# History of U.S. Military Contributions to the Study of Viral Hemorrhagic Fevers

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**The viral hemorrhagic fever viruses represent a unique group of viruses that can produce large outbreaks of both animal and human disease and produce severe, highly fatal, human illnesses. The viral hemorrhagic fever viruses display a great deal of diversity in their genetic organization, vectors for transmission, and geographic distribution. They share common features in being able to induce a great deal of cellular damage and to elicit an immune response among humans that can result in severe hemorrhage, coagulopathy, shock, and death. The characteristics of the viral hemorrhagic fever viruses as arthropod-borne or rodent-borne viruses that can result in human illnesses with high morbidity and mortality rates make these viruses a unique threat, historically, currently, and in the future, to deployed soldiers around the world. In response to this threat, U.S. military scientists have been world leaders in the development of knowledge on the viral hemorrhagic fever viruses, from extensive fieldwork in areas in which these viruses are endemic, outbreak investigations of epidemics, and careful clinical studies elucidating the pathogenesis of severe disease. Defining the disease threat and creating practical countermeasures through the development of drugs and vaccines has been the major mission of military scientists and has resulted in numerous candidate vaccines currently in animal and human clinical trials.**

## Introduction

The viral hemorrhagic fever viruses are arthropod-borne or rodent-borne viral infections that can result in hemorrhage and shock. In the case of the filoviruses, the transmission to humans and the natural reservoir are not known. The viral hemorrhagic fever viruses can produce a clinical syndrome that is characterized by fever, severe systemic symptoms such as headache, myalgias, arthralgias, nausea, vomiting, and diarrhea, and varying degrees of coagulopathy. Coagulopathy is a distinguishing feature of the viral hemorrhagic fever viruses and is manifested by hemorrhage into the skin as petechiae or ecchymoses, oozing at puncture sites, epistaxis, gingival bleeding, hematemesis, melena, and severe vaginal bleeding. Cardiovascular collapse and shock syndrome can occur through blood loss or intravascular plasma leakage into the extravascular space.

The viral hemorrhagic fever viruses are represented by a variety of RNA viruses with varying vectors of transmission, epidemiology, pathogenesis, and case fatality rates. The RNA viruses are highly susceptible to point mutations, in the range of $10^{-4}$ to $10^{-5}$ substitutions per nucleotide copied, and undergo homologous and heterologous recombination, gene reassortments, and formation of quasispecies during replication.[1,2] The high mutation and recombination rates observed explain in part the great deal of genetic diversity seen among the RNA viruses.[3] The result is a virus that undergoes rapid evolution and that can become highly adaptable to the host and the environment. The diversity of the viral hemorrhagic fever viruses and their adaptability to the host and the environment result in a group of pathogens that have been in the past, are currently, and potentially will be in the future major disease threats to military personnel deployed in virus-endemic areas. This article is a review of the military significance of the viral hemorrhagic fever viruses and the contributions of military scientists toward understanding the viruses.

## Dengue

Dengue is an expanding public health problem in the tropics and subtropics. Reports suggest that 2.5 billion people are at risk for dengue, with up to 100 million dengue virus infections occurring each year and more than 60,000 reported deaths.[4–6] Dengue transmission occurs in Central and South America, South and Southeast Asia, Africa, and the Caribbean and Pacific regions. There have been recent outbreaks in Texas, Florida, and Hawaii.[7–9] Population growth, urbanization, and regional and international travel sustain the continually worsening global dengue situation.[10,11]

The U.S. military has made great contributions to the understanding of the etiology, epidemiology, immunology, and pathogenesis of dengue virus infections. Numerous dengue vaccine candidates have been developed by the U.S. military and are being evaluated in Phase I/II clinical trials.

Dengue viruses belong to the genus *Flavivirus* and the family Flaviviridae.[12] The virion is a single-strand, positive-sense, RNA genome coding for capsid, membrane, and envelope proteins and seven nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5).[13] The dengue viruses exhibit substantial genetic diversity, exemplified by the existence of four distinct serotypes (DEN-1–4).[13,14] The genetic diversity and phylogenetic relationships of dengue virus strains isolated from different parts of the world suggest the existence of numerous DEN-1, -2, -3, and -4 genotypes.[15–25]

The pathogenesis and pathophysiology of severe dengue virus infections (dengue hemorrhagic fever) remain incompletely understood. Early theories were based on clinical observations and seroepidemiological studies.[26–28] An extensive body of work describing clinical and basic science observations on pathogenesis has been completed by U.S. military scientists and their collaborators at the Walter Reed Army Institute of Research (WRAIR)

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(Silver Spring, Maryland) and the Armed Forces Research Institute of Medical Sciences (Bangkok, Thailand).[29–35]

Most dengue hemorrhagic fever cases occur among persons sustaining secondary dengue virus infections with heterologous dengue virus serotypes.[36] The immune-enhancement theory of dengue pathogenesis states that cross-reactive non-neutralizing antibodies from a previous heterologous dengue virus infection facilitate dengue virus entry into Fc receptor-bearing cells such as monocytes and macrophages. This increase in the number of infected cells leads to more severe disease.[37] The sequence of infecting dengue virus serotypes,[38,39] genetic diversity among genotypes, race, gender, age, and preexisting chronic diseases may all affect the clinical severity of infection.[40–58]

### U.S. Military Significance

Dengue's place in U.S. history occupies two significant time periods, namely, a 1780 outbreak in Philadelphia, Pennsylvania, and the years encompassing World War II. Emerging from these experiences was what many believe is the first accurate clinical description of dengue illness and a plethora of observations on the etiology of dengue, immune system responses to dengue infection, and early vaccine development efforts.

Dr. Benjamin Rush provided an in-depth description of a dengue fever epidemic after observing numerous patients during the 1780 outbreak in Philadelphia, Pennsylvania.[59] He noted that the August/September febrile exanthem was confined to persons residing along the Delaware River waterfront. Both genders and all ages were afflicted. Rush described the symptom complex accompanying the fever, The pains . . . were exquisitely severe in the head, back and limbs. The pains in the head . . . occupied . . . the eyeballs. A few complained of their flesh being sore to the touch . . . nausea universally, and . . . vomiting, accompanied by a disagreeable taste in the mouth . . . a rash often appeared on the third and fourth days . . . a profuse hemorrhage from the nose, mouth, and bowels, on the tenth and eleventh days, preceded a fatal issue of the disease . . . its . . . name among all classes of people was, the Break Bone Fever (pp 92 and 93).[59]

Dengue was a disease of great military importance during World War II. Soldiers stationed in the Pacific theater introduced dengue viruses throughout Southeast Asia, Japan, and the Pacific Islands. The deployment of nonimmune troops to dengue-endemic areas with unchecked vector populations resulted in large epidemics of disease and significant decreases in combat-effective troop strength. Between 1942 and 1945, the highest annual attack rates were in the Southwest Pacific (32 cases per 1,000 troops), Central and South Pacific (21 cases per 1,000 troops), and China-Burma-India (18 cases per 1,000 troops) regions. The highest yearly rate among all overseas troops occurred in 1944 (13 cases per 1,000 troops); the rate sharply decreased the following year (4 cases per 1,000 troops), with the implementation of effective vector control.[55]

McCoy and Sabin[55] described the U.S. Army's dengue experience. Epidemics among troops were recorded in the Northern Territory and Queensland (1942), Espíritu Santo (1943), New Caledonia (1943), New Guinea (1944), and the Philippines (1945). The Japanese port cities of Nagasaki, Kure, Sasebo, Kobe, and Osaka also suffered significant disease during World War II. Reports suggest there were more than 2 million cases of dengue between 1942 and 1945 in Japan.

An extensive dengue outbreak among U.S. forces occurred in 1944 in the Marianas Islands. The rainy season (August) in Saipan brought abundant populations of *Aedes aegypti* and *Aedes albopictus* mosquitoes. The dengue rate among Army, Navy, and Marine Corps personnel living in the barracks was 300 cases per 1,000 troops per annum and rose to 3,560 cases per 1,000 troops per annum by September. Despite the use of dichlorodiphenyltrichloroethane (DDT) to control vector populations, more than 20,000 dengue cases are thought to have occurred in Saipan by late October.[55] Dengue continued to have an adverse impact on the U.S. military during operations in Vietnam, Somalia, and Haiti, detracting from combat readiness.[44,60–64]

### U.S. Military Contributions

Early research into the etiology of dengue fever-like illnesses implicated bacteriological, protozoan, and spirochetal causes.[65–67] Ashburn and Craig[68] provided evidence for the viral etiology of the disease, making dengue virus the second human viral pathogen identified, after the yellow fever (YF) virus.[69] Siler et al.[70] researched the role of *A. aegypti* as a vector in the transmission of dengue virus, building on the work of Graham[66] in Lebanon. Research performed by Hotta[71] and Sabin[259] during World War II isolated virus types 1 and 2, identified the presence of homotypic immunity following infection, and described the clinical and diagnostic significance of neutralizing antibodies. Dengue hemorrhagic fever was recognized during hemorrhagic fever outbreaks in the Philippines and Thailand during the 1950s.[72,73] In 1956, the dengue epidemic in Manila resulted in the identification and naming of DEN-3 and DEN-4 viruses by Hammon et al.[72] In the early 1980s, scientists working out of U.S. Naval Medical Research Unit 2 demonstrated that dengue infections remained an important cause of pediatric hospitalizations in the Philippines and dengue outbreaks among U.S. military personnel (Clark Air Base) continued to occur.[74,75] U.S. Naval Medical Research Unit 3 in Peru has continued to characterize dengue epidemiology in Iquitos since the 1990 outbreak. Epidemiological observations and basic science research in Southeast Asia have continued at the Armed Forces Research Institute of Medical Sciences in Bangkok since its creation following the Thai hemorrhagic fever outbreak.[76,77]

The U.S. military has had a consistent presence at the forefront of dengue vaccine development (for a comprehensive review of the U.S. Army dengue vaccine development effort.[260] Early efforts date back more than 70 years, with attempts to prevent virus transmission using infectious human plasma treated with ox bile or virus grown in live mosquitoes and inactivated with Formalin.[78] Schlesinger et al.[79] and Sabin and Schlesinger[80] undertook the first attempts at immunization using mouse-passaged, live, attenuated DEN-1 and -2 viruses. The U.S. Army dedicated more than a decade to further development of these vaccine candidates.[80] In 1962, a field efficacy trial in Puerto Rico using the DEN-1 vaccine candidate demonstrated partial protection during an outbreak of predominantly DEN-3 virus.[81] In 1971, the U.S. Army Medical Research and Development Command launched a program at the WRAIR to develop a cloned, live, attenuated vaccine candidate for each DEN virus type.[10] The subsequent 4 years of clinical trials yielded disappointing results from both reactogenicity and immunogenicity standpoints.

Halstead and colleagues revealed that dengue viruses could be attenuated for humans by passage in primary dog kidney cell

culture.[82–86] WRAIR subsequently received seed viruses adapted to grow in primary dog kidney cells from the University of Hawaii. Monovalent vaccine candidates were tested and subsequently combined into tetravalent formulations. Tetravalent dengue vaccine candidates are now in Phase II clinical testing.[85,87,88]

The U.S. military is also involved in the development and testing of several second-generation dengue vaccines. Inactivated whole-virus and recombinant subunit vaccines are being pursued.[89,90] DNA vaccines show promise as a stand-alone approach or as a "prime-boost" with inactivated vaccines.[91–94] The U.S. Naval Medical Research Center has conducted work on improving DNA vaccine immunogenicity by using novel methods of vaccine delivery and adjuvants to stimulate the immune response.[91,95,96] An inactivated, monovalent, dengue vaccine candidate has been tested in nonhuman primates and is ready for Phase I clinical testing in humans.[89,261]

The lack of an appropriate animal model for dengue infection and vaccine immunogenicity has delayed the development and testing of candidate vaccines. Recent U.S. Army attempts to reintroduce a human challenge model offer an approach to evaluating the efficacy of modern vaccines before field efficacy testing with large numbers of susceptible volunteers.[125,126] Experimental infection of human volunteers with dengue virus has been produced among many hundreds of volunteers since the beginning of the last century, without untoward effects.[68,70,71,78–80,97–105]

## Yellow Fever

Since the 17th century, YF epidemics have strained public health and economic systems in the Americas, Europe, and Africa.[106] Today, YF continues to affect communities in tropical South America and sub-Saharan Africa. There are estimates of 200,000 cases occurring annually, with a case fatality rate of 20 to 50%.[107] YF virus has the distinction of being the first virus to be demonstrated as a cause of human disease transmitted by an arthropod, the *A. aegypti* mosquito. Griffin Hughes is given credit for being the first to use the term "yellow fever" in his book "Natural History of Barbados" (1750).[108] However, reports of diseases similar to YF date back to 1498 (San Domingo), 1585 (West Africa), and 1647–1649 (Barbados, Cuba, and Mexico).[109]

The YF story is synonymous with U.S. military medicine and scientific achievement. The discoveries of Maj Walter Reed and the Yellow Fever Commission established precedence for the conduct of epidemiological studies and human use research.

YF virus takes it name from the Latin word for yellow (*flavus*) and is of the family Flaviviridiae and the genus *Flavivirus*.[108] In 1927, workers of the Rockefeller Foundation's West African Yellow Fever Commission isolated a YF virus from a young Ghanian named Asibi, the parent strain of the 17D vaccine.[106] In 1985, Rice et al.[110] published the nucleotide sequence of the 17D virus. Molecular sequencing predicted the existence of five genotypes (three in Africa and two in South America).[111] Phylogenetic analyses suggest that YF virus originated in Africa and divided into West and East African lineages, with the West African lineage being the progenitor virus for the strains subsequently imported into South America and the New World via the slave trade.[108]

The clinical spectrum of YF varies from a mild, nonspecific, febrile illness to a fulminating, sometimes fatal, disease.[112] Following infection and 3 to 6 days of viral incubation, two phases of clinical illness ensue. The first (acute phase) is characterized by fever, muscle pain (with prominent backache), headache, rigors, anorexia, nausea, and/or vomiting. Viral titers in blood peak at $10^5$ to $10^6$ infectious particles/mL.[111] Laboratory abnormalities include leukopenia and elevated serum transaminase levels. The majority of patients show clinical improvement 3 to 4 days following the onset of illness. In contrast, approximately 15 to 25% of patients enter a "toxic phase." Within 24 hours, fever recurs and jaundice develops, accompanied by abdominal pain with vomiting. Hemorrhage may begin from the mouth, nose, eyes, and/or gastrointestinal tract, and renal failure may occur concurrently. Fatality rates reach 50% within 10 to 14 days.[113]

### U.S. Military Significance

More than 135 YF epidemics occurred in the United States between 1668 and 1893. In 1793, a YF epidemic in Philadelphia killed 1 in 10 residents, resulting in a complete disruption of society.[106,107] A devastating YF epidemic in 1878 swept up the Mississippi River valley from New Orleans, killing 20,000 people.[107] One year later, Tulane University Professor Stanford E. Chaille led a commission to Havana, Rio de Janeiro, and the West Indies. The commission's conclusion that the etiology of YF was possibly an entity living in the atmosphere set commission member Carlos J. Finlay onto the mosquito transmission theory.[114]

Following the U.S. military occupation of Cuba in 1898 during the Spanish-American War, hundreds of soldiers succumbed to YF virus infections. News of the deaths prompted U.S. Army Surgeon General and Chaille Commission participant George Miller Sternberg to assemble the Yellow Fever Commission. Four experienced physicians and scientists composed the commission, including U.S. Army pathologist and bacteriologist Maj Walter Reed and Maj James Carroll, Maj Aristides Agramonte, and Maj Jesse Lazear.

### U.S. Military Contributions

#### *The Yellow Fever Commission*

Early efforts of the commission focused on validation of the 1897 theory of Italian bacteriologist Giuseppe Sanarelli that *Bacillus icteroides* caused YF. This work was conducted much to the dismay of Lazear, who wrote to his wife on August 23, 1900, ". . . I would rather try to find the germ without bothering with Sanarelli."[115] The dismissal of *B. icteroides* as a cause of YF diminished support for the fomite theory of transmission (e.g., contaminated clothing and sheets) and built evidence for the transmission to humans via an intermediate host. This led to testing of the mosquito theory of the Cuban scientist Carlos J. Finlay and the famous human use trials to discover the answers described below.[116]

From August 11 to 25, 1900, the equivalent of YF mosquito inoculation challenge experiments were conducted on nine different occasions, all failing to reproduce clinical YF. Lazear challenged Carroll on August 27, 1900, and soldier William J. Dean 4 days later; both quickly developed YF.[117] The contrasting circumstance of the latter challenges was that the mosquitoes for Carroll and Dean had fed on YF patients during the first 3 days of illness (peak viremia) and were used to challenge after 12 days. The delay in mosquito challenge allowed the required viral

"extrinsic incubation" period to occur. Lazear also developed YF; the circumstances of his infection are unknown but almost certainly represent an experimental infection. He died of his YF on September 25, 1900.[117]

In November 1900, on the rolling fields of the Finca San Jose, the commission established Camp Lazear, dedicated to their deceased colleague. The camp consisted of two wood-frame buildings and seven tents to accommodate and support the study subjects. Robert P. Cooke, a U.S. Army Medical Corps physician, conducted three trials using 20 study subjects to disprove the fomite theory of YF transmission.[117]

The commission subsequently proceeded with four series of mosquito experiments. In the first, study subjects developed YF following mosquito inoculation challenge. In the second, Reed used case-control experiments to demonstrate, ". . . the essential factor in the infection of a building with yellow fever is the presence therein of mosquitoes, and nothing more."[118] The third series of experiments used successive inoculations by the same mosquitoes, over time, to pinpoint the "extrinsic incubation" period, the time required for a newly infected mosquito to become a competent vector of YF, which was no less than 11 days. These findings complemented the epidemiological observations of Henry Rose Carter of the Marine Hospital Service, who noted a lag time of 2 to 3 weeks between the identification of a YF index case and subsequent secondary cases.[107] The fourth experiment complemented the third by approximately defining the period over which YF-infected mosquitoes retained their vector competency, i.e., more than 40 days.[117]

The final experiments involved subcutaneous inoculations of blood taken from YF-infected patients into YF-naive study subjects. YF was produced in four study subjects; subsequent blood cultures from the study subjects failed to grow *B. icteroides*, finally laying Sanarelli's theory to rest.

In all, 14 nonfatal cases of YF were produced during the Camp Lazear experiments.[117] Reed et al.[69] published the results of their experiments in the Journal of the American Medical Association. The U.S. Army ordered a skeptical Maj William C. Gorgas, the Army's sanitarian in Havana, to complete mosquito source reduction. In 90 days, Havana was free of YF.[107]

### *YF Vaccine*

The Rockefeller Foundation laboratories (New York, New York) developed the 17D live, attenuated, YF vaccine in the 1930s.[106,119–123] In more than 60 years, approximately 400 million people have safely received the vaccine.[111] There have been rare (21 cases) but fatal adverse events (encephalitis) associated with use of the 17D YF vaccine, primarily among young children.[124] Viscerotropic infection following vaccination, similar to natural disease, is thought to reflect atypical host responses rather than genomic instability of the vaccine.[111] Durable immunity, in the form of protective neutralizing antibodies, is found for 90% of vaccinees within 10 days and for 99% within 30 days after vaccination.[141]

## Arenaviruses

The arenaviruses constitute the family Arenaviridae, small, enveloped, single-strand, RNA viruses with a unique genetic arrangement. The genome is divided into two segments, designated short and long, each coding for final protein products in an ambisense orientation. Ribosomes contained within virions give the characteristic sandy appearance of the virus by electron microscopy, thus, the origin of the name arena (Latin, sand).[127] New arenaviruses are being discovered at a rate of one every 2 to 3 years. The 20 known species of the family belong to a single genus, *Arenavirus*, but are taxonomically divided into Old World and New World (Tacaribe complex) groups.[127–129] All arenaviruses have close associations with specific rodent hosts, and humans can become infected when exposed to these rodents or, more particularly, their excreta.[130]

### U.S. Military Significance

A minority of arenaviruses cause significant human disease, and only a select few are of military importance. Hemorrhagic fever arenavirus infections pose a profound threat to troops deployed to disease-endemic areas, or potentially as biological weapons; these are Lassa fever and the South American hemorrhagic fevers. Of the latter, Argentine hemorrhagic fever (caused by Junin virus) and Bolivian hemorrhagic fever (caused by Machupo virus) are the most common. Lassa virus infection is frequently mild or subclinical but, of the 5 to 10% of infected persons who develop disease serious enough to require hospitalization, 15 to 25% perish. Lassa fever is differentiated from the other arenavirus hemorrhagic fevers by its low incidence (17%) of frank hemorrhage or petechiae and its proclivity for person-to-person spread. The South American hemorrhagic fevers are more severe, with overall mortality rates of 15 to 30%. Hemorrhagic features are much more common in severe cases, and neurological symptoms are frequently prominent. Death from arenavirus hemorrhagic fever is thought to result from shock and circulatory collapse, although the precise mechanism of these events is still unclear.[127,130] The hemorrhagic fever arenaviruses are highly contagious by aerosol and require Biosafety Level 4 containment for laboratory work.

### U.S. Military Contributions

Military medical scientists have been at the forefront of researching these diseases. Because of the biocontainment issues related to arenavirus research, most military contributions have originated from the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) in Fort Detrick, Maryland. Work performed at USAMRIID was responsible for much of our present understanding of arenavirus infections. In fact, the original descriptions of two members of the Tacaribe complex, Oliveros virus and Guanarito virus (the agent of Venzeuelan hemorrhagic fever) are attributable to work done at USAMRIID.[131,132] Key contributions were made in animal model development, pathophysiology and pathogenesis, human and rodent reservoir epidemiology, and vaccine and therapeutic agent development. Names associated with USAMRIID research are among the most prominent in the field of arenavirus study, including C.J. Peters, Gerald Eddy, Peter Jahrling, Karl Johnson, and others.

In 1972, then-LTC Gerald Eddy left the National Institute of Allergy and Infectious Diseases Mid-American Research Unit, where he had been involved in early groundbreaking research with Karl Johnson on South American arenaviruses, to become the chief of virology at USAMRIID. Early arenavirus research under Dr. Eddy focused on Machupo virus. The most important

contributions from this time were the establishment of guinea pig and primate models for further study using a variety of monkey species.[133,134] Pathology and pathogenesis studies in these models greatly advanced our understanding of the arenavirus hemorrhagic fevers.[135–138] Dr. Eddy's group was the first to demonstrate that immune serum protected primates from acute disease in Machupo virus infection, confirming early reports of successful passive immunization in other arenavirus infections.[139] Their studies also described the late neurological disease that is now a well-known sequela of arenavirus hemorrhagic fever treated with immune serum.[135,140]

In the early days of USAMRIID, arenavirus work was limited to the South American hemorrhagic fevers, because the Centers for Disease Control and Prevention (CDC) had laid claim to all research with Lassa virus (P. Jahrling, personal communication). This arrangement came to an abrupt end when a laboratory accident involving a tube of serum from infected *Mastomys* rodents exposed two CDC laboratory workers to a potentially fatal dose of Lassa virus. The two patients were brought to USAMRIID's clinical biocontainment facility, affectionately called "The Slammer." Their presence and the CDC's request for assistance launched USAMRIID's work on Lassa fever. Taking direction mostly from Peter Jahrling and C.J. Peters, Lassa fever research at USAMRIID made tremendous strides in passive immunization studies, drug discovery, and pathogenesis models.

Perhaps USAMRIID's most profound contribution was in therapeutic interventions for Lassa fever. Research at USAMRIID demonstrated that ribavirin could successfully treat Lassa virus infection in primate models.[141–143] Based on this research, the CDC and the Sierra Leone Ministry of Health conducted a series of human trials that definitively established ribavirin as the treatment of choice in severe Lassa fever.[144]

Further work at USAMRIID looked at passive immune therapy. Animal model studies (many done at USAMRIID) and clinical experience indicated limited efficacy of immune serum in treating Lassa fever, possibly because of the late development and low titer of neutralizing antibodies after infection.[130,145,146] However, animal studies conducted by Peter Jahrling and C.J. Peter's group did demonstrate efficacy of passive immune therapy (alone or in combination with ribavirin) if the immune serum was strain-specific and of high titer.[143] Using a technique developed at USAMRIID, serum plasmapheresed from Lassa fever patients in Liberia was selected for high therapeutic efficacy.[147] Unfortunately, the immunoglobulin in those specimens was destroyed during processing at a biotechnology company, and efficacy in humans was never tested (P. Jahrling, personal communication).

The therapeutics research was far from USAMRIID's only output regarding Lassa fever and arenavirus infections in general. Throughout this period, USAMRIID investigators produced instrumental studies on the pathogenesis and immunology of Lassa virus, in a variety of rodent and primate models.[142,145,146] Many of these examples are still the prototypical models for the study of Lassa virus today. Significant contributions were made in developing diagnostic serological tests and studying the aerosol stability and infectivity of Lassa virus.[148,149] Studies of Lassa fever patients in Liberia yielded discoveries about serological and biological diversity among Lassa virus isolates, timing of viremia, and humoral immune responses in human disease.[147,150] Finally, model development of lymphocytic choriomeningitis virus infection in rodents and primates provided valuable tools for studies of Old World arenavirus infections. For New World arenavirus studies, a lethal guinea pig model of Pichinde virus (a member of the Tacaribe complex) infection was developed at USAMRIID, allowing lethal arenavirus animal model research to be conducted with minimal biocontainment.[145,151,152]

While Lassa virus research progressed, USAMRIID continued to make strides in the field of the South American hemorrhagic fevers that were equally impressive. Guinea pig and primate model research continued, demonstrating pronounced strain-specific virulence, the tendency for New World arenaviruses to cause neurological disease, high infectivity by the aerosol route, greater importance of humoral immune responses (especially antibody-dependent cell-mediated cytotoxicity) for recovery compared with Lassa virus infection, and independence from immune responses of the physiological and organ system derangements caused by lethal infection.[145,153–158]

Significant progress was also made in therapeutic research for the South American hemorrhagic fevers. A double-blind trial performed in Argentina in 1979 definitively proved that immune serum treatment was effective against Argentine hemorrhagic fever, but the mechanisms of its efficacy and of the relatively frequent late neurological syndrome that followed remained a mystery.[139] USAMRIID studies of guinea pig infections found that protection was based on antibody-dependent clearance of infected cells, rather than neutralization of virus.[159,160] The neurological disease following immunotherapy was studied extensively in primate models. Animals with late neurological disease were found to have evidence of virus in brain tissue, a feature not found in the course of untreated infections. Although late neurological disease was seen more often with later initiation of treatment, it sometimes manifested in animals treated early and with higher doses of antiserum. Therefore, the mechanism of this syndrome was never completely elucidated.[134–136,158] In addition to immune serum, guinea pig and primate studies of Junin infection showed beneficial responses to ribavirin, although late neurological manifestations were seen among some survivors.[153,161,162] These studies paved the way for clinical trials of ribavirin in Argentine hemorrhagic fever, and this therapy is now considered a useful, if secondary, treatment.

Perhaps the most remarkable story in USAMRIID's arenavirus research history is the development of the effective Candid 1 vaccine for Argentine hemorrhagic fever. In the middle 1970s, a prominent Argentine scientist, Dr. Julio Barrera Oro of the Instituto Malbran, Buenos Aires, came to USAMRIID through a collaborative effort with the Pan American Health Organization. He was advised through frequent contacts and visits to USAMRIID by Dr. Julio Maiztegui of the Laboratorio Nacional de Fiebre Hemorrhagica, Pergamino, Argentina. Both of these men worked closely with USAMRIID scientists Drs. Gerald Eddy and George French. Dr. Barrera Oro started with the XJ clone 3 isolate, a partially attenuated, mouse brain-passaged strain of Junin virus originally obtained from Dr. Jordi Casals at Yale University. It was adapted to diploid fetal rhesus lung cell culture at USAMRIID for use as a possible candidate vaccine by repeated cloning through limiting virus dilution and plaque selection. All subsequent passages, in-

cluding primary and secondary seed preparation and vaccine production, were conducted in rigorously certified diploid cell cultures (G. Eddy, personal communication). This new virus proved to be genetically stable and was called "Candidate #1." It had no detectable peripheral virulence or neurovirulence in cell culture or rodent models. Dr. Barrera Oro truncated the name to "Candid 1," and study progressed toward human trials. Safety and immunogenicity studies were successful in primates, and Candid 1 proved to be 100% protective in a rhesus model of Junin infection, even at a very low dose of vaccine, when administered intramuscularly.[156,163] Candid 1 vaccine was produced at the Salk Institute facility in Swiftwater, Pennsylvania, and, after Phase I and II studies at USAMRIID and in Argentina, a large, placebo-controlled, randomized, blinded trial was conducted in Argentina.[164] In 1988, USAMRIID researchers collaborated with Dr. Maiztegui at the Argentine National Institute for Hemorrhagic Fever Viruses, the Johns Hopkins University School of Public Health, and the Salk Institute to enroll 6,500 participants in a highly disease-endemic region in Argentina. Of the 23 participants who developed Argentine hemorrhagic fever during the next two harvest seasons, 22 had received the placebo vaccine, yielding a vaccine efficacy of 95%. Rodent and nonhuman primate studies have since shown probable efficacy against Machupo virus infection as well. Since the Argentine trial, more than 175,000 doses of Candid 1 have been administered in Argentina, dramatically reducing the incidence of Argentine hemorrhagic fever. Unfortunately, lack of financial incentives and lack of production facilities in Argentina have made future prospects for Candid 1 tenuous.[127]

## Filoviruses

Although never having caused a symptomatic infection in an American, the filoviruses have become widely recognized names in this country, carrying with them a menacing stigma shared by few, if any, other diseases. The two members of the family Filoviridae, Marburg virus and Ebola virus, are prototypes of emerging infectious diseases. Both have been discovered in the past 40 years, with human outbreaks arising at irregular intervals, from human settlements in the deepest regions of sub-Saharan rain forests, and with increasing frequency in the past 10 years. The mysterious reservoir of these viruses, the unpredictable nature of outbreaks, the high mortality rates associated with infection, the gruesome descriptions of deaths from these viruses in the popular press, the unveiling of Soviet filovirus biological warfare programs, and the appearance of Ebola virus on American soil in 1989 have fueled an increased research effort over the past 15 years. Many of the most significant advances in filovirus research have come from U.S. military scientists.

As the name implies (*filo* is thread in Latin), the Filoviridae are uniquely structured viruses, having a rope-like, often filamentous appearance under electron microscopy. The virions consist of a helical nucleocapsid of closely associated RNA and protein, with a tight-fitting envelope derived from the host cell and studded with viral proteins. Genomes are composed of a single segment of negative-sense RNA of approximately 19 kilobases, with a number of unique features.[165] Recent taxonomic convention has clarified the relationship between the filoviruses. Because of a lack of serological cross-reactivity and the existence of differences in structure and genomic sequence, Ebola virus and Marburg virus have been classified as separate genera. Although Marburg virus has only one species, Ebola virus has four, i.e., Zaire, Sudan, Reston, and Cote d'Ivoire.[166] In addition to genetic heterogeneity, the filoviruses are differentiated by epidemiological and clinical (specifically, mortality rates) features.[167] Because all filoviruses cause hemorrhagic fevers with high mortality rates and are transmissible by the airborne route, they are classified as Biosafety Level 4 agents.

### U.S. Military Significance

Filoviruses continue to be a focus for military biomedical research for two reasons, namely, the threat to troops deployed to disease-endemic areas and the threat of use as biological weapons. Filoviruses are stable in aerosol and are highly infectious by this route, making them attractive candidates for weaponization.[168] Research for biodefense against filoviruses was spurred in the early 1990s by reports that the Soviet Union had active programs researching biowarfare applications of filoviruses and had potentially produced weapons with Marburg virus (P. Jahrling, personal communication).

The threat to deploying troops is centered in equatorial Africa. Marburg virus was discovered in 1967, when monkeys shipped from Uganda caused infection among laboratory workers in Germany and Yugoslavia. Since then, western/central Africa has remained the epicenter of human and nonhuman primate outbreaks of both Marburg virus and Ebola virus.[70,165,169,170]

A disturbing new development in the history of filoviruses occurred in 1989, when a new strain of Ebola virus was discovered in laboratory monkeys in Reston, Virginia.[171] These animals, as well several other shipments of monkeys later found to be infected with the newly discovered Ebola-Reston strain, were traced to a single distributor in the Philippines.[165] The origins and location of Ebola virus in the Philippines remain a mystery.

Filovirus infections among deployed troops are of concern for several reasons. First, without working knowledge of a reservoir and route of acquisition in index cases, it is difficult to implement protective measures. Second, the mortality rates of these hemorrhagic fevers are potentially catastrophic for a deployed force. Mortality rates for Marburg hemorrhagic fever are generally quoted at 25%, although the recent outbreak in the Democratic Republic of the Congo had a reported mortality rate of more than 80%.[130,169] Disease from Ebola virus infection is fatal in 50 to 90% of cases, with disease from Ebola-Zaire occupying the high end of this spectrum. Only one case of Ebola-Cote d'Ivoire infection, which was not fatal, has been documented. Ebola-Reston, although highly pathogenic in monkeys, has yet to cause disease among humans, although four people have had serological evidence of infection.[165] However, the facts that Ebola-Reston appears to be easily transmitted through an airborne route, has a close relationship to Ebola-Zaire, and apparently exists outside Africa make it a concerning potential threat.

### U.S. Military Contributions

Dr. Eugene Johnson was the impetus behind the earliest significant filovirus research in the U.S. military. A legendary eccentric, Gene Johnson was a civilian scientist at USAMRIID in the late 1970s and 1980s. He was well known in the field of

filovirus research, evoking tremendous respect for his laboratory research and field epidemiology and at the same time causing profound frustration because he rarely published any of his work. In 1982, Karl Johnson moved from the Special Pathogens Division of CDC to become chief scientist at USAMRIID. He brought with him samples of Ebola-Zaire and gave them to Gene Johnson (P. Jahrling, personal communication). Using this seed, Gene Johnson, veterinary pathologist Nancy Jaax, and a group of researchers at USAMRIID produced some of the earliest and most important primate pathogenesis studies. Among the most important discoveries were the demonstration of aerosol transmission of filoviruses and the extensive pathological description of disease in primates.[172,173]

In the middle/late 1980s, Gene Johnson's work focused on field epidemiology in an attempt to find the natural reservoir and transmission source for filoviruses, particularly Marburg virus. With the assistance of C.J. Peters and others, Dr. Johnson led USAMRIID researchers across the heart of Africa, trapping and testing thousands of insects, rodents, birds, and primates (P. Jahrling, personal communication). Particular efforts centered on Kitum cave, a common exposure site among some sporadic Marburg virus cases located near the border of Kenya and Uganda. These expeditions did not find a natural reservoir for filoviruses, adding to the mystery surrounding these viruses. They did provide valuable practice in field biocontainment procedures, and Richard Preston's recounting of Gene Johnson's team entering Kitum cave adorned with military gas masks and flower-patterned pillowcases makes for classic reading.[174]

Military filovirus research was launched in earnest by a convergence of two phenomena at the start of the 1990s (P. Jahrling, personal communication). Intelligence reports of Soviet filovirus biowarfare research, fueled by the testimony of defected scientist Ken Alibek, caused the sudden recognition of a significant military and national security threat. As this came to light, USAMRIID dove into filovirus research when electron-microscopist Tom Geisbert stared at the image of an obvious filovirus isolated from a fatal epizootic that was ravaging in a primate housing facility in Reston, Virginia.

Made famous in Richard Preston's book,[174] the Reston outbreak was monumental for several reasons. Under the direction of Gene Johnson, C.J. Peters, and Peter Jahrling, USAMRIID researchers discovered that this was a new species of Ebola virus, which they named Ebola-Reston. The new virus was highly pathogenic in monkeys but apparently not in humans. The researchers dispelled the idea that filoviruses were found only in Africa, because the monkeys had been imported from the Philippines.[130,175] The investigators documented a high likelihood of aerosol transmission outside a controlled laboratory setting, because the virus appeared to pass between rooms to infect susceptible monkeys.[165] Specimens from animals that died or were killed to eradicate the outbreak yielded fertile ground for research in new Ebola virus detection and identification techniques and the virological and pathological events associated with infection.[171,176–181]

The Reston experience spurred a boom of Ebola virus research at USAMRIID. Important progress was made in understanding the transmission, pathophysiology, and immunology in primate models of infection.[172,181–183] USAMRIID developed enzyme immunoassays for improved and timely diagnosis of Ebola virus infections, and USAMRIID researchers remained involved in field studies in Africa, attempting to isolate vectors or reservoirs.[184–187]

USAMRIID has continued to produce some of the most important advances in filovirus research in the past decade, with the goal of finding preventive or therapeutic interventions for filovirus infections. Mouse and guinea pig models developed by USAMRIID researchers have been invaluable tools in vaccine and therapeutic research.[188–191] Although humoral immunity is thought to play a secondary role in protection and recovery, studies of passive immune therapy at USAMRIID have shown some beneficial effect that may be amplified if selected monoclonal antibodies are used.[181,190,192–194] The first vaccine to protect primates from Marburg virus challenge, a Venezuelan equine encephalitis replicon vaccine, was developed at USAMRIID.[195] Finally, USAMRIID has been intimately involved in the development of DNA- and adenovirus-vectored vaccines for Ebola virus, the combination of which has provided the first protection against Ebola virus infection in primates and will start human testing within the next several years.[166,196,197]

Because of mission and biocontainment requirements, military research with filoviruses has been confined to USAMRIID. From the laboratory and field research of Gene Johnson to the Reston outbreak to vaccine development, USAMRIID has been at the forefront of discovery in the study of Marburg and Ebola viruses over the past 20 years. Efforts continue across a wide spectrum of filovirus research and, as biodefense becomes a more urgent need in the 21st century, USAMRIID should continue to be an integral part of this filovirus research.

## Hemorrhagic Fever with Renal Syndrome

The viruses that produce hemorrhagic fever with renal syndrome (HFRS) are in the family Bunyaviridae, genus *Hantavirus*. The Bunyaviridae share similar morphological features, with a spherical virion and a size between 80 and 120 nm.[198] They contain a lipid envelope with two glycoproteins, which determine cell tropism and host pathogenicity and are sites for viral neutralization by antibody.[189,190,199,200] The genetic organization of the Bunyaviridae consists of a single negative strand of RNA organized into three segments, i.e., large, medium, and small segments, which code for the virus nucleocapsid, glycoproteins, and polymerase proteins, respectively.[201,202] Viral factors for the Bunyaviridae that are associated with human disease are medium segment-encoded polyproteins that contain a mucin-like domain and a furin cleavage site.[203] Mucin-like domains and furin cleavage sites have been implicated in causing endothelial damage, cellular cytotoxicity, and interferon antagonism.[204] The Bunyaviridae may exert a direct effect on host gene regulation during infection, as evidenced by the hantaviruses' ability to suppress cellular interferon responses.[205]

There are more than 20 genotypes of the genus *Hantavirus*, which are maintained in the environment by specific rodent species.[206] Specific viruses in the genus *Hantavirus* are the Hantaan, Puumala, Seoul, Dobrava Belgrade, and Saarema viruses. All have specific geographic locations, as determined by the rodent host. Hantaan virus occurs in eastern Asia, Puumala virus in northern and eastern Europe, Seoul virus in Asia, and Dobrava Belgrade and Saarema viruses in central Europe.[203,206,207] Human infection occurs by inhalation of infected

rodent excreta. The genotype of *Hantavirus* determines the severity of clinical disease. Puumala virus, for example, produces nephropathia epidemica, which is a milder form of clinical illness, with a mortality rate of less than 1%.[208,209] Seasonal occurrence is largely determined by the rodent species and human behavior leading to exposure. In Korea, two peaks occur, in the spring and autumn, with the latter being the largest peak of disease occurrence.[210] Persons at risk for infection are those with the greatest risk of exposure to rodent excreta, including farmers and soldiers. The age and gender distributions of cases reflect this population at risk, with cases occurring primarily among adult men.[211]

The initial presentation of HFRS can be an abrupt onset of fever with severe headache, myalgia, and back and abdominal pain. Following an initial febrile period, there is an onset of hemorrhage, elevation of liver enzyme levels, and acute renal failure; renal failure is initially oliguric, followed by a polyuric phase.[212] Unlike with the other viral hemorrhagic fever viruses, leukocytosis is a common laboratory finding. Renal biopsies revealed acute tubular necrosis, with interstitial cell infiltration and edema. Common causes of death are shock, respiratory failure, and pulmonary hemorrhage.[212] In a retrospective analysis of HFRS among 26 U.S. soldiers, two patients had an initial presentation of severe shock and hemorrhage, with rapid onset of death.[213] Eighteen patients presented with acute renal failure lasting approximately 21 days, and five patients presented with mild renal dysfunction. Several patients developed acute pulmonary edema requiring hemodialysis, and retroperitoneal hemorrhage was a major complication in this group. Six patients had a febrile illness with normal renal function, thrombocytopenia, abnormal urinalysis results, and transient elevation of liver enzyme levels.[213]

### U.S. Military Significance

HFRS has been described since the early 1900s in China, the former Soviet Union, Scandinavia, and eastern Europe.[214,215] On December 22, 1951, a symposium was held on epidemic hemorrhagic fever in the Far East Command, convened by BG William E. Shambora, Chief Surgeon, Far East Command (Smadel Library Collection, WRAIR, Silver Spring, Maryland). COL Joseph H. McNinch, Chief, Preventive Medicine Division, Office of Chief Surgeon, Far East Command, introduced the symposium and wrote,

> Today we have before us for discussion a disease entity which has created intense interest among both medical and lay personnel in the Far East Command. During the month of June 1951 there were admitted to the U.S. medical installations in Korea several patients with an acute febrile disease presenting a combination of symptoms and signs not previously encountered among United Nations personnel in Korea. The symptoms included malaise, weakness, chills, fever, headache (especially retrobulbar ache), blurred vision, nausea, and vomiting. These symptoms were associated with manifestations of hemorrhagic diathesis: petechial rash, marked injection of the conjunctiva, hematuria, and hematemesis (unpublished data).

COL McNinch went on to discuss the possible etiologies that were investigated. The first was drug sensitivity to chloroquine for the treatment of malaria. It soon became apparent that many soldiers who developed this condition had no history of chloroquine treatment. The next focus was on leptospirosis as a possible etiology. Leptospirosis was eliminated as a cause because of negative serological tests and pathology findings on autopsy that were not typical for leptospirosis. Between June 28 and July 23, 1951, 55 cases of this syndrome were observed, all from combat units of the 8th Army and the majority from a single division located northeast of Seoul. In July 1951, Surgeon General George Armstrong and MG Edgar Hume, Chief Surgeon of the Far East Command, were on an inspection visit in Korea and noted the high incidence of the leptospirosis-like illness. They arranged for COL Arthur Long, Far East Command consultant in preventive medicine, to investigate. He observed the clinical illness and called in COL Richard Mason, clinical pathologist from the Army Medical Service Graduate School, who confirmed that the disease entity was unlikely to be leptospirosis. Reports of the presence of a large number of field rodents in the areas from which cases were originating were confirmed. At that time, attention was directed toward a disease that the Japanese Army had encountered in Manchuria in 1939–1941. This disease was called by a variety of names, including Songo fever, Kokka disease, Korin fever, Nidoko disease, and epidemic hemorrhagic fever. Working with Japanese associates, a comparison of pathology slides for Japanese soldiers and U.S. soldiers confirmed that epidemic hemorrhagic fever, as described in the Japanese literature, was the same disease as that occurring among United Nation soldiers in Korea. A second peak to the epidemic occurred during 1951, with 28 cases, and confirmed the observations by Japanese military physicians. Case-fatality rates ranged from 13 to 18%. The Japanese described epidemic hemorrhagic fever as being attributable to a filterable virus maintained in the field rodent *Apodemus agrarius*. During this symposium, results of the investigation were presented by a number of Army scientists, including then-CPT Edward Buescher from the 406th Medical General Laboratory.

### U.S. Military Contributions

The initial outbreak of hemorrhagic fever among United Nation soldiers was followed by the creation, by the Armed Forces Epidemiological Board (AFEB), of a Commission on Hemorrhagic Fever in 1952, to investigate the outbreak among United Nation forces in Korea.[262] Members of the AFEB Epidemic Hemorrhagic Fever Field Research Unit included Dr. Ross L. Gauld, Dr. Quentin M. Geiman, Dr. Marshall Hertig, Dr. Joseph E. Smadel, Dr. Kenneth Smithburn, and LTC Robert Traub. Results of the investigation at that time were reviewed, including human cell culture results, egg passage, inoculation in guinea pigs and nonhuman primates, ecological studies, and pathology of renal tissue. CPT Carleton Gajdusek reported his results reviewing hemorrhagic fever in the Soviet Union. The AFEB continued to support the Commission on Hemorrhagic Fever and directed the scientific investigation of this disease by both Army and U.S. civilian scientists. Scientific personnel increased to more than 50 members, who directed the scientific investigation of the epidemic, the training of physicians (including a 45-minute movie on the clinical care and epidemiology of the disease), and the publication of results in a special supplement of the *American Journal of Medicine*.[263] By the end of 1954, more than 29 scientific publications on this disease had been presented by military scientists. The Korean War resulted in more

than 3,000 United Nation soldiers developing hemorrhagic disease, with renal failure, shock, and death in 10 to 15% of cases.[214,215] The etiological agent of this disease in Korea was isolated in 1967 from the rodent *A. agrarius* and was named Hantaan virus after the Hantaan River.

Military scientists continued to investigate HFRS, with numerous publications and the development of a vaccine program that continues in the Military Infectious Disease Research Program. Intravenous ribavirin (Viratek Pharmaceuticals) therapy was found to be effective for HFRS, and this is currently an Investigational New Drug with the U.S. Food and Drug Administration (U.S. Department of Defense Investigational New Drug 16,666). The only double-blind, placebo-controlled, clinical trial of intravenous ribavirin therapy was conducted among 242 patients with HFRS in the People's Republic of China by scientists from the USAMRIID.[216] Mortality rates were reduced sevenfold for the ribavirin-treated patients, and ribavirin therapy resulted in significant reductions in the risks of entering the oliguric renal phase and of developing hemorrhagic manifestations.[216]

Today military scientists continue to address the hantaviruses as a military threat and have published seminal articles on the epidemiology, pathogenesis, and virology of these viruses.[202,211,217–236] The Department of Defense Military Infectious Disease Research Program continues in its efforts to develop an effective vaccine against HFRS.

## Rift Valley Fever

Rift Valley fever (RVF) virus is in the family Bunyaviridae, genus *Phlebovirus*. As previously noted, the Bunyaviridae share similar morphological features, with a spherical virion and a size between 80 and 120 nm.[198]

### U.S. Military Significance

RVF is an acute zoonotic disease that affects both ruminant animals and humans and occurs as an epizootic, with transmission to humans primarily from infected mosquitoes (*Culex*, *Aedes*, and *Anopheles* species) and secondarily from the handling of infected animal carcasses.[214] RVF virus was isolated in 1930 in the Rift Valley of Kenya, in East Africa, and has been responsible for more than 30 large outbreaks of animal and human disease in East Africa since the 1930s. Weather pattern analysis demonstrated that RVF outbreaks followed periods of abnormally high rainfall, which were predictive up to 5 months in advance of outbreaks.[237] In 1977, RVF was responsible for a large outbreak of animal and human disease in Egypt, involving more than 18,000 clinical cases and 598 deaths.[238] Subsequent outbreaks occurred in Mauritania in 1987, again in Egypt during 1993, and recently in Yemen and the Kingdom of Saudi Arabia in 2000. Saudi Arabia reported a total of 886 cases and a case fatality rate of 13.9%.[239] The majority of cases in Saudi Arabia occurred among adult men with significant risk factors for exposure to mosquito bites and infected animals. Age-specific mortality rates were greatest for the elderly, with an overall mortality rate in the population of 14%.[240] RVF virus is considered an emerging pathogen, causing considerable economic loss among domestic animals and human disease. Factors responsible for its emergence include the movement of infected livestock and mosquito vectors, global weather pattern changes, and economic development resulting in environmental conditions favoring mosquito breeding, such as periods of heavy rainfall or the building of dams, with associated flooding of plains.[239]

The major clinical characteristics of RVF include hepatocellular failure, acute renal failure, and hemorrhagic manifestations.[241] Development of retinitis and meningoencephalitis is a late complication of the disease. Death has been observed in 33.9% of cases. Hepatorenal failure, shock, and severe anemia were all factors associated with death.[241]

### U.S. Military Contributions

Military scientists have contributed to the understanding of the epidemiology, pathogenesis, and diagnosis of RVF. The full potential of RVF as a human pathogen and military threat was determined by military scientists from the Naval Medical Research Unit in Cairo, Egypt, during a large outbreak of RVF in Egypt in 1977.[238] Four clinical syndromes were documented during that outbreak, i.e., a febrile illness, encephalitis, ocular complications, and hemorrhagic disease. Other contributions have been characterization of the virus and its vector and transmission cycle and the development of diagnostic assays.[239,242–244] One of the most significant achievements by military scientists was in the analysis of weather patterns as a predictive model for RVF outbreaks.[237,245–247]

## Chikungunya

Chikungunya (CHIK) virus is classified in the family Togaviridae, genus *Alphavirus*. The alphaviruses contain a nucleocapsid enclosed within a lipoprotein envelope containing a single strand of positive-polarity RNA. Two viral envelope glycoproteins, termed E1 and E2, exist and function as a heterodimer and the site for antibody neutralization.[248] CHIK is antigenically closely related to other alphaviruses, including O'nyong-nyong, Mayaro, and Semliki Forest viruses, and is serologically indistinct from O'nyong-nyong virus. CHIK is transmitted to humans by *Aedes* mosquitoes, primarily *A. aegypti* and *Aedes africanus*.[249,250]

Human infection with CHIK is manifested by the sudden onset of fever, myalgia, headache, ocular pain, sore throat, nausea, and vomiting.[250] A maculopapular rash develops and is accompanied by enlarged tender lymph nodes. Severe joint arthralgias are common; they occur during the acute period and can last for several months into convalescence. Hemorrhagic manifestations can occur during acute CHIK infection and were observed during outbreaks in India and Southeast Asia. The hemorrhagic manifestations of CHIK were first observed during the early 1960s in Bangkok, Thailand, where approximately 10% of children admitted for dengue hemorrhagic fever were in fact suffering from CHIK virus.[250–254] Outbreaks of CHIK have been identified in Southeast Asia, India, Zambia, southeastern Zimbabwe, and Zaire.

### U.S. Military Significance

CHIK was not a major problem among U.S. forces deployed in Vietnam and was not a significant factor in previous military operations. As an arbovirus with epidemic potential that can produce a sudden debilitating disease, its potential as a serious military threat is considerable.

### U.S. Military Contributions

Military scientists have contributed to our understanding of the epidemiology, transmission, and pathogenesis of CHIK virus infections. The significance of CHIK virus as a human pathogen was demonstrated during the early part of the dengue hemorrhagic fever outbreak in Southeast Asia, where its clinical manifestations and potential as a hemorrhagic fever virus were described. Military scientists also have contributed significantly in the development of both killed and live attenuated CHIK vaccines, demonstrating low reactogenicity and high immunogenicity in Phase I clinical studies.[255–258]

## Summary of Key U.S. Military Contributions

The viral hemorrhagic fever viruses are a unique group of viruses that can produce severe, highly fatal human illnesses following the bites of mosquitoes or ticks, infected rodent or domestic animal exposure, or contact with other infected humans. They share common features in being able to directly induce cellular damage and to elicit an immune response among humans that can result in severe hemorrhage, coagulopathy, shock, and death. The characteristics of arthropod-borne or rodent-borne transmission, combined with illnesses that result in high morbidity and mortality rates, make the viral hemorrhagic fever viruses a unique threat to deployed soldiers around the world. The viral hemorrhagic fever viruses have historically been a major cause of disease for both U.S. and foreign soldiers, are currently a major cause of morbidity among U.S. soldiers, and will certainly be an ever-present disease threat for the U.S. military. The key military contributions are as follows: (1) leaders in the development of knowledge on the epidemiology and pathogenesis of dengue fever and dengue hemorrhagic fever, (2) development of numerous candidate dengue vaccines and a live attenuated dengue tetravalent vaccine currently in human clinical trials, (3) instrumental in gaining knowledge on the epidemiology and pathogenesis of YF, including the discovery of its transmission to humans from the bites of mosquitoes, (4) development of fundamental knowledge on the epidemiology and pathogenesis of the arenaviruses, (5) contributions in the characterization and testing of the Junin vaccine, (6) development of fundamental knowledge on the epidemiology and pathogenesis of filoviruses and filovirus vaccine development, (7) leaders in the development of knowledge on the epidemiology and pathogenesis of HFRS, (8) development of an effective antiviral drug against HFRS, ribavirin, and the vaccine development program, and (9) development of fundamental knowledge on the epidemiology and pathogenesis of RVF and CHIK.

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