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14. ABSTRACT

The purpose of this contract is to carry out emerging infectious disease surveillance in Kenya. Specific areas in which work is performed include respiratory illness surveillance (particularly influenza), acute febrile illness surveillance, malaria resistance surveillance, diarrhea etiology and antimicrobial resistance surveillance, sexually transmitted illness surveillance, and capacity building. KEMRI maintained surveillance sites in Ministry of Health clinics and hospitals throughout Kenya. KEMRI operated reference laboratories for this work in Nairobi, Kericho, and Kisumu, including the arbovirus reference laboratory, the antimalarial resistance laboratory, entomology facilities, the Center of Excellence in Microscopy, the microbiology reference laboratory Outbreak investigation and response constituted a significant portion of the efforts in this contract year.

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INTRODUCTION:

KEMRI supports USAMRU-K's establishment of an emerging infectious disease surveillance network by providing contract personnel, laboratory and administrative facilities, capacity development capabilities for contracted personnel and partner organizations, regulatory oversight, and other required functions for the performance of infectious disease surveillance and research. The areas of research/surveillance performed are categorized by the pillars as defined by the US Department of Defense's Armed Forces Health Surveillance Center Department of Global Emerging Infectious Disease Surveillance and Response (DoD-GEIS). These pillars include respiratory illnesses, acute febrile illnesses, malaria, enterics, sexually transmitted infections and antimicrobial resistance, and capacity building. KEMRI maintains both surveillance sites and central laboratories to accomplish this mission.

BODY: For clarity's sake, this report will be divided by DoD-GEIS pillar.

Respiratory Illness:

The World Health Organization has made it clear that global influenza surveillance is a critical priority to detect viral antigenic shift and drift and to detect outbreaks. The Global Emerging Infections Surveillance and Response System (GEIS) concurs with this priority. Historically there has been no consistent influenza surveillance in east and central Africa despite the fact that there is strong anecdotal evidence for frequent acute upper respiratory infections. Kenya is a nation of approximately 30 million people with large urban centers in Nairobi and Mombasa that experience significant international traffic. It is possible that influenza viruses may be introduced into or emerge from Kenya. Only active surveillance can detect such events. This past year the USAMRU-K GEIS program has developed and implemented the first consistent influenza surveillance system in Kenya. All samples are tested at the National Influenza Center laboratory in Nairobi, Kenya. With a new protocol, the AFI surveillance project will now parallel the current sentinel influenza surveillance program by using integrated questionnaires and by using the NIC in Nairobi to process all of the respiratory samples.

Following the establishment of routine influenza surveillance with AI/PI funding in FY07, 1742 good quality sample specimens were collected from the seven active sites between July 2006 and June 2007. Most of these specimens have been processed for virus isolations through the three cell lines. A total of 161 virus isolates have been obtained from these specimens since the commencement of surveillance activity. The viruses isolated include nine influenza A?s (H3N2), twenty two influenza B?s, parainfluenza viruses, RSV, HSV1, adenoviruses, echoviruses, enteroviruses including coxsackievirus subtype B and other uncharacterized enteroviruses. Seventy three (73) of these virus isolates have been shared with AFIOH.

There is currently a large gap about the circulating strains of influenza, in sub-Saharan Africa as most outbreaks of influenza in the region go undetected. There are only a few surveillance studies and outbreak reports in the literature; a finding that stems, in part, from the lower priority given to influenza in comparison to endemic diseases such as malaria and HIV/AIDS. Kenya and other sub-Saharan African countries are plagued by lack of resources. Lab facilities and public health systems are lacking, the disease reporting system is unreliable at best and does not include influenza. Global surveillance is needed to: 1) Detect viral antigenic shift and drift 2) Select the appropriate components for inclusion in future influenza vaccines 3) Detect outbreaks. Most of the other areas of the world - Asia, Europe, much of the Americas, Australia, have laboratories and public health agencies that are actively conducting influenza surveillance. However, there has not been any consistent influenza surveillance in sub-Saharan Africa, despite the fact that acute respiratory infections are one of the leading causes of morbidity. This capability now exists in Kenya with the first site brought on line in July 2006. Three additional sites will be brought on line before the end of August 2006 with

FY06 AI funding. Kenya is a country of over 32 million people with significant international trade and tourism. It is rated among the developing countries and territories categorized as High-Risk Countries for H5N1 avian influenza (World Bank, 2005). Even though, poultry farming in the sub-Saharan countries of interest is free range, there are a few commercial poultry farms near most of the urban centers. Free range holdings have transmission implications because of the increased chances of mixing between domestic and wild birds. Kenya is classified as high risk by the World Bank because of the migratory flyways, local wild birds, high population density, free-range poultry, commercial poultry, and low hygienic standards. A similar situation exists in Uganda, Cameroon and other countries in the region. Wild birds have been implicated in the spread of avian influenza. In wild birds, such as water fowl, there usually is no manifestation of the disease and the infection often goes undetected. Poultry, on the other hand, do manifest the disease. It is the mixing of poultry and wild birds with the potential for virus transmission that is cause for the most concern. Hence, surveillance is important along the migratory flyways, to learn more about infection in the wild birds, such as water birds, and implications for spillover into domestic birds and more importantly, human populations. Migratory bird surveillance in Kenya started in 2005, by NAMRU-3, and is a collaboration between NAMRU-3, USAMRU-K, the National Museums of Kenya, and CDC-IEIP and will continue into FY 07.To date over 750 wild birds have been trapped and sampled. Veterinary research technicians have been trained in a recent workshop held in Kenya with FY06 AI funds.

Acute Febrile Illness:

To increase the knowledge about the epidemiology and etiology of acute febrile illnesses in Kenya through identification of outpatient and inpatient presentations with influenza, malaria, arboviruses, viral hepatitities, viral hemorrhagic fevers, rickettsial disease, brucellosis and leptospirosis in various regions in Kenya. To aid the Ministry of Health in its public health surveillance mission, formulation of public health policy, and early recognition of disease outbreaks. This surveillance project will utilize USAMRU-K GEIS surveillance sites across Kenya. The study population will include persons of all ages presenting to surveillance centers with an acute febrile illness. Volunteers will be consented, interviewed, and phlebotomized. Clinical and epidemiological information will be collected by questionnaire. The enrollment target is a maximum of ten patients per site per week. The enrollment target is a maximum of ten patients per site per week. Samples will be sent to various USAMRU-K laboratories for testing.

The primary goal is to identify the etiology of acute febrile illness (AFI)or fever of unknown origin (FUO)in Kenya. Malaria has been considered the primary etiological agent in the differential diagnosis of acute febrile illness in most of Sub-Saharan Africa leading to excess empiric treatment and over-reporting of malaria with the resultant under-reporting of other causes of febrile illness. Numerous bacterial, parasitic and viral pathogens are known or suspected to cause disease and presentations of febrile illness. Respiratory disease including viral causes like influenza is a frequent cause of FUO in Africa where relatively modern means of diagnostics are not readily available. Little work has been done in Kenya or the region during the past decade to determine the true incidence or prevalence of many of these diseases. Since many of these diseases present with similar symptoms and are indistinguishable on clinical grounds, a detailed laboratory evaluation is the only means of identifying the pathogen.

Mosquitoes, sand flies and ticks will be samples using standard collection methods, identified to species, pooled, homogenized and stored at ultra low temperatures. Homogenates will be analyzed for the presence of arboviruses by cell culture inoculation and/or RT-PCR. Isolated viruses will be characterized using serologic and molecular techniques. Minimum infection rates per vector species will be determined.

The rickettsiae and related diseases (such as ehrlichiosis, Q fever) make up a family of gram-negative coccobacilli that grow strictly in eukaryotic cells. Characteristics of these organisms include their obligate intracellular location and persistence. The pathogenic rickettsiae that cause diseases (called rickettsioses) move through mammalian reservoirs, such as domestic farm animals, and most are transmitted by insect or tick vectors. Except for louse-borne typhus, humans are incidental hosts. Among rickettsiae, Coxiella burnetii (the agent of Q fever) is notorious for its ability to survive for an extended period outside of the reservoir or vector and for its extreme infectiousness: inhalation of a single microorganism can cause pneumonia. Blood samples and ticks will be collected from domesticate animals presented for slaughter. Ten uL of blood will be collected from each animal and used for serum and genomic DNA (gDNA) preparation. Antibody titers to Spotted Fever Group (SFG) and Typhus Group (TG) rickettsiae will be done by ELISA. Briefly, R. typhi and R. conorii antigen (representing the two groups) will be passively adsorbed onto microtiter plates. Horseradish peroxidase-conjugated antibodies will then be added and presence of antibodies revealed by adding ABTS and read at 405 nm. Quantitative real-time PCR will be used for detection of different rickettsia agents. The prevalence of Rickettsia infections in the different animal species and regions will be compared using ANOVA with Bonferroni adjustment for multiple comparisons. For each quarter of FY08 we hope to collect and perform Rickettsia assays from 450 specimens.

West Nile virus is an arthropod virus in the family Flaviviridae and genus flavivirus. It is a member of the Japanese Encephalitis (JE) virus serocomplex. The complex includes the Japanese Encephalitis virus, ST. Louis Encephalitis (SLE) virus and Murray Valley encephalitis virus 1. The virus possesses a single stranded plus sense RNA genome of approximately 11,000 nucleotides. West Nile Virus circulates in natural transmission cycles involving mainly the culex species of mosquitoes and birds. Humans are incidental hosts. Human infections in West Nile endemic areas are common. However, the infections are usually mild or sub clinical. Severe disease is usually associated with the elderly. Wild birds will be collected from six field surveillance sites from three distinct geographic regions in Kenya. Birds will be trapped using standard methods. A cloacal swab, throat swab, and blood sample will be taken before bird release back to the wild. Birds will be handled by trained ornithologists and technicians in a humane manner. Samples will be analyzed using a rapid antigen test, RT-PCR, and virus isolation.

The leishmaniases are a diverse group of diseases caused by protozoan parasites of the genus Leishmania that are transmitted by the bite of infected female phlebotomine sand flies (Magill AJ. 1995. Epidemiology of the leishmaniases. Dermatol Clin. 13: 505- 23.) An estimated 12 million people in 88 countries on five continents suffer from the leishmaniases, with an estimated 350 million persons at risk (Kravchenko et al 2004). More than a million new cases of human leishmaniasis are "reported" annually. The leishmaniases include a wide spectrum of diseases ranging from a relatively benign, ultimately self-healing cutaneous form (cutaneous leishmaniasis) to a potentially fatal systemic disease (visceral leishmaniasis). The cutaneous form of the disease can sometimes cause highly disfiguring lesions or, with certain species of Leishmania present in the New World, can spread to the nasal or oral mucosa causing a form of the disease called mucosal leishmaniasis. Visceral leishmaniasis is the most serious form of the disease and attacks the reticuloendothelial system, including the spleen, liver, bone marrow, and lymph nodes. Left untreated, visceral leishmaniasis is usually fatal (Guerin PJ, Olliaro P, Sundar S, Boelaert M, Croft SL, Desjeux P, Wasunna MK, Bryceson AD. 2002. Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. Lancet Infect Dis. 2: 494-501). Sand flies also transmit other diseases such as sand fly fever, bartonellosis, phleboviruses, and certain flavivruses, arboviruses and vesiculoviruses causing health problems to humans and domestic animals. Sand flies are also a nuisance pest, so control measures to suppress large populations are required even in areas where leishmaniasis does not occur. However, control

measures are complicated, because sand flies occupy a variety of habitats and many species may feed on humans as well as a number of reservoir host animals. Sand flies will be collected from areas occupied by DoD personnel deployed to Africa under the Combined Joint Task Force Horn of Africa (CJTF-HOA). These may include base camps and other garrison type environments, forward operating bases, observation posts, residential areas, and recreation sites. Such sites will be selected from leishmaniasis endemic countries including Ethiopia, Eritrea, Djibouti, Kenya, Sudan, and Egypt. Sand flies will be collected using standard CDC light traps deployed at night as well as stick traps. Samples will be transported to a central laboratory for identification and pathogen detection using real-time PCR. When possible parasites will be cultured to improve understanding of Leishmania strains unique to the region. Data will be georeferenced and presented as risk map indicating areas where infected sand flies were collected.

Malaria:

In-vitro (new, non-radiological methods) and in-vivo malaria drug resistance testing will occur at selected sites based on predefined, uniform criteria that will be agreed upon by all participating sites (WRAIR, Kenya and Thailand). Surveillance will constitute, under an approved protocol, obtaining malaria smears and blood samples from individuals presenting with symptoms of malaria. Careful historical and epidemiological data will be obtained to support in-vitro testing. To complement human sampling, Anopheles mosquitoes will be screened for malaria parasites possessing genetic polymorphisms known to confer drug resistance.

Enterics:

Acute gastroenteritis, including diarrhea, is a debilitating illness that can rapidly compromise the regular daily functions of an individual. Historically, diarrhea remained the most common illness reported by U.S. military service members during numerous military exercises or mobilizations to regions/theaters where the sanitation condition was poor [1-5]. During Operation Bright Star (Egypt, 2001) in which 15,000 U.S. military personnel participated, more than 500 service members were affected by acute diarrhea [2], forcing them to stay off work for at least one day or more. Such a high loss of manpower during an operation would adversely affect unit readiness and morale. As the U.S. Armed Forces continue to deploy troops to Africa for strategic training and contingency operations, the service members represent an immunologically naive group to the various enteric pathogens in the region and are likely to be at higher risks for contracting acute infectious diarrhea. An outbreak of acute gastroenteritis will significantly impact the capabilities of the service members to accomplish their missions. The efforts it takes to combat this force health protection issue become more challenging with the worldwide emergence of multiple drug resistant strains among the bacterial pathogens. Therefore, a thorough examination on the etiological agents and the respective antibiotic resistance pattern of the enteric pathogens in the region is of great strategic and tactical advantages to the U.S. military regional command as well as the Department of Defense.

This study will survey the bacterial causes of diarrhea in Kenya; Perform the biochemical identification, antibiogram determination, and molecular typing on each isolate; potential pathogens such as Escherichia coli, Vibrio, Salmonella, Shigella, Yersinia, and Campylobacter spp. will be screened; Implement a data reporting network with the PulseNet Middle East (CDC) to aid future epidemiological investigations, domestic or global.

KEY RESEARCH ACCOMPLISHMENTS:

Characterization of etiologies of influenza-like illness in Kenya

Identification of circulating strains of Influenza virus in Kenya

Characterization of selected viral, bacterial, and rickettsial etiologies of febrile illness in Kenya

Continued elucidation and tracking changes in antimalarial resistance patterns in Kenya

Ongoing characterization of etiologies of diarrheal illnesses in Kenya

REPORTABLE OUTCOMES: Not available

CONCLUSION:

KEMRI provides critical support to USAMRU-K's emerging infectious disease surveillance program in Kenya. Without KEMRI, USAMRU-K would not be able to execute its mission. KEMRI provides the legal and regulatory framework, personnel, and laboratory structure necessary to carry out scientific work. The organizations exist in partnership, with USAMRU-K working fully under the KEMRI umbrella in Kenya. Together, we have made great strides in establishing surveillance capabilities in the areas of respiratory illnesses, acute febrile illnesses, malaria, enterics, and capacity building. KEMRI maintains both surveillance sites and central laboratories to accomplish this mission.

REFERENCES: Not available