6-month deployments of the Forward Deployed Preventive Medicine Unit East to Kuwait. Working with NAMRU-3, AFRICOM, and Naval Forces Europe, NEPMU-2 staff evaluated new sites in Ghana, Liberia, Cote d'Ivoire, Burkina Faso, Djibouti, and Cameroon. NEPMU-2 also enhanced its partnership

with NAMRU-3 by sending personnel to gain experience in avian influenza laboratory processing techniques and protocols and assisting NAMRU-3 in the December 2007 investigation of human-to-human avian influenza transmission in Pakistan.

Navy Environmental and Preventive Medicine Unit Six

During 2008, the Navy Environmental and Preventive Medicine Unit 6 (NEPMU-6) based in Pearl Harbor, Hawaii, supported febrile respiratory illness surveillance efforts in the western Pacific. Staff visited Singapore, Palau, Guam, and Saipan to identify and resolve surveillance problems and maintain organizational knowledge across staff redeployments. Travel to these sentinel sites identified challenges involved in the maintenance of corporate knowledge and the transport of specimens. These concerns were addressed via additional training and site visits. Furthermore, the availability of subject matter experts facilitated the review of parasitology laboratory methods, biological and fume hood certification, and aspects of laboratory response network capacities pertinent to H5 influenza surveillance.

Staff collaborated with local public health service representatives to evaluate the utility of infrared cameras as mass screening devices during periods of febrile disease activity. NEPMU-6 staff also completed an evaluation of respiratory illness rates in all Navy ships and MTFs for the 18-month period commencing January 2006.

Brooke Army Medical Center

Brooke Army Medical Center (BAMC) continued to contribute to the DoD-GEIS network with its work in leptospirosis, multidrug-resistant bacterial pathogens, and respiratory viruses. As one of the busiest military medical centers and the home of the US Army Institute of Surgical Research with its burn center, BAMC is vital in the treatment of wounded servicemembers and the large local military and beneficiary population. In addition, BAMC evaluates and tracks local infection patterns.

During FY08, BAMC continued to develop a repository of leptospirosis isolates from around the world and to characterize those isolates by genetic fingerprinting using PFGE and antimicrobial resistance patterns. BAMC has worked closely with the overseas DoD research laboratories in Peru, Egypt, and Thailand and in collaboration with the CDC. BAMC is continuing to evaluate new and to optimize current PCR primers on various platforms to improve the accurate and timely diagnosis of acute leptospirosis. These diagnostic technologies offer the potential to better study the global epidemiology of leptospirosis.

Since the beginning of Operation Iraqi Freedom and Operation Enduring Freedom, combat-related injuries have developed infections with multidrug-resistant bacteria such as *Acinetobacter baumannii-calcoaceticus* complex, ESBL-producing *Klebsiella pneumoniae*, and methicillin-resistant *Staphylococcus aureus*. The source of Gram-negative multidrug-resistant bacteria has not been clearly elucidated. However, *A. baumannii-calcoaceticus* complex appears to be primarily associated with nosocomial transmission in and out of the combat zone. The source of methicillin-resistant *S. aureus* is likely multifactorial, reflecting colonization of the casualty, inoculation of the bacteria into the wound at the time of injury, or nosocomial transmission. These resistant bacteria affect warrior care and the care of patients not associated with combat who are being managed within DoD inpatient and outpatient MTFs.

The multidrug-resistant molecular epidemiology laboratory at BAMC, in collaboration with partners, studies the epidemiology of multidrug-resistant bacteria to better understand these bacteria and their spread and to recommend and support infection control efforts. FY08 studies and investigations building upon existing technologies and infrastructure at BAMC resulted in the following:

- Improved patient care;
- Directed ongoing infection control and outbreak investigations;
- Comparable results from PFGE typing of bacteria.

Wound Infections Confronted throughout Military Health System

In the early stages of Operation Iraqi Freedom in 2003, military infectious disease physicians noted a high proportion of casualties returning from theater with wound infections caused by the soil bacterium *Acinetobacter baumannii*. These infections were spread through the MHS, including the USNS *Comfort* and military treatment facilities that received wounded from Operation Iraqi Freedom. Soon, physicians observed that patients other than those involved in combat operations were also acquiring *A. baumannii* infections.

The Army Surgeon General's office initiated an Epidemiological Consultation by USACHPPM with laboratory support from the WRAIR Department of Bacterial Diseases. This consultation revealed that *A. baumannii* isolates from wounded servicemembers were genetically similar to isolates recovered from environmental sampling at combat support hospitals in Iraq, suggesting nosocomial infection in theater leading to spread into the MHS during evacuation. These results led to the implementation of preventive measures at MHS facilities, such as isolation and screening of new patients admitted from theater. The new isolation and screening protocols have reduced the number of infections associated with noncombatant *A. baumannii* within the MHS.

***A. baumannii* infections can be acquired in combat areas and spread to hospitals**

While wound infections with *A. baumannii* continue, the MHS faces a growing need to control rising numbers of multidrug-resistant organisms reported by military treatment facilities. Multidrug-resistant organisms cause increased morbidity in servicemembers and lead to increased health care costs given the prolonged hospitalizations and use of expensive antibiotics required to treat the infections.

DoD-GEIS recognized the need to adopt a systems approach to MHS-wide health risks such as *Acinetobacter* and multidrug-resistant organism wound infections, both of which occur at multiple sites throughout the MHS. Consequently, GEIS is funding Walter Reed Army Medical Center, National Naval Medical Center, Landstuhl Regional Medical Center, and Brooke Army Medical Center, the principal medical centers receiving wounded from Operation Iraqi Freedom, in addition to the Department of Bacterial Diseases at WRAIR to standardize genetic characterization protocols and regularly share and pool data on these infections. DoD epidemiologists then analyze these data and disseminate the pertinent results about current trends of *Acinetobacter* and multidrug-resistant organisms wound infections throughout the MHS.

In addition, GEIS funded an FY08 project at Forward Operating Base Delta in Iraq to collect environmental samples during construction of a new surgical hospital on the base. These samples were processed for multidrug-resistant organisms to better ascertain their origin, with the goal of facilitating measures to first control and then stop these infections.

Landstuhl Regional Medical Center

The Landstuhl Regional Medical Center (LRMC) Infectious Disease Laboratory in conjunction with USACHPPM-Europe operates a robust surveillance and reporting program for influenza and other respiratory virus throughout the EUCOM and CENTCOM areas of responsibility. In FY08 LRMC enhanced its surveillance capabilities by establishing new surveillance sites and expanding to include several infectious diseases in addition to influenza. This increased the overall number of sample submissions and permitted a more efficient use of established program assets. Through its strengthened surveillance infrastructure, LRMC is now prepared to participate in the early identification of prepandemic influenza outbreaks and, subsequently, in rapid control and surge responses.

The LRMC surveillance sites throughout Europe collect nasopharyngeal swabs from patients presenting with symptoms consistent with the DoD influenza-like illness case definition using BinaxNOW Rapid Flu antigen testing and a viral culture. Starting in May 2008, all respiratory specimens from influenza-like illness cases were automatically analyzed by real-time PCR and/or the Luminex Respiratory Virus Panel. The Luminex panel allowed the testing of 19 respiratory viruses including types A (H1, H3) and B influenzas

During FY08 LRMC received 2,120 specimens from 40 individual surveillance sites. This represents a 12% increase in the number of specimens and an 11% increase in the number of submitting sites over FY07.

Acinetobacter infections have a major impact on hospital operations, and LRMC continued to establish a two-tiered *Acinetobacter* surveillance and control program. The first tier focuses on the rapid identification of nosocomial outbreaks through an improved integration of LRMC surveillance efforts into the hospital's infection control program. The second tier helps determine the sources of *Acinetobacter* infection and resistance patterns by tracking colonized Operation Iraqi Freedom and Operation Enduring Freedom patients.

All incoming patients from Operation Iraqi Freedom, Operation Enduring Freedom, and Africa are placed under contact precautions and tested for colonization with *Acinetobacter* at the time of hospital admission. LRMC adopted the practice of conducting microbial genotyping of all detected *Acinetobacter* isolates using two platforms: PFGE and repetitive-element PCR analysis. LRMC is comparing the accuracy and effectiveness of the two platforms and will use the results in future acquisition decisions. PFGE permits the comparison of genotyped strains with other isolates from DoD medical centers such as WRAMC, BAMC, and NNMC.

LRMC uses a relatively new system, Bacterial Barcodes, to rapidly identify the causative organism of outbreaks, track contamination sources, and determine antimicrobial resistance patterns. Testing with this automated bacterial strain typing system is performed according to the Clinical Laboratory Standards Institute Standard M2-A7. Using Bacterial Barcodes, LRMC compared FY08 *A. baumannii* resistance patterns with those from 2003–2007. The preliminary results suggest that a diverse population of *A. baumannii* strains has been isolated at LRMC over the years, although 73% of the isolates can be placed into one of four genotypes. Results suggest that no large unique strain of *Acinetobacter* has contributed to the nosocomial transmission over the course of surveillance.

65th Medical Brigade

The 65th Medical Brigade (formerly 18th Medical Command) partnered with GEIS and CHPPM to provide threat assessments and surveillance on selected vector-borne and infectious diseases in the Republic of Korea. These data will be used to identify vector populations and their distributions, prevalence of human and zoonotic infections in arthropods and small mammals, and dynamics of seasonal distribution of disease potential on the Korean peninsula. Disease surveillance involves routine coordination with the Korea National Institute of Health, the Korea Centers for Disease Control and Prevention, Korean universities, and US agencies.

Influenza Surveillance

The 65th Medical Brigade and US Army Medical Department Activity-Korea submits influenza-like illness specimens for testing to USAFSAM. Specimens come from select US MTFs and medical clinics throughout South Korea. In FY08, upgraded laboratory equipment

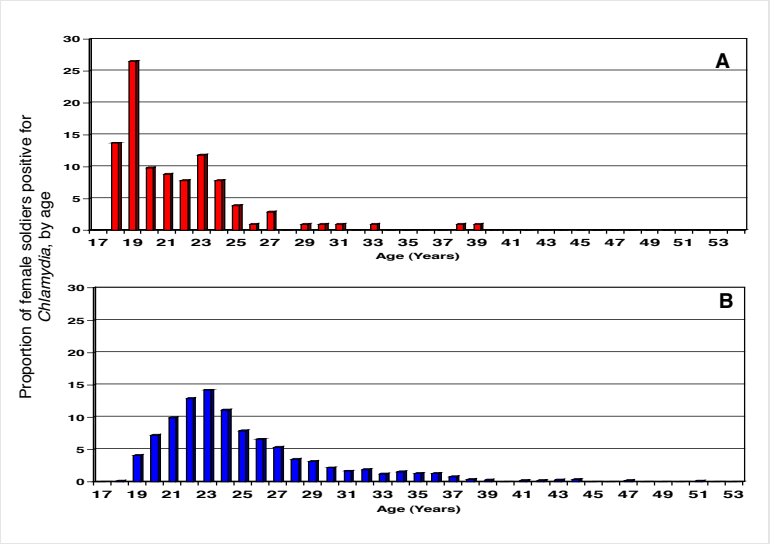
Surveillance for Sexually Transmitted Infections in Korea

Chlamydia is the most frequently reported and prevalent sexually transmitted disease among US civilian and military populations. Among US females, *Chlamydia trachomatis* infection rates ranged from 4 to 14%, with 4 million new cases reported annually. In females, both symptomatic and asymptomatic (estimated at 70% of all sexually transmitted infections in females) *C. trachomatis* infections may result in ectopic pregnancy, pelvic inflammatory disease, and chronic reproductive complications including infertility.

To reduce these severe and sometimes life-threatening complications, Headquarters and the 65th Medical Brigade established a screening program in FY08 in Korea in which all female Eighth United States Army soldiers are tested for *C. trachomatis* infection and gonorrhea during inprocessing. Because ~50% of all clinical infections reportedly result from soldier-to-soldier transmission, ~10% of all male Eighth United States Army soldiers were voluntarily screened for *C. trachomatis* infection in FY08 to access transmission among US soldiers deployed in Korea.

Since November 2007, 1,592 female and 1,687 male Eighth United States Army soldiers at the First Recruitment Center, Yongsan Garrison, Korea, were tested during inprocessing using Gen-Probe urine kits for *C. trachomatis* infection. Of those screened, 6.4% of females and 5.6% of males were positive. Female soldiers aged 17–25 years had a higher risk (9.7%) than those aged 25–53 years (1.6%). Seven asymptomatic female soldiers were also positive for gonorrhea, four of whom were also positive for *C. trachomatis* infection. Follow-up of positive *C. trachomatis* cases revealed one patient infected with syphilis.

Screening all female soldiers in FY08 resulted in earlier detection of *C. trachomatis* infection, because the mean age at diagnosis among females during inprocessing was 22.8 + 0.4 compared with 25.9 + 0.4 for clinical examinations. Screening female and male soldiers during inprocessing will facilitate early identification and treatment of sexually transmitted infections, reduce the impact of serious complications in female soldiers, and contribute to awareness of sexually transmitted disease for all inprocessing soldiers.



Proportion of female Eighth US Army soldiers positive for *C. trachomatis* by age at inprocessing, November 2007–September 2008 (A) and at clinical (well women and symptomatic) examinations, January–September 2008 (B).

was acquired, including freezers, enabling local influenza specimen collection, virus identification and characterization, and the provision of timely responses to medical providers working in Korea.

Chlamydia Surveillance

As of November 2007, the 65th Medical Brigade implemented Gen-Probe testing for *Chlamydia trachomatis* infection and gonorrhea for all female Eighth United States Army female soldiers (age 17–53 years) during inprocessing to Korea. Follow-up investigations of cases positive for C. *trachomatis* and gonorrhea included testing for syphilis. Results demonstrated that 6.4% of all female soldiers aged 18–39 years were positive for C. *trachomatis* and that female soldiers aged 18–19 years accounted for the highest proportion of all C. *trachomatis* infections (13.6–26.5%). Additionally, C. *trachomatis* screening

detected infections in 5.6% of volunteer male soldiers during inprocessing to Korea. Because *C. trachomatis* infection can cause serious problems among females with long-term asymptomatic infections, early diagnosis and intervention are critical to reducing complications, particularly the risk of infertility and pregnancy complications. As a result of this investigation, the 65th Medical Brigade began testing all inprocessing Eighth United States Army female soldiers in FY08.

Mosquito-borne Disease Surveillance

Malaria

The 65th Medical Brigade continued its efforts to characterize the distribution of anopheline mosquitos throughout Korea and assess the threat of mosquito-borne disease transmission. During 2007, >15,000 *Anopheles* larvae were collected from three widely dispersed selected locations at Warrior Base (near the demilitarized zone), Cheongseong, and Hayang (Figure 25). Because of the backlog, these mosquitoes were assayed during 2008–2009. Overall, adult mosquito populations were low in June (2.4%), peaked in July and August (45.7% and 31.1%, respectively), and decreased in October (1.5%). The epidemiology of malaria in Korea indicates that *A. pullus* and *A. kleini* are the primary vectors and that their low population densities south of training sites contribute to reduced transmission risk south of Seoul. This work supports the development of malaria risk and mosquito distribution models. Larval overwintering by *A. lindesayi japonicus* was identified.

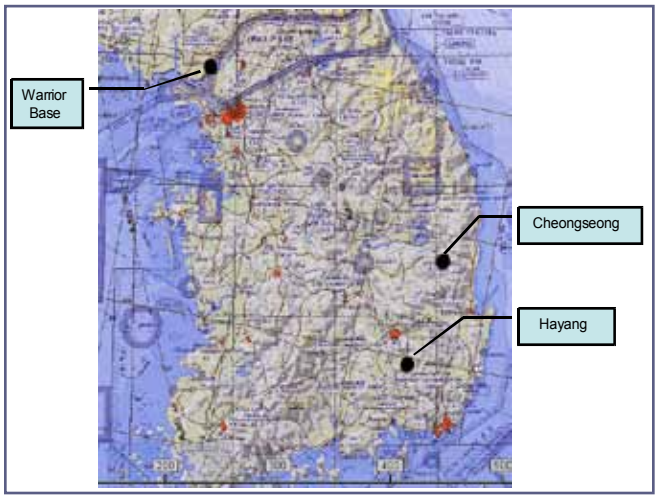


Figure 25. Primary mosquito larval collection sites in South Korea during 2007.

Japanese Encephalitis

Japanese encephalitis is carried and transmitted in Korea by *Culex tritaeniorhynchus*. Distribution models of *C. tritaeniorhynchus* were made after these mosquitoes were captured during routine *Anopheles* mosquito trapping near the demilitarized zone. *C. tritaeniorhynchus* populations peaked early in August, and populations were relatively low in September. These mosquitoes were sent to AFRIMS for assay by PCR for Japanese encephalitis virus.

Rodent-borne Disease Surveillance

During FY08, serosurveillance of small mammals and rodents for Hantavirus, scrub typhus, murine typhus, and leptospirosis was conducted at Camp Humphreys, Yongsan Army Garrison, Nightmare Range, Chipori Range, Osan Air Base, and Gunsan Air Base (Table 6). Overall, 1,373 striped field mice (*Apodemus agrarius*) were captured, of which 93 (6.8%) were positive for Hantavirus. A total of 748 *A. agrarius* were captured at Chipori and Nightmare Ranges with 9.4% ($n = 70$) seropositive for Hantavirus (Table 7). Overall, 638 *A. agrarius* were captured at Camp Humphreys with 3.4% ($n = 20$) seropositive for Hantavirus (Table 8). The surveillance program also identified a new Hantavirus, Imjin virus, carried by the insectivore rodent *Crocidura laciura*.

A total of 249 human serum samples were obtained from the Microbiology Department, School of Medicine, Seoul National University, and analyzed by indirect fluorescent antibody test and nested PCR for selected rickettsial pathogens. A summary of sera samples with IgG titers 1:320 or greater demonstrated that seven (2.8%) were seropositive for scrub typhus, and four (1.6%), five (2.0%), and two (0.8%) were seropositive for *Rickettsia japonica*, *R. sibirica*, and *R. typhi*, respectively. Six from 25 sera that had low antibody titers (IgM) (1:40, 1:80, or 1:160) were found to be positive for rickettsial pathogens by PCR. Sequence analysis further demonstrated the presence of *R. conorii* ($n = 1$), *R. japonica* ($n = 3$), and *R. monacensis* ($n = 2$). One of 11 sera that were seropositive for scrub typhus group *Rickettsia* was positive by PCR for *Orientia tsutsugamushi* (Boryong strain). *R. typhi* was not detected by PCR. These ongoing studies will include rodent tissues from the rodent-borne disease surveillance program and characterization of rickettsial and other infectious agents.

Table 6. Results of Hantavirus and Other Rodent-borne Disease Surveillance at Chipori Range, Nightmare Range, Camp Humphreys, Osan Air Base, and Gunsan Air Base, October 2007–September 2008

Species	Seropositive rate (%)				RT-PCR for Hantavirus
	Hantavirus	Scrub typhus	Murine typhus	Leptospirosis	
Apodemus agrarius	93/1,373 (6.8)	161/1,373 (11.7%)	30/1,373 (2.2)	29/1,206 (2.4)	61/93 (65.6)
Apodemus peninsulae	0/3	1/3 (33.3)	0/3	0/3	—
Crocidura laciura	0/76	0/76	1/76 (1.3%)	1/73 (1.4%)	—
Myodes regulus	0/22	1/22 (4.5)	0/22	0/22	—
Microtus fortis	0/26	1/26 (3.8)	0/26	0/15	—
Micromys minutus	2/29 (6.9)	1/29 (3.4)	0/29	2/27 (7.4%)	0/2
Mus musculus	0/4	1/4 (25.0)	0/4	0/4	—
Total	95/1,533 (6.2)	166/1,533 (10.8)	31/1,533 (2.3)	32/1,350 (2.4)	61/95 (64.2)

Table 7. Results of Hantavirus and Other Rodent-borne Disease Surveillance at Chipori and Nightmare Ranges near Demilitarized Zone, October 2007–September 2008

Species	Seropositive rate (%)				RT-PCR for Hantavirus
	Hantavirus	Scrub typhus	Murine typhus	Leptospirosis	
Apodemus agrarius	70/748 (9.4%)	136/748 (18.2%)	29/748 (3.9%)	11/721 (1.5%)	52/70 (74.3%)
Apodemus peninsulae	0/3	1/3 (33.3%)	0/3	0/3	—
Crocidura laciura	0/62	0/62	1/62 (1.6%)	1/73 (1.4%)	—
Myodes regulus	0/22	1/22 (4.5%)	0/22	0/22	—
Microtus fortis	0/3	0/3	0/3	0/3	—
Micromys minutus	1/16 (6.3%)	1/16 (6.3%)	0/16	2/14 (14.3%)	0/1
Mus musculus	0/4	1/4 (25%)	0/4	0/4	—
Total	71/858 (8.3%)	140/858 (16.3%)	30/858 (3.5%)	14/827 (1.7%)	52/71 (73.2%)

Table 8. Results of Hantavirus and Other Rodent-borne Disease Surveillance at Camp Humphreys, October 2007–September 2008

Species	Seropositive rate (%)				RT-PCR for Hantavirus
	Hantavirus	Scrub typhus	Murine typhus	Leptospirosis	
Apodemus agrarius	20/590 (3.4%)	20/590 (3.4%)	1/590 (0.2%)	15/450 (3.3%)	7/20 (35.0%)
Crocidura laciura	0/13	0/13	0/13	0/12	—
Micromys minutus	1/12 (8.3%)	0/12	0/12	0/12	0/1
Microtus fortis	0/23	1/23 (4.3%)	0/23	0/12	—
Total	21/638 (3.3%)	21/638 (3.3%)	1/638 (0.2%)	15/486 (3.1%)	7/21 (33.3%)

Figure 26. Collection sites for *I. phasiana* in Jeollanam Province and Cheju Island, 2007.

Tick-borne Disease

In collaboration with the Seoul National University and Korea National Institute of Health, the 65th Medical Brigade identified a tick-borne encephalitis virus from two species of ticks collected on Cheju Island. Tick collections were conducted during FY07–FY08 in the southern area of the Republic of Korea, Cheju Island, and near the demilitarized zone. *Ixodes phasiana*, first identified in Japan, was first reported in Korea during this survey (Figure 26). In addition, five individual/pools of ticks, *Haemaphysalis flava* ($n = 1$, Korea National Institute of Health, collection site 25) and *H. longicornis* ($n = 4$, Seoul National University) were positive for tick-borne encephalitis by PCR. Limited numbers of an infrequently collected tick, *H. pomerazevi*, previously reported only from US military collections throughout much of Korea, were collected from rodents at Nightmare Range (elevation ~500 m) near the demilitarized zone.

Tick-borne Encephalitis Surveillance Protecting US Military in Korea

Sites on Cheju Island where ticks *(H. flava and H. longicornis)* carrying encephalitis virus were trapped *(red circles)* and where tick-borne encephalitis was identified *(stars)*.

Tick-borne encephalitis is an emerging disease in Korea and has the potential to be a health threat for US servicemembers and their dependents. Information regarding the epidemiology and vectors of the causative agent, tick-borne encephalitis virus, in Korea is scarce, and the impact of the changing ecology on faunal distributions and relative abundance of the virus is unknown. Nevertheless, this information is critical for understanding the potential for tick-borne encephalitis virus infections in US military personnel.

The 65th Medical Brigade collected ticks during 2007 to begin to fill these gaps in knowledge. Collections along the southwestern tip of Korea and on Cheju Island resulted in the identification of a tick species, *Ixodes phasiana*, previously not found in Korea. Another infrequently collected tick in Korea, *Haemaphysalis pomerazevi,* was collected near the demilitarized zone from the striped field mouse *Apodemus agrarius.* In addition, assay of ticks from Cheju Island resulted in five tick-borne encephalitis virus-positive pools of ticks: one pool of *H. flava* and four pools of *H. longicornis.* Findings from this investigation improve the military's capacity to determine where tick-borne encephalitis infections can be expected in Korea and to take appropriate preventive measures.

Engorged nymph and adult H. pomerazevi *(top)* and female ovipositing *(bottom).*

Attempts to collect this tick by tick drag were unsuccessful. *H. pomerazevi* was only collected February–May from rodents. Most collected ticks are awaiting assay at Seoul National University and Konkuk University.

This and previous reports suggest that this tick only occurs at higher elevations in Korea, because none were collected at elevations of <50 m (>6,000 small mammals surveyed).

Uniformed Services University of the Health Sciences

The Uniformed Services University of the Health Sciences (USUHS) conducts tropical medicine training for uniformed personnel through an agreement with DoD-GEIS. The training serves the educational needs of medical students, residents, infectious disease fellows, master and doctoral candidates, and junior staff in all uniformed services. During FY08, 53 officers benefited from this program, an increase of 19 from FY07. Personnel from three services received training in 15 countries including rounds at the five DoD overseas laboratories. Most trainees (72%) were physicians with an interest in tropical medicine and/or public health or with working in the OCONUS laboratories. Most of the remaining participants were USUHS medical students who expressed an interest in pursuing a career in infectious disease or preventive medicine.

The following examples of trainee experiences illustrate the breadth of education offered by the USUHS-GEIS program. A PhD candidate worked with the CDC in Puerto Rico studying *Aedes aegypti* breeding sites. Of three USUHS Master of Tropical Medicine and Hygiene candidates in the program, one used geographic information systems mapping and DNA analysis to determine the regional prevalence of a known Babesia strain and attempted to ascertain the presence of any other strains in Thailand; the second participated in a diarrheal outbreak investigation on a Navy ship in Peru; and the third gained clinical experience treating patients with various tropical diseases in the Dominican Republic, learned about dengue and malaria surveillance, and assisted in an outbreak investigation. Other physicians trained through this program included family practice residents in a structured Graduate Medical Education program in Honduras,

two infectious disease fellows in the NAMRU-3 laboratory, and a dermatology resident in Guyana.

As in past years, second year medical students participated in a training rotation at NMRCD in Peru. During this structured rotation, eight students learned about various surveillance and laboratory research projects, assisted entomology technicians in the collection of adult and larvae mosquitoes, saw hospitalized patients with various tropical diseases, and experienced the challenges of practicing medicine in a developing country by working with board-certified physicians during a Medical Readiness Training Exercise. Additionally, seven fourth-year medical students participated in various other overseas training opportunities.

Center for Disaster and Humanitarian Assistance Medicine

The Center for Disaster and Humanitarian Assistance Medicine (CDHAM) maintains and builds relationships within the DoD and with other organizations active in disease surveillance and response. CDHAM assists the COCOMs in development, planning, and execution of education and training programs for pandemic and avian influenza surveillance and response.

CDHAM expanded its COCOM support role in FY07 to include surveillance and response training for emerging infectious diseases other than pandemic and avian influenza. As a result, in FY08 CDHAM developed a program that assessed the civilian-military preparedness for emerging infections outbreaks within the COCOM area of responsibilities. Additionally, CDHAM initiated a needs assessment for pandemic and avian influenza surveillance and response activities. A CDHAM emerging infectious disease liaison was placed at Headquarters to strengthen communications among CDHAM, Headquarters, and the COCOM surgeons and to serve as the project manager for the emerging infections needs assessments.

CDHAM supported the following 17 COCOM-oriented activities in FY08:

- NORTHCOM conferences: Agroterrorism , 23 January 2008; Coordinating Existing National Biosurveillance Capabilities, 12–13 February 2008; Disaster Mental Health, 7–10 July 2008, all Colorado Springs, CO.
- PACOM training events and conferences: Command Surgeon's Conference and Workshop on Avian and Pandemic Influenza, 26–29 November 2007, Bangkok, Thailand; Cambodian Laboratory Technician Training, January 2008; pandemic and avian influenza breakout at the Asia Pacific Military Medicine Conference, 11–14 April 2008, Singapore; public health emergency officer course, 29 April–1 May 2008, Honolulu, HI; Australia pandemic and avian influenza multilateral workshop, 2–7 June 2008, Cairns, Australia.
- SOUTHCOM: laboratory needs evaluations of Caribbean Epidemiology Center in Trinidad and Gorgas Institute in Panama; placement of the Alerta surveillance system in Panama; multilateral educational symposium on the USDA compensation program, July 2008, Panama City, Panama.
- EUCOM/AFRCIOM conferences: EUCOM Command Surgeon pandemic influenza tabletop exercise, September 2008, Germany; host nation pandemic and avian influenza conferences and tabletop exercises, July 2008, Burkina Faso and August 2008, Cote d'Ivoire.
- CENTCOM conferences: Regional Health Security Workshop cohosted with Marshall Center, 11–14 March 2008, Bishkek, Kyrgyzstan; Regional Disaster Management Center of Excellence Health Security Workshop, June 2008, Addis Ababa, Ethiopia; host nation pandemic influenza and emerging infectious diseases conference and tabletop exercise, 24–28 March 2008, Dushanbe, Tajikistan.

DoD Veterinary Service Activity

The DoD Veterinary Service Activity (DoDVSA) and DoD-GEIS partnered to sponsor a Veterinary Food Analysis and Diagnostic Laboratory workshop at Fort Sam Houston (Texas) on 31 March–April 2008. The meeting addressed limitations of information management/information technology systems and improvements in networking to develop initiatives and advance DoD Veterinary Service laboratory surveillance programs. The

conference also provided a valuable forum to advance military public health efforts, review and update pathogen surveillance programs in food and animal populations, and identify opportunities to improve systems and enhance participant networking opportunities. The 41 participants included US Army Veterinary Service laboratory leaders and key operational staff from DoD Veterinary Food Analysis and Diagnostic Laboratory and representatives from Veterinary Laboratory Europe, Pacific Regional Veterinary Command Food Safety Laboratory, 106th Medical Detachment Veterinary Services Food Safety Laboratory, DoDVSA, US Army Veterinary Command, US Army Medical Department Center and School, US Army Natick Soldier Research, Development and Engineering Center, NORTHCOM, USACHPPM, and Headquarters. Participants discussed the following areas:

- Improved data analysis to provide more military public health information;
- Methodologies for foodborne virus identification;
- Greater laboratory capacity for avian influenza surveillance and outbreak response;
- Validation of avian influenza methodologies on JBAIDS.

In addition DoDVSA and DoD-GEIS cohosted an Avian Influenza Response Conference in Crystal City, Virginia, in September 2008. Experts from the Department of Agriculture Animal Plant Health Inspection Service, Colorado State University, DoD-GEIS, DoDVSA, USAMRIID, and US Army Veterinary Service presented information and trained outbreak response teams from the DoD Veterinary Service. The interagency partnerships fostered by this meeting and the associated training enables DoDVSA to participate with other federal agencies in surveillance efforts and to respond appropriately in avian influenza outbreaks.

United States Northern Command

During FY08, DoD-GEIS continued to fund United States Northern Command (NORTHCOM) to foster mission-building relationships among Canada, Mexico, and the United States. Through the Trinational Pandemic Influenza Preparedness and Modeling the Effect of Pandemic Influenza on Military Forces project, NORTHCOM strengthened relationships among Canada, Mexico, and the United States and identified areas of common concern for pandemic influenza planning and response. NORTHCOM conducted a series of preseminar planning meetings that were held among a small US delegation that represented the Department of Defense, Department of State, Department of Homeland Security, and the Department of Health and Human Services with representatives of Mexico and Canada.

These efforts culminated in a trinational seminar and workshop on pandemic influenza 16 June 2008 in Colorado Springs, Colorado, that served as a follow-up to last year's historic conference. Sponsored by GEIS, this year's Trinational Pandemic Influenza Tabletop Exercise and Seminar (Figure 27) was attended by more than 100 officials from militaries and departments of health, public safety, security, and emergency response in Canada and Mexico and North American Aerospace Defense Command-NORTHCOM, Department of State, Department of Homeland Security, and Department of Health and Human Services. The objectives were to improve the ability to respond to a pandemic in North America, identify key organizations in Canada, Mexico, and the United States, advance a North American common operating picture, and establish a way ahead regarding coordination, integration, and strategic communication.

Figure 27. Representative from Mexican ministry of health discusses government actions during a hypothetical pandemic influenza in North America during Trinational Pandemic Influenza Tabletop Exercise and Seminar 16 June 2008.

The pandemic influenza scenario for the tabletop exercise and seminar was designed to address the potential threat of an influenza pandemic in North America. The three national authorities collaborated to stop, slow, or otherwise limit the spread of a hypothetical pandemic throughout the continent. The scenario matured with expatriate and border challenges, alleged unethical

antiviral stockpiling, and sick cruise ship passenger issues. An after-action report was completed and approved for distribution by the NORTHCOM Commander. A postconference compact disk containing all presentations and materials used at the tabletop exercise will be made available.

The tabletop exercise and seminar provided an excellent opportunity to initiate and maintain a cohesive North American team approach to preventing, containing, and mitigating the impact of pandemic influenza in North America. All participants clearly supported this concept and agreed with the aphorism that "disease knows no borders." A successful response to the next influenza pandemic will require all countries to work and communicate collaboratively.

Armed Forces Institute of Pathology

Medical Mortality Registry

The Mortality Surveillance Division of the Armed Forces Medical Examiner System at the Armed Forces Institute of Pathology (AFIP) is the only centralized agency in DoD with the mission and authority under federal law (10 USC 1471) to investigate and determine accurately and specifically the medical cause of death for all active duty personnel. Also, the Mortality Surveillance Division is the only DoD agency with the authority, experience, and capability to accurately track autopsy-determined medical causes of death in mass casualty events.

Emerging infections surveillance and testing are core activities of the Mortality Surveillance Division. The Mortality Surveillance Division operates the DoD Medical Mortality Registry and its Alert Component to achieve real-time visibility on all servicemember deaths worldwide. The Alert Component actively monitors deaths for infectious or potentially infectious etiologies, notifies appropriate authorities in the event of infection clusters, unusual infections or clinical presentations, and, if needed, obtains tissue specimens for more extensive testing to identify the causation or contributing agent(s). Infectious disease-related deaths are reported at the twice-monthly DoD-GEIS Epi-Chiefs' teleconferences.

The Mortality Surveillance Division collects mortality and death circumstance information from DoD, federal, and civilian investigative agencies and collaborates with DoD and civilian medical examiners. The Mortality Surveillance Division enters comprehensive files containing the medical, personnel, special investigation, safety, and legal reports into the DoD Medical Mortality Registry. The Mortality Surveillance Division then reviews files for relevant medical diagnostic information, risk factors, and circumstances of death. A physician reviews all complex cases to validate the medical cause of death. The searchable database of the DoD Medical Mortality Registry contains 15,156 records from all services since 1 October 1997.

The DoD Medical Mortality Registry database is integrated into the Armed Forces Medical Examiner Tracking System, the data center of all casualty documentation in the Armed Forces Medical Examiner System. Military medical examiners can access the web-based system worldwide, and military autopsy reports are directly uploaded into the system along with demographic, autopsy, toxicology, and DNA reports, overseas death certificates, and incident information. The timely death notifications provided by the system to the Armed Forces Medical Examiner have resulted in more thorough active duty death investigations.

During FY08, 1,547 active duty fatalities occurred, a 29% decrease from FY07. Combat in Iraq and Afghanistan during FY08 accounted for 26% of all active duty deaths, a marked drop from the 47% of the FY07 military fatalities. Army personnel were disproportionately represented in the total FY08 deaths (60%) and combat-related deaths (84%). The Mortality Surveillance Division received reports of 399 "illness" or "determination pending" deaths during FY08. Of these cases 39 merited in-depth review. Thirteen were determined to have an infectious disease cause of death: two from respiratory illnesses, two from blood-borne pathogens, three from sepsis, and three from myocarditis/pericarditis. Two neurological cases presented: meningitis and brain abscess. The thirteenth confirmed infectious disease death was attributed to a vector-borne disease. The associated infectious agents identified in 9 of the 13 cases included influenza virus, hepatitis B and C viruses, *Ehrlichia* species, *Neisseria meningitidis, Staphylococcus aureus*, and *Streptococcus pneumoniae*. The Mortality Surveillance Division consulted 1) NHRC for undiagnosed respiratory disease cases, 2) AFIP infectious disease, pulmonary and cardiovascular branches when the cause of death was not otherwise apparent, and 3) USAMRIID when Hantavirus testing was indicated.

DoD Directory of Public Health Laboratory Services

AFIP maintains a password-protected DoD Directory of Public Health Laboratory Services in a Virtual Public Health Laboratory network accessible to DoD beneficiaries worldwide (http:// afip-geis.afip.osd.mil). The directory includes information on more than 180 biological agents and corresponding diseases and lists contact information for military laboratories able to test for these agents. Each agent and disease have a unique description page containing detailed information regarding symptoms, epidemiology, pathogenicity, modes of transmission, communicability, immunizations, diagnostic tests, treatment, and safety precautions. Clinical illustrations and histological images are provided along with links to additional information from the CDC, National Institutes of Health, WHO, and more than 65 other federal and state public health laboratories.

DoD laboratories have access to the directory via registration. More than 250 users have registered with 20 during the past year alone. Users receive monthly electronic newsletters containing brief descriptions of relevant items involving infectious agents along with links to more detailed information. Military environmental laboratories are being added to the website using a Environmental Protection Agency format modified for the DoD, and so far nine Army, Navy, and Air Force laboratories are listed. Contributing laboratory directors enter and edit their on-line information to keep the directory current. An annually updated version of the directory is available on compact disk.

AFIP has maintained the directory monthly since October 2002 to identify clustering of inquiries and requesting organizations. The five most popular topics in FY08 were adenovirus, salmonella, influenza, malaria, and dengue fever. These clusters may be from general user interest or media-focused reports rather than concerns regarding specific disease outbreaks.

Nonmilitary Organizations

National Aeronautics and Space Administration

DoD-GEIS and the National Aeronautics and Space Administration (NASA) have been monitoring, mapping, and predicting outbreaks of Rift Valley fever since 1998 using the Global Inventory Mapping and Monitoring System (GIMMS) at the Goddard Space Flight Center (Greenbelt, MD). This year GIMMS focused on Africa and the Arabian peninsula and predicted the emergence of climatic and ecological conditions associated with Rift Valley fever activity in Sudan, eastern and southern Africa, and Madagascar. In addition, GIMMS provided active surveillance support to the Rift Valley fever outbreak investigations in Sudan, South Africa, and Madagascar (June 2007–May 2008).

GIMMS predictions are founded in the detailed analyses of global scale indicators of interannual climate variability such as El Niño Southern Oscillation and satellite-generated data sets on long-term vegetation and related global climate variations, including sea surface temperature, outgoing long-wave radiation, and rainfall sourced from National Oceanic and Atmospheric Administration archives. As in past years, GIMMS detected climatic and ecological dynamics and anomalies that could lead to the emergence and resurgence of disease insect vectors and, using its Rift Valley fever risk mapping model, derived predictive information about geographic areas at risk for outbreaks. The monthly Rift Valley fever risk assessments were published on the DoD-GEIS website at http://www.geis.fhp.osd.mil/GEIS/SurveillanceActivities/RVFWeb/indexRVF.asp (Figure 28). In addition, GIMMS produced on-demand risk maps and summaries, such as cumulative rainfall anomaly maps, to meet the information needs of DoD-GEIS partners conducting other vector-borne disease monitoring activities worldwide.

During FY08, the GIMMS satellite-based monitoring of climatic and ecological conditions raised alerts and provided response support to various vector-borne disease outbreaks. In addition, climate anomalies (floods and droughts) related to El Niño were validated for association with Rift Valley fever outbreaks in Africa in 2006–2008 and with the chikungunya outbreaks in 2004–2006 in coastal eastern Africa and on the islands of Seychelles and Comoros in the western Indian Ocean.

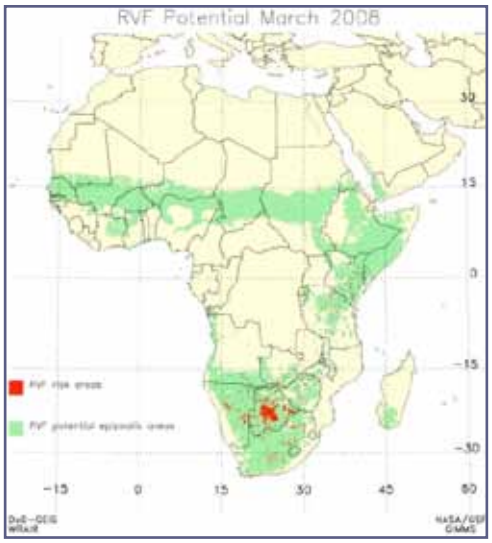


Figure 28. Rift Valley fever risk map for Africa, March 2008.

GIMMS published early warning alerts for an elevated risk of Rift Valley fever activity in Sudan starting in June 2007 on the GEIS website. Based on this information the WHO mobilized technical and logistics support for a joint Sudanese Federal Ministry of Health and Ministry of Animal Resources and Fisheries field investigation. The field team included staff from the Food and Agriculture Organization of the United Nations (FAO) and field diagnostics capacity from NAMRU-3. The first human cases of Rift Valley fever were reported in Sudan in October 2007.

In a similar process, GIMMS detected a transition from warm (El Niño) to cold (La Niña) conditions in mid-2007, which shifted the center of above-normal rainfall from eastern to southern Africa. The result of this shift was that from September 2007 to January 2008, most areas over southern Africa received an excess of rain of 100–400 mm. This excess led to ideal ecological conditions for the emergence of mosquito vectors associated with Rift Valley fever (Figure 29) in southern Africa. Based on the GIMMS Rift Valley fever risk assessment model that incorporates data from October through December 2007, an early warning of elevated risk for Rift Valley fever activity was issued for southeastern Botswana, southern Zimbabwe, northern South Africa, and Madagascar, and subsequent cases occurred between February and May 2008. This early warning prompted the National Institute for Communicable Diseases of

South Africa to deploy a team to target vector surveillance activities in South Africa and FAO/WHO to jointly conduct investigations in Madagascar. The National Institute for Communicable Diseases reported its first South African cases of Rift Valley fever in livestock and humans in February 2008. In March 2008, the World Organization for Animal Health /WHO/FAO reported Rift Valley fever cases in Madagascar.

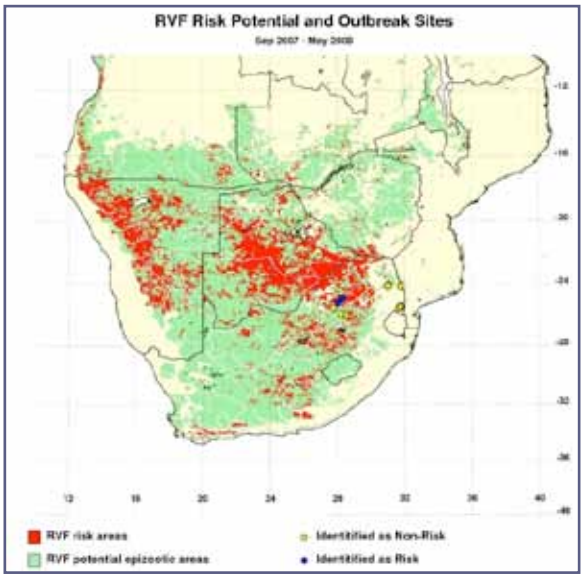


Figure 29. Summary Rift Valley fever risk map, September 2007–May 2008.

Unlike Kenya in 2006, where early warnings led to a timely response by host country authorities (1.5–2 months earlier than in 1997–1998), the 2007 response in Sudan was delayed and resulted in many human fatalities. By comparison, in 2008 in South Africa, authorities responded quickly and mobilized resources to control Rift Valley fever outbreaks there. The Rift Valley fever outbreak foci turned out to be concentrated in commercial dairy farms and domestic livestock and buffalo ranches. No cases were reported in the areas without formal herding or farming, such as open ranges and savannahs with wild animals and few humans. Because Rift Valley fever tends to be recognized in humans before it is seen in wild and domestic animals, the lack of reports from these less formal pastoral areas suggests that Rift Valley fever activity in South Africa might have been more widespread than detected and reported.

The Rift Valley fever outbreaks between 2006 and 2008 in eastern and southern Africa illustrate that the GIMMS remote sensing and predictive modeling can trigger timely and appropriately planned and conducted responses that lead to effective control of vector-borne disease outbreaks. However, valid predictions also require ground truthing by entomologic and/or animal disease field surveillance that detects competent mosquito vectors and diseased reservoirs in sufficient numbers to make disease transmission to humans probable. When used appropriately, remote sensing information guides surveillance activities to areas most at risk for outbreaks. Such targeting permits intensive surveillance where it is most needed and where outbreaks are most likely to be detected early and hence controlled efficiently.

Events in FY08 demonstrated the need for effective communication and coordination among a diverse spectrum of DoD-GEIS partners, including USAMRU-K, NAMRU-3, WHO, FAO, World Organization for Animal Health, and, crucially, host country ministries. Good communication and collaboration can ensure that early warnings are maximally exploited and lead to planned, coordinated responses. Based on the FY08 experience, the system is being refined to enhance predictive capability, including a revision and adjustment of the Rift Valley fever potential epizootic area mask. This will facilitate the ability of GIMMS to characterize different ecologic zones where recent Rift Valley fever activity has occurred. It will also allow GIMMS to incorporate locally generated rainfall data into the model. Additionally, specific sentinel monitoring sites will be added. These improvements will add continuous, systematic surveillance of ground conditions to help direct the activities of entomologic teams and will provide site-specific early warning of outbreak emergence or recrudescence in known Rift Valley fever epicenters.

During FY08, DoD-GEIS continued to support efforts at the Johns Hopkins University Applied Physics Laboratory (JHU/APL; Laurel, MD) in two primary areas: the development of early warning electronic disease surveillance tools applicable to resource-limited settings and improvements in disease modeling for novel respiratory viruses. Both denote components of a growing suite of global electronic surveillance tools and represent a continuing expansion and refinement of tools developed in FY06–FY07 (Figure 30).

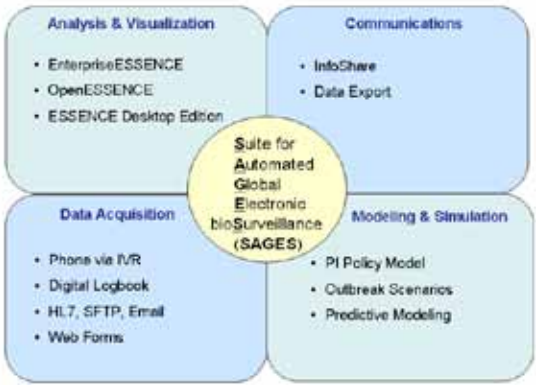


Figure 30. Suite of global electronic surveillance tools developed by JHU/APL and GEIS.

Early Warning Systems in Overseas Settings

Collaborating with DoD-GEIS overseas partners, JHU/APL utilized its surveillance and response system evaluation framework developed in 2006, and its modeling studies and field evaluations completed in 2007, to implement and enhance early warning surveillance capabilities in resource-limited settings chosen by DoD-GEIS. FY08 activities at JHU/APL comprised four projects:

- Enhanced data collection and early event detection in the Philippines (ESSENCE desktop edition);
- Mathematical modeling (ARIES model) for guiding policy and planning decisions by host country authorities in the event of an acute respiratory disease epidemic;
- Development of an affordable and easily accessible communications tool for remote data capture;
- Disease surveillance workshop in Lima, Peru, in conjunction with NMRCD.

The four JHU/APL activities benefited US government and host country infections surveillance and global pandemic influenza preparedness activities. The development by JHU/APL of early warning systems for use in resource-limited settings will eventually benefit, either directly or indirectly, all DoD-GEIS public health partners by strengthening global capacity to more quickly identify and respond to emerging outbreaks at the earliest point of detection.

Enhanced Surveillance Capabilities in the Philippines

Building upon a July 2007 site visit to the Philippines by a JHU/APL, NAMRU-2, and DoD-GEIS team and a 2007 meeting in Thailand with public health practitioners, primarily from Asia, who use various surveillance systems that incorporate aspects of the EWORS, JHU/APL developed ESSENCE desktop edition, which delivers enhanced analysis capability for early outbreak detections (Figure 31). ESSENCE desktop edition is based on the web-based version of the ESSENCE software currently used by DoD Tricare military treatment facilities worldwide and many US civilian public health departments. Unlike traditional ESSENCE, which relies on automated data feeds, ESSENCE desktop edition enables public health officials to load data into the program's analysis tool without relying on Internet connectivity. To augment this capability, JHU/APL also developed clinic data entry system software in FY08 to study the efficiency and effectiveness of electronic data collection at the clinic level. In FY09, JHU/APL intends to pilot and test the clinic data entry system and ESSENCE desktop edition at the Guadalupe-Barangay Health Center in Cebu City, Philippines, and at each level of the Philippine ministry of health, including the National Epidemiology Center in Manila. This pilot deployment will be used to evaluate and refine the new data entry practices and the analysis tool.

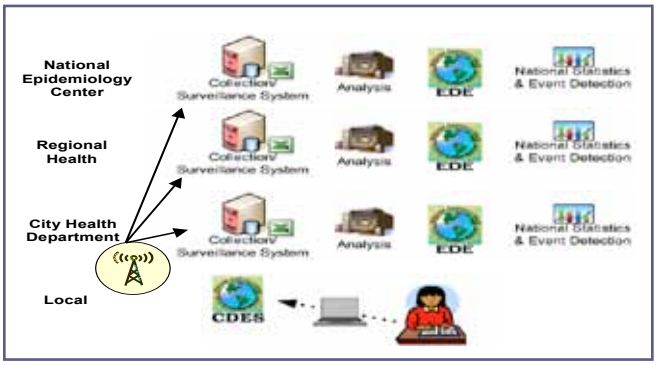


Figure 31. ESSENCE desktop edition (EDE), version 1.0.0. In this pilot version, data are entered into ESSENCE via Microsoft Access or SQL. A future enhancement will be the ability to read data directly from a Microsoft Office database (.odb) or text (.txt) file. CDES, clinic data entry system.

ARIES Mathematical Modeling

JHU/APL conducted site visits to selected national and provincial public health offices and hospitals in Laos in 2006 to evaluate the applicability and expandability in Laos of EWORS, which was developed a decade ago by NAMRU-2. The result of the visits was the recognition that, although Lao health monitors spoke highly of EWORS and gave anecdotal evidence of its benefits, a more science-based assessment of the system's capability and sustainability was required before further investments could be made. Wanting to retain the subjective situational awareness capability achievable with EWORS and recognizing that such situational awareness is universally difficult to quantify, JHU/APL developed the ARIES model as a tool to analyze surveillance systems. ARIES estimates the benefit of disease surveillance policy decisions.

Remote Data Capture

JHU/APL started development of a prototype data capture system that will enable rural health workers to immediately report data on detected health events to central authorities using touch-tone phones as a communications platform (Figure 32). When completed, this system will be open-source so that it can be self-hosted, maintained, and modified by health authorities throughout the world. JHU/APL is writing the voice menu-driven subsystem, known commercially as interactive voice response. Menus will be in the health worker's local language. Based on a prompting system, health workers will be able to enter aggregated population health information, leave voicemail messages for central authorities, and directly connect with a system operator for the immediate reporting of unusual observations or disease activity. Conversely, remote health workers will eventually be able to obtain data from the central system to compare their observations with geographic and historic trends.

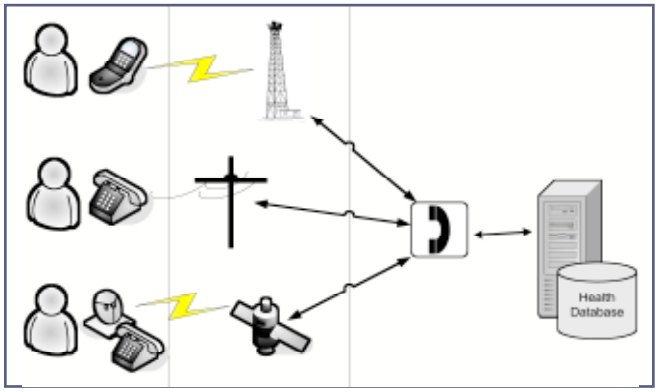


Figure 32. Remote data capture system that allows village health workers to instantly file reports to central authorities with any touch-tone telephone (e.g., cell, landline, or satellite).

Disease Surveillance Workshop

NMRCD, JHU/APL, and DoD-GEIS hosted a 2-day workshop in Lima, Peru, on 29–30 April 2008, to discuss electronic disease surveillance. The first day comprised presentations by practitioners from regional countries, predominantly from Central America, South America, and the Caribbean, and leading electronic disease surveillance researchers from the United States and the region describing their experiences with public health informatics tools for disease surveillance and focusing on potential applications in resource-poor areas. The second day featured small group breakout sessions to discuss current activities in their respective countries and the potential for using electronic surveillance systems in resource-poor settings.

Disease Modeling Efforts

The pandemic influenza policy model developed by JHU/APL and various DoD installations underwent refinements in FY08 that included improving the system's user interface. Tailored for the military, the model allows intervention strategies to be evaluated based on disease models. Efforts included building on the mathematics of prior models, using military-specific social network epidemiology, accurately characterizing the population at-risk, and including the impact of interventions not examined by previous models. The model has been introduced at nearly all (>90) CONUS installations. GEIS expects functionality at all CONUS installations to be underway in FY09.

JHU/APL hosted the Infectious Disease Modeling Meeting at its campus in Laurel, Maryland, on 12–13 May 2008 (http://www.jhuapl.edu/IDMM2008). More than 30 participants from US government agencies, including the Defense Threat Reduction Agency, Sandia National Laboratories, and DoD-GEIS, attended. The first day consisted of presentations detailing past and current work in infectious disease modeling at the participating organizations. The second day consisted of a roundtable discussion about optimizing infectious disease modeling efforts and maximizing opportunities for collaboration and coordination while minimizing unintended redundancy. Procedures to eliminate duplication and improve coordination of modeling efforts were discussed, and future meetings will be required to further address these issues.

University of Iowa

In early FY07 DoD-GEIS began collaboration with the Center for Emerging Infectious Diseases at the University of Iowa (Coralville, IA) to institute a training program for epidemiologists in the field. A certificate program is now in place, and the first class was held this year. This program also produces effective collaboration that led to symposia in Iowa and Mongolia and a zoonotic avian influenza prospective epidemiologic transmission study that includes sites in five countries.

Certificate Program

The certificate program in emerging infectious diseases epidemiology training provided advanced training in emerging infectious diseases to public health practitioners engaged in influenza and respiratory disease surveillance, with the goals of building sustainable epidemiology and research capacity and promoting future collaborations with the DoD overseas laboratories. This program combines preliminary distance-learning assignments and subsequent on-campus study. Training includes lectures, tutorials, field experience, laboratory exercises, public health demonstrations, and written examinations. The web-based, asynchronous, distance-learning module allows participants to complete enrollment and participate in introductory coursework at their home institutions, making the overall program cost-efficient. Students then spend an intensive 2 weeks in formal coursework at the University of Iowa. DoD-GEIS sponsored 23 foreign-national students to attend the course (Figure 33), and Headquarters staff were involved as instructors (Figure 34).

Figure 33. Course participants in newly launched certificate program at the University of Iowa represented 12 nations, May 2008.

Figure 34. Headquarters staff lecturing about zoonotic diseases in first certificate program at University of Iowa, May 2008.

Symposia on Emerging Infectious Diseases

The emerging infectious diseases symposia were held at the University of Iowa in December 2007 and in Ulaanbaatar, Mongolia, in May 2008 (Figures 35 and 36). These symposia served as opportunities for participants to share experiences in influenza and other emerging infectious diseases and initiate collaborations in influenza-related surveillance and epidemiology. A 2-day follow-up symposium in Mongolia is scheduled for July 2009.

Figure 35. Headquarters staff lecturing at University of Iowa symposium, December 2007.

Figure 36. Participants in two emerging infectious disease symposia in FY08. Upper, University of Iowa, December 2007; lower, Ulaanbaatar, Mongolia, May 2008.

Zoonotic Avian Influenza Transmission Study

The third University of Iowa initiative, begun in FY07 and continued in FY08, is an avian influenza zoonotic prospective epidemiologic transmission study that was expanded from two to five countries on three continents (Figure 37). The study's goal is to epidemiologically describe local, clinical, and subclinical zoonotic influenza transmission factors so that public health and veterinary officials can design country-relevant pandemic influenza control measures and practices.

Figure 37. Countries participating in University of Iowa avian influenza study.

To date 1,600 adult study subjects have enrolled at sites in Thailand and Cambodia, and data and serologic analyses are underway. An additional 1,450 adults will be enrolled in early FY09 at sites in Nigeria, Romania, and Mongolia. Among the 800 Cambodian enrollees, 699 (87%) have a history of exposure to poultry. In contrast, among the 800 Thai enrollees, 435 (54%) reported such exposure. Among cohort members, 41 Cambodians had influenza-like illness, with 14 typed as influenza A (all H3) and two typed as influenza B; 19 Thais had influenza-like illnesses, with nine typed as influenza A (three H1 and six H3) and three as influenza B and one influenza A (H3)/influenza B coinfection. The Center for Emerging Infectious Diseases is also running an initial set of 400 Cambodian baseline sera against low-pathogen avian influenza viruses and plans to workup the remaining 1,200 enrollment samples in FY09.

University of California at Los Angeles, Global Viral Forecasting Initiative, and University of Buea

In FY08 the University of California at Los Angeles (UCLA), the Global Viral Forecasting Initiative (Los Angeles, CA), and DoD-GEIS initiated infectious disease studies in Cameroon by taking advantage of existing infrastructure from a previous collaboration between the WRAIR Division of Retrovirology and the Johns Hopkins Walter Reed Cameroon Project and a new partnership with the University of Buea (Buea, Cameroon). Cohort studies for influenza-like illness and other respiratory infections were initiated at several sites in Cameroon (Figure 38); refurbishment of BSL-2 laboratories in Buea and Yaounde began (Figures 39 and 40); and surveillance activities were established at the HEVECAM industrial rubber plantation in southern Cameroon near the border with Equatorial Guinea.

This collaboration provides the framework for expanding DoD-GEIS surveillance for influenza-like illnesses and other respiratory diseases in central African countries and for exploring poultry farming practices and other human-animal interface scenarios. Sites for wild bird sampling have been established in Cameroon that will allow wild bird infections to be investigated as risk factors for interspecies transmission and human infection with avian influenza (Figure 41). Plans for this collaboration in FY09 include the following:

- Influenza-like illness surveillance, similar to Alerta, at HEVECAM rubber plantation in southern Cameroon;
- Capacity for real-time RT-PCR of human and animal samples in Yaounde;
- Virus culture and immunofluorescence assay diagnostic capability at University of Buea;
- Genetic sequencing at USAMRU-K and the WHO National Influenza Center-Kenya supported by NAMRU-3;
- Sentinel influenza-like illness surveillance at up to eight clinics in northern, central, and western provinces in coordination with the Cameroonian Ministry of Health;
- Influenza-like illness surveillance at Military Hospital in Yaounde, Cameroon.

This project's strengthening of the Cameroonian diagnostic laboratory infrastructure provides local and other public health authorities with much needed influenza surveillance data in central Africa. Continuing engagements among local ministry of health officials and DoD-GEIS partners will enable better understanding of seasonal human influenza transmission and of the importance of wild birds and the domestic bird trade to the spread of avian influenza in Africa. All these activities give DoD an increased understanding of the emerging infections threat in this region.

Figure 38. Network for influenza-like illness surveillance throughout Cameroon. Blue dots, University of Buea sites; red dots, UCLA/Global Viral Forecasting Initiative sites.

Figure 39. Renovated laboratory and project house at University of Buea, October 2008.

Figure 40. BSL-2 laboratory at Yaounde undergoing renovation, October 2008.

Figure 41. Sites for wild bird sampling in Cameroon, 2007–2008.

DoD-GEIS Headquarters

During FY08, DoD-GEIS Headquarters continued to identify and address force health protection vulnerabilities in emerging infectious disease preparedness and developed and implemented corrective solutions through collaborations with GEIS partners. Headquarters support for and participation in international meetings and smaller discussion groups were used as opportunities to identify and assess the FY08 strategic goals of GEIS: surveillance and detection, response and readiness, integration and innovation, and cooperation and capacity building. Presentations by Headquarters staff about GEIS-supported programs and activities at professional meetings allowed GEIS work to go through peer review.

Through a defined proposal review and assessment process, Headquarters provided financial support and scientific expertise to current and potential partners who demonstrated interest and an ability to initiate and conduct emerging infectious disease programs, projects, and activities beneficial to force health protection and global public health, particularly as they relate to the worldwide community of DoD health care beneficiaries. Headquarters staff used its professional expertise and its daily programmatic activities to mentor students, interns, and residents, which built a cadre of future emerging infections specialists and DoD-GEIS partners. In addition, Headquarters staff undertook general consultation and program and project review activities to assist numerous DoD and civilian organizations. Often requests for assistance were for participation in lectures and seminars about emerging infectious diseases.

Influenza and respiratory disease surveillance and response remained the highest GEIS priority. With Headquarters funding and consultative guidance, significant enhancements have taken place within the past 3 years (Table 9). The strategic location of nine BSL-3 laboratories around the world gives DoD a unique capability that is leveraged by GEIS partners in each of the five COCOM regions. This network of partners and collaborators now spans 72 countries with more than 350 sites and complements the WHO Global Influenza Surveillance Network. Influenza surveillance activities expanded in the five COCOM areas of responsibility:

- PACOM—Bangladesh, Bhutan, Cambodia, Indonesia, Nepal, Palau, Philippines, Republic of Korea, Saipan, Singapore, Thailand, and 7th Fleet;
- SOUTHCOM—four Central American militaries, Panamanian ministry of health, Colombian military;
- CENTCOM—Middle East, former Soviet Union republics, and Joint Task Force-Horn of Africa;
- AFRICOM—Cameroon, Ghana, Kenya, Nigeria, Tanzania, Uganda, North Africa;
- EUCOM—Landstuhl Regional Medical Center, USACHPPM -Europe, 5th Fleet.

Table 9. Pandemic and Avian Influenza Surveillance Enhancements, FY05–FY09

Capability	FY05	FY06–FY07	FY08–FY09	Comment
No. of countries in network	30	56	72–75	Increases in South America, Africa, Middle East, and Southeast Asia
No. of specimens/year	~9,000	~18,000	~21,000	Increased surge capacity and improved analysis capability
BSL-3 laboratories in network	4	8	9	*Existing:* USAMRIID, Thailand (animal), Egypt, USAFSAM, Peru *In development:* Germany, NHRC, Thailand (human), South Korea
Data integration	No	Yes	Yes	Data analysis, synthesis, and distribution for leadership

Key among these influenza surveillance activities was the expansion of military-military collaborations that represented a mechanism for consistent analysis of respiratory disease trends in allied foreign military forces and a collaborative framework for close coordination and sharing of results within similar populations at risk. Expansion of collaborations was dictated by the ongoing need to identify circulating influenza strains, to conduct informed influenza threat assessments, and to follow the priority of interactions of the COCOM surgeons. Relevant military-military collaborations included establishment of the following:

- Sentinel surveillance with the Kenyan and Nigerian militaries and Tanzanian Peoples' Defense Forces;
- Recruit surveillance efforts and pandemic influenza response capacity with the Polish Military Institute of Hygiene and Epidemiology;
- Sentinel surveillance with the Israeli, Hungarian, and Singaporean defense forces;
- Disease surveillance system capabilities with the Peruvian and Colombian militaries.

The Headquarters Communications Center was used to disseminate timely and reliable information on current issues regarding respiratory, gastrointestinal, febrile and vector-borne infections, antimicrobial resistance, and sexually transmitted infections. The Communications Center transmitted alerts to GEIS partners by electronic mail, daily when necessary, and continued to conduct twice-monthly epidemiology chiefs' telephone conferences for interactive reviews and discussions of current

surveillance detections, identification of recent pertinent publications and presentations at professional meetings, and consultations on DoD emerging infections response activities. Headquarters also provided support, guidance, and coordination assistance to DoD epidemic and pandemic influenza planning and preparedness assessment exercises.

Having reliable state-of-the-art diagnostic laboratory capability available to DoD-GEIS partners whenever and wherever needed remained a high priority in FY08. A potential significant vulnerability to deployed servicemembers was revealed when Headquarters discovered that a militarily appropriate laboratory procedure for identifying highly pathogenic avian influenza, subtype influenza A/H5, infection was unavailable. To fill this gap in the MHS, Headquarters promptly entered into an interagency agreement with Chemical Biological Medical Systems-Joint Program Executive Office (Frederick, MD) and the CDC. The resulting initiative is bringing the influenza A/H5 assay, which was recently cleared by the FDA, into the DoD JBAIDS laboratory testing platform. In addition, Headquarters leveraged its funds and professional expertise to promote force health protection for deployed servicemembers through improved standardization of clinical laboratory diagnostic capability. Headquarters worked with MHS partners to develop and implement standardized laboratory procedures for identifying important pathogens associated with battle wound infections.

DoD-GEIS contributed to the 11th Annual Force Health Protection Conference in Albuquerque, New Mexico, on 11–15 August 2008 by planning and implementing preconference workshops on influenza preparedness and the exchange of epidemiologic information using the CDC Epi-X System. Headquarters also contributed to various conference sessions about diverse emerging infectious disease issues.

Figure 42. Opening session of 2nd Saint Petersburg International Ecological Forum, 1–4 July 2008, Saint Petersburg, Russia.

DoD-GEIS staff participated significantly in two large international meetings: the International Conference on Emerging Infectious Diseases in Atlanta, Georgia, on 16–19 March 2008 and the 2nd Saint Petersburg International Ecological Forum in Saint Petersburg, Russia, on 1–4 July 2008 (Figures 42 and 43). At the International Conference on Emerging Infectious Diseases, Headquarters staff served on the Scientific Program Committee; other staff assisted as session conveners and moderators; and Headquarters and GEIS partners presented ten peer-reviewed oral papers and posters. An estimated 500 conference attendees visited the DoD-GEIS booth in the exhibitor's hall during this conference.

Figure 43. DoD-GEIS director (front left) and director of Research Institute for Influenza, Russian Academy of Medical Sciences (front right), with Headquarters staff at 2nd Saint Petersburg International Ecological Forum, 1–4 July 2008, Saint Petersburg, Russia.

The July 2008 meeting in Saint Petersburg resulted from a Headquarters initiative that began in FY03 when DoD-GEIS played a major role in a unique North Atlantic Treaty Organization-WHO planning workshop about civil-military cooperation in influenza pandemic preparedness. The Russian Academy of Medical Sciences in Saint Petersburg served as the workshop host. After participants of the 2003 workshop called for follow-up to continue communication and cooperation, the director of the Research Institute for Influenza of the Russian Academy of Medical Sciences requested that DoD-GEIS partner with the institute in developing influenza sessions at the 2nd Saint Petersburg International Ecological Forum. Headquarters staff gave seven didactic presentations and engaged in several respiratory disease seminars and discussion groups at the 2008 meeting.

Headquarters supported smaller meetings during FY08 to assess emerging infectious disease priorities and to foster cooperative interaction among existing and potential GEIS partners. These included special information and discussion sessions at the Force Health Protection Conference. Headquarters arranged for a special session on the challenges associated with the adenovirus 14 emergence at military training centers. Headquarters staff later assisted with the preparation and publication of session proceedings. At the 2007 American Society of Tropical Medicine and Hygiene Meeting in Philadelphia, Headquarters staff hosted sessions addressing standardization of malaria drug resistance testing.

Headquarters staff also participated in two interactive roundtable discussions for government decision-makers hosted by the Institute of Federal Health Care (IFHC) in

Accomplishments of DoD-GEIS Influenza Surveillance

The DoD-GEIS influenza surveillance system is a triservice effort that strengthens force health protection and global health preparedness. Begun in 1997, the system now includes almost 400 sites in 72 countries and consolidates earlier efforts by USAFSAM and NHRC. It has expanded the knowledge base concerning the epidemiology of seasonal influenza, particularly H5N1 highly pathogenic avian influenza, in Africa and Southeast Asia. Specimens collected have contributed to global vaccine efforts and continue to provide training and reference services for many regions where little infrastructure exists.

Distribution of DoD-GEIS influenza surveillance sites in FY08.

Populations under surveillance include active duty US military and dependent populations, government employees at US embassies, and host country civilian and military personnel. Findings from GEIS influenza surveillance comprise a large part of the US government's contributions to the WHO Global Influenza Surveillance Network and have helped fill longstanding surveillance gaps in Asia, Africa, and Latin America. With increased support in FY06 for avian and pandemic influenza preparedness, the GEIS system expanded significantly through FY08.

The two goals of DoD influenza surveillance are 1) to identify and characterize antigenically diverse strains that affect DoD beneficiary populations and threaten global public health and 2) to collaborate with relevant organizations such as the CDC, FDA, and WHO to develop vaccines and other effective public health countermeasures. The foundation of the system is the network of DoD overseas laboratories, military treatment facilities, and specialized diagnostic and reference laboratories in the continental United States. This laboratory-based surveillance is augmented by significant epidemiologic training and preparedness initiatives.

The laboratory-based component of the DoD-GEIS influenza surveillance system is coordinated through USAFSAM and NHRC. USAFSAM serves as the Air Force reference laboratory and maintains a global network of more than 70 MTF sentinel sites, and NHRC conducts population-based febrile respiratory illness surveillance at the eight largest domestic recruit training facilities for the three armed services and the Coast Guard and on 20 Navy ships in the 2nd, 3rd, and 7th Fleets.

Because they are highly immunized and well documented, active duty servicemembers provide a valuable population base for studying annual influenza vaccine effectiveness against laboratory-confirmed cases of influenza and nonspecific influenza-like illness or febrile respiratory illness. NHRC conducts population-based vaccine effectiveness studies at the eight recruit training facilities, and USAFSAM constructs a cohort from the immediate family members of active-duty, influenza-positive index cases in the Air Force.

Annual results from GEIS studies of global strain diversity and vaccine effectiveness are presented to the FDA Vaccine and Related Biologic Products Advisory Committee for selection in the annual trivalent influenza vaccine and assist the DoD in evaluating the effectiveness of its influenza vaccination efforts. For the 2008–2009 season, an isolate from the GEIS network was utilized as a strain in the live attenuated influenza vaccine, and several other isolates have been either vaccine strains or reference antigens in past years. Accomplishments of the DoD-GEIS influenza effort at the overseas laboratories and in capacity building follow.

Continued

Accomplishments, *Continued*

DoD Overseas Research Laboratories

- Identification of novel strains of influenza A (H3N2) by USAMRU-K that helped fill the gap of strain surveillance from Africa.
- Extensive support to Africa, the Middle East, and south central Asia by NAMRU-3 in its capacity as WHO H5 Regional Reference Laboratory.
- Assistance with investigations of H5N1 outbreaks in Egypt and Pakistan by NAMRU-3.
- Ongoing influenza surveillance by NAMRU-2 in Laos and Cambodia, countries in the region that has been hit hardest by H5N1.
- Assistance with response to H5N1 outbreak in Cambodia by NAMRU-2.
- Development of a unique cohort for influenza surveillance among US embassy personnel by AFRIMS, an effort that has been expanded in 26 embassies through collaboration with the Department of State.
- Description of the first strains of avian influenza among migratory birds in the Andean region by NMRCD.
- Elucidation of climatic and environmental factors associated with influenza epidemiology in Peru by NMRCD and Peruvian ministry of health.
- Novel surveillance efforts such as community- and household-based cohort studies to study influenza transmission from poultry, swine, and equines to humans in high-exposure settings in Southeast Asia (Thailand and Cambodia), Central Asia (Mongolia), Africa (Nigeria), and Europe (Romania).
- Expansion of Alerta, a real-time electronic disease reporting system optimized for resource-limited settings, in four South American countries.
- Reduction of DoD's reliance on influenza rapid tests in austere settings through joint venture with the CDC and FDA to add an influenza A/H5-specific real-time PCR assay to a ruggedized field-deployable JBAIDS package already in use at more than 300 US military clinics and hospitals worldwide.

Capacity Building

- Upgrade of seven network laboratories to BSL-3 to bring the network BSL-3 total to nine by the end of 2009.
- Support of BSL-2 laboratories in Afghanistan, Jordan, Libya, Ghana, Kenya, Uganda, Cameroon, and Cambodia.
- Use of PCR-based screening platforms to accurately identify potentially highly pathogenic strains before growing them in culture.
- Regional training seminars for DoD health planners and public health professionals through CDHAM to optimize worldwide pandemic preparedness.
- Establishment of certificate program for emerging infectious diseases at the University of Iowa and support for attendance of 24 international public health professionals at first course in FY08.

Recent Significant DoD-GEIS Contributions to Seasonal Influenza Vaccine

Isolation year	Vaccine strain	Virus	Detection population (institution)	Contribution
1999		A/Panama/2007	Panama (USAFSAM)	Seed strain, Northern and Southern Hemispheres: 2000–2004
1999	A/New Caledonia-like		Peru (NMRCD)	Reference strain: 2000–2007
2006	A/Wisconsin/67/2005 (H3N2)-like	A/Nepal/921/2006	Nepal (AFRIMS, WARUN)	Reference strain: 2007
2007	A/South Dakota/ 4344/Ellsworth	A/South Dakota/6/07	South Dakota (USAFSAM)	Seed strain, LAIV: 2008–2009

Washington, DC. The first roundtable (October 2007) addressed the future of syndromic surveillance programs by assessing their costs and benefits. Participants included representatives from federal agencies, Capitol Hill, state and local health departments, and academia. This roundtable identified controversial aspects of current syndromic surveillance programs and advocated the development of a clear roadmap for syndromic surveillance expectations, well-defined national and international uses, and the provision of meaningful assessments. Reports from this roundtable by Headquarters staff were published in *Military Medicine* and the *American Journal of Public Health* and posted on the IFHC website.

The second roundtable (June 2008) focused on sexually transmitted infections. This roundtable addressed the need for collaboration between the CDC and the MHS to strengthen US sexually transmitted infection programs overall. Discussions identified several areas in which DoD and CDC can work together. This roundtable, which was summarized on the IFHC website, resulted in the formation of a joint CDC-GEIS working group on sexually transmitted infections and a collaborative effort to promote the use of a modified CDC questionnaire to assess the availability of clinical laboratory tests for sexually transmitted infections in Army military treatment facilities.

Headquarters staff provided consultation for GEIS partner projects covering febrile illnesses, public health laboratory services, surveillance, and acute febrile respiratory diseases. A DoD-GEIS-supported review of the reemergence and persistence of *Plasmodium vivax* malaria in Korea during 1993–2007 is being published in two articles in *Military Medicine* in 2009.

A longstanding priority for DoD-GEIS has been to increase the percentage of at-risk military females screened annually for *Chlamydia trachomatis*. The percentage has increased from 35% in 2001 to 72% in December 2007. During FY08, MHS *Chlamydia* screening captured a much higher percentage of at-risk females than the civilian health care counterparts. However, the MHS still faces surveillance challenges. A Headquarters review of military medical databases revealed that *Chlamydia* infection rates remain high in military personnel, even though a systematically applied program for screening and treating new recruits (not a universal practice in DoD) is associated with a reduced rate of pelvic inflammatory disease from *Chlamydia* infections. Headquarters-supported sexually transmitted infection consultations also included the development and assessment of two *Chlamydia* screening programs and the development of a model for assessing the cost-effectiveness of screening military males for *Chlamydia*.

Headquarters-supported training programs and defined projects for individuals are generally effective vehicles for introducing students, interns, and residents to emerging infectious diseases and stimulating interest in careers in infectious diseases. FY08 presentations by Headquarters staff included discussions of DoD-GEIS training opportunities. Headquarters facilitated the initiation of mentorships for three servicemembers, a preventive medicine resident, a first-year postgraduate intern, and a Doctorate of Public Health candidate.

In summary, Headquarters staff identified vulnerabilities in emerging infectious disease preparedness and provided resources and ongoing consultation to partners in developing and implementing effective programs. With 76 projects in 87 countries and locations (Figure 44), pandemic and avian influenza surveillance and response activities received the highest priority for Headquarters in FY08. Headquarters staff supported work with GEIS partners that delivered impressive improvements in the screening of DoD beneficiaries for *Chlamydia* infections, undertook efforts to move clinical laboratory testing for pandemic and avian influenza closer to deployed forces, and instituted a system to standardize laboratory procedures for the identification and characterization of agents causing war wound infections.

Afghanistan
Algeria
Argentina
Australia
Azerbaijan
Bahrain
Bangladesh
Belgium
Benin
Bhutan
Bolivia
Burkina Faso
Burma
Cambodia
Cameroon
Colombia
Cote d'Ivoire
Djibouti
Ecuador
Egypt
El Salvador
Ethiopia
Georgia
Germany
Ghana
Guam
Guatemala
Honduras
Hungary
Indonesia
Iraq
Israel
Italy
Japan
Jordan
Kazakhstan
Kenya
Kuwait
Kyrgyzstan
Laos
Libya
Malaysia
Maldives
Mali
Mexico
Moldova
Monaco
Mongolia
Morocco
Nepal
Nicaragua
Nigeria
Northern Mariana Islands
Oman
Pakistan
Panama
Paraguay
Peru
Philippines
Poland
Qatar
Romania
Saudi Arabia
Senegal
Sierra Leone
Singapore
South Korea
Spain
Sri Lanka
Sudan
Syria
Tajikistan
Tanzania
Thailand
Togo
Tonga
Tunisia
Turkey
Turkmenistan
Uganda
Ukraine
United Arab Emirates
United Kingdom
United States
Uzbekistan
Venezuela
Yemen

Figure 44. FY08 GEIS-supported activities in pandemic and avian influenza surveillance (72) and response (15) comprised 76 projects in 87 countries and locations *(brown)*.

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Acronyms

AFB	Air Force base
AFHSC	Armed Forces Health Surveillance Center
AFIP	Armed Forces Institute of Pathology
AFRICOM	United States Africa Command
AFRIMS	Armed Forces Research Institute of Medical Sciences (Bangkok, Thailand)
APMMC	Asia Pacific Military Medicine Conference
BAMC	Brooke Army Medical Center
BSL	biosafety level
BSL-3E	biosafety level 3 (enhanced)
BUD/S	Basic Underwater Demolition/SEAL
CBMS-JPEO	Chemical Biological Medical Systems of the Joint Program Executive Office
CDC	Centers for Disease Control and Prevention
CDHAM	Center for Disaster and Humanitarian Assistance Medicine
CENTCOM	United States Central Command
CGTC	Coast Guard Training Center
CHPPM	*see* USACHPPM
CI	confidence interval
COCOM	Combatant Command
CONUS	continental United States
DMDC	Defense Manpower Data Center
DMSS	Defense Medical Surveillance System
DoD	Department of Defense
DoDVSA	Department of Defense Veterinary Service Activity
EAgg	enteroaggregative
ELISA	enzyme-linked immunosorbent assay
EPEC	enteropathogenic *Escherichia coli*
Epi-X	Epidemic Information Exchange
ESBL	extended-spectrum beta- lactamase
ESSENCE	Electronic Surveillance System for the Early Notification of Community-Based Epidemics
ETEC	enterotoxigenic *Escherichia coli*
EUCOM	United States European Command
EWORS	Early Warning Outbreak Recognition System
FAO	Food and Agriculture Organization of United Nations
FDA	Food and Drug Administration
FRI	febrile respiratory illness
FY	fiscal year
GEIS	Global Emerging Infections Surveillance and Response System
GIMMS	Global Inventory Mapping and Monitoring System
HL7	Health Level Seven
IFHC	Institute of Federal Health Care
Ig	immunoglobulin
KEMRI	Kenya Medical Research Institute
JBAIDS	Joint Biological Agent Identification and Diagnostic System
JHU/APL	Johns Hopkins University Applied Physics Laboratory
LAIV	live attenuated influenza virus
LRMC	Landstuhl Regional Medical Center
MCRD	Marine Corps Recruit Depot
MDCoE	Malaria Diagnostic Center of Excellence
MHS	military health system
MLST	multilocus sequence typing
MLVA	multilocus variable number tandem repeat analysis
MTF	military treatment facility
NAMRU-2	Naval Medical Research Unit No. 2 (Jakarta, Indonesia)

NAMRU-3	Naval Medical Research Unit No. 3 (Cairo, Egypt)
NASA	National Aeronautics and Space Administration
NCLE	National Center for Laboratory and Epidemiology (Laos)
NEPMU	Navy Environmental and Preventive Medicine Unit
NHRC	Naval Health Research Center
NMCPHC	Navy and Marine Corps Public Health Center (formerly Navy Environmental Health Center)
NMRC	Naval Medical Research Center
NMRCD	Naval Medical Research Center Detachment (Lima, Peru)
NNMC	National Naval Medical Center
NORTHCOM	United States Northern Command
NSTC	National Science and Technology Council
OCONUS	outside the continental United States
PACOM	United States Pacific Command
PAHO	Pan American Health Organization
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
PHEO	public health emergency officer
ROK	Republic of Korea
RSV	respiratory syncytial virus
RT-PCR	reverse transcriptase-polymerase chain reaction
SARS	severe acute respiratory syndrome
SEAL	sea, air, land
SOUTHCOM	United States Southern Command
TMP-SXT	trimethoprim sulphamethoxazole
USACHPPM	United States Army Center for Health Promotion and Preventive Medicine
USAFSAM	United States Air Force School of Aerospace Medicine (formerly Armed Forces Institute of Operational Health)
USAID	United States Agency for International Development
USAMRIID	United States Army Medical Research Institute of Infectious Diseases
USAMRU-K	United States Army Medical Research Unit-Kenya
USDA	United States Department of Agriculture
USUHS	Uniformed Services University of the Health Sciences
WARUN	Walter Reed/AFRIMS Research Unit-Nepal
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research
WRAMC	Walter Reed Army Medical Center






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