Homeland Defense & Security Information Analysis Center

Countermeasures Against the Degradation of Warfighter Capabilities due to Infectious Disease Threats

STATE OF THE ART REPORT

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ABOUT THIS PUBLICATION:

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Dr. Endy is an international expert in the field of dengue and dengue hemorrhagic fever and emerging viral pathogens. Dr. Endy has conducted basic scientific research in the field of virology, developed vaccine field and epidemiological study sites in Southeast and Central Asia, conducted phase I and II clinical vaccine trials and is active in the development and management of research programs that are product oriented towards developing vaccines and diagnostics that meet U.S. Food and Drug Administration (FDA) regulatory requirements. Currently, Dr. Endy is the principal investigator for the US Army's Dengue Human Infection Model (DHIM), being developed at The State University of New York (SUNY) Upstate and a co-principal investigator for the clinical development of a heroin vaccine. Dr. Endy is an active reviewer for peer reviewed journals, a subject matter expert on dengue and dengue vaccine development for industry partners, has published over 120 manuscripts and book chapters, and is a National Institutes of Health (NIH) funded researcher conducting studies on dengue in Thailand. He is a board certified physician in the subspecialty of infectious diseases and internal medicine and did his residency and fellowship training at the Walter Reed Army Medical Center, Washington, DC. Dr. Endy obtained his medical school training at the Uniformed Services University, F. Edward Herbert School of Medicine, Bethesda, MD; received a Master of Public Health in Epidemiology from the University of Michigan, School of Public Health, Ann Arbor, MI; and a degree from the Pennsylvania State University, University Park, PA. He served with distinction in the United States Army for 24 years and retired as a Colonel in 2006.

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Dr. Paolino attended the SUNY Buffalo School of Medicine under the Armed Forces Health Professions Scholarship Program and was trained at Walter Reed Army Medical Center in Washington, DC, becoming board certified in both internal medicine and infectious diseases. He then spent over 5 years working as a clinician researcher at the Walter Reed Army Institute of Research where he served as Chief of the Clinical Trials Center (CTC), overseeing over 24 clinical trials to include the first site to vaccinate a patient with the current leading ebola vaccine, as well as several other leading vaccine candidates against tropical disease treats. He is currently part of the infectious disease faculty at SUNY Upstate Medical University, faculty mentor for the University's Resident Research Council and Director of Clinical Research at the Upstate Institute for Global Health and Translational Sciences. He has developed a clinical and research interest in Lyme disease and other tick-borne diseases.

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Dr. Thomas is a Professor of Medicine, Professor of Microbiology & Immunology, and Infectious Diseases physician-scientist from the State University of New York (SUNY), Upstate Medical University in Syracuse, NY. He is the Chief, Division of Infectious Diseases and Director, Institute for Global Health and Translational Science (IGHTS). Prior to joining SUNY Upstate, Dr. Thomas spent twenty years in the U.S. Army Medical Corps serving at the Walter Reed Army Institute of Research (WRAIR) and completing his career as the Chief Operating Officer of the institute. Dr. Thomas is a virologist and vaccinologist who spent more than 5 years of his early career living and working in Thailand and Southeast Asia. He played a key leadership role advising senior DoD leadership during the West Africa Ebola outbreak and leading his team's planning and execution of Ebola vaccine trials. He was instrumental in developing and implementing his institution's first in human MERS-CoV vaccine trial and Zika vaccine development efforts. At SUNY, Dr. Thomas leads the Infectious Diseases Division responsible for providing in- and out-patient infectious disease consultation. As the IGHTS Director, he provides strategic and technical leadership to multiple initiatives in the US and abroad encompassing education, research, and clinical service opportunities. Current research activities include work on dengue human infection models, development of vaccines against opioid use disorders, Latin America, and Asia-based field-based studies exploring transmission and pathogenesis of arboviral diseases, and early and advanced phase testing of a number of vaccines and therapeutics against tropical diseases. Dr. Thomas earned his Bachelor of Arts with Honors in Biomedical Ethics from Brown University, his Medical Degree from the Albany Medical College, and completed his internship and residency in Internal Medicine and Infectious Diseases fellowship at the Walter Reed Army Medical Center.

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Mara Kiernan is the lead analyst at the Homeland Defense and Security Information Analysis Center (HDIAC) where she contributes expert writing, analysis, and synthesis to research and training products across the HDIAC's 8 technical focus areas. Prior to working for the HDIAC, Mara specialized as a mixed method researcher and has extensive quantitative and qualitative research experience. Her past work spans a number of focus areas, with publications on continuing productivity during presidential administration transitions as well as drone warfare. Mara is currently pursuing her M.S. in Terrorism & Homeland Security Policy at American University, and holds her B.A. in Foreign Affairs and Middle Eastern Languages & Literatures from the University of Virginia.

Executive Summary

The Homeland Defense and Security Information Analysis Center (HDIAC) develops regular state of the art reports in order to provide a compendium of scientific/technical articles that summarize the most current state of research in topic areas of importance to the Department of Defense. These state of the art reports are a means of satisfying user needs for authoritative information directly applicable to their ongoing work. Infectious disease is a subset of the HDIAC medical technical focus area and was chosen due to its historical and ongoing impact on military operations; as of the date of this publication, the world is consumed with the ramifications of infectious disease in light of the 2019 Novel Coronavirus (COVID-19) pandemic, clearly demonstrating the importance of this topic.

Infectious diseases are disorders caused by pathogenic microorganisms such as bacteria, viruses, fungi, or parasites that can be passed by human-to-human contact, by insects or other animals, or by contaminated surfaces, food, or water. By its very nature, warfare lends itself to the spread of such disease, and contagions have had an impact on every conflict. During the Napoleonic wars, eight times more soldiers in the British Army died from disease than from wounds sustained in battle, and Napoleon turned back during a Mediterranean campaign due to plague; in the U.S. Civil War, fully two-thirds of the 650,000 casualties were due to infectious disease. As will be discussed in the following pages, the very conditions anticipated in combat - operating in close quarters in spartan conditions, deployments to remote areas, exposure to unknown pathogens, the rigors and wounds associated with combat, and the potential for encountering weaponized agents - are known to be precursors to the spread of diseases that can and have decimated combat readiness.

That said, improved understanding of the causes of these diseases has led to improved preventative measures over time, leading to decreases in non-combat losses. Accounting for the potential of infectious diseases during peacetime training, contingency planning, and combat operations can mitigate the effects of disease and lead to increased combat readiness through a reduction in non-combat casualties. The purpose of this report is to provide the medical practitioner, the medical planner, and the commander with knowledge that will aid in balancing the myriad competing demands in the most trying of human endeavors.

This report explores the impact of infectious disease on military personnel, providing both an historical and ongoing risk profile of the various infectious diseases that put the warfighter at risk. It includes a look at the historical impact of infectious diseases on past conflicts before going on to detail current and future infectious disease risks, their impact on the warfighter, and challenges in prevention or treatment, and concludes with a quick-look summary of state of the art developments and recommended countermeasures to aid leaders during training and planning.

1

Introduction

1.1 Need for and Definition of Countermeasures Against Infectious Disease

Infectious disease has long been a threat to military forces. Prior to World War I, more soldiers died from infectious disease than from battle injury [1]; during World War II, Gen. Douglas MacArthur complained that for every healthy division, he had two more in either the hospital with or recovering from malaria [2]; and during the Vietnam War, approximately 580,000 work days were lost due to infectious disease in just one year [3].

Despite significant improvement in prevention and treatment measures, infectious diseases have continued to pose a threat to military forces. Their spread can impact not only morbidity and mortality but also significantly erode force readiness. As a recent example, on a two-week mission in Liberia in 2003, mission-capable forces were cut nearly in half when 44% of deployed Marines were incapacitated by malaria [4]. Infectious diseases can also create costly operational and logistical challenges. Early deployments in Operation Iraqi Freedom were heavily impacted by cutaneous leishmaniasis. Of the 1,700 impacted soldiers, many had to be evacuated for treatment [4], an unplanned and expensive operation. And as seen in Figure 1-1, even with advanced therapeutic treatments, disease has continued to be a cause of mortality among deployed U.S. forces.

The primary factor driving the risk of infectious disease to military personnel is deployment to areas with endemic infectious disease. However, several additional factors combine to increase the overall risk. Tight quarters with communal living and training spaces, and deployment to conflict or disaster

zones with compromised food and water safety increase personnel exposure to potential illness. Operational environment can also play a role; harsh climates, high altitudes, and both physical and psychological stress can cause immunological changes that suppress immune response [6]. Forces also face the unique and ongoing threat of exposure to weaponized infectious agents.

War	*Total served*	*Disease deaths*	*Combat deaths*	*Disease to combat deaths (ratio)*	*Disease death rates (%)*
		DISEASE ERA			
Revolutionary (1775–83)	ca.290,000	ca.18,500[a]	7,174	2.6:1 (2.44, 2.73)	6.4 (6.20, 6.56)
War of 1812 (1812–15)	286,730	ca.17,000[a]	2,260	7.5:1 (6.90, 8.25)	5.9 (5.75, 6.10)
Mexican (1846–48)	78,718	10,986	1,548	7.1:1 (6.40, 7.94)	13.9 (13.47, 14.44)
Civil War[b] (1861–65)	2,213,363	224,586	110,070	2.0:1 (2.01, 2.07)	10.2 (10.07, 10.23)
Spanish-American[c] (1898)	280,564	2,565	345	7.4:1 (6.03, 9.54)	0.91[d] (0.84, 0.99)
Philippine (1899–1902)	127,068	2,748	1,037	2.7:1 (2.30, 3.08)	2.2 (2.00, 2.32)
World War I[c] (1917–18)	4,057,101	57,460	50,280	1.1:1 (1.12, 1.17)	1.4 (1.39, 1.44)
		TRAUMA ERA			
World War II[c] (1941–45)	11,260,000	14,904	229,823	0.06:1 (0.063, 0.067)	0.13 (0.128, 0.137)
Korean[c,e] (1950–53)	2,834,000	509	27,709	0.02:1 (0.015, 0.022)	0.02 (0.015, 0.021)
Vietnam (1964–73)	8,744,000	935[f]	47,322	0.02:1 (0.017, 0.022)	0.01 (0.009, 0.012)
Persian Gulf[g] (1990–91)	688,702	30[h]	147	0.2:1 (0.061, 0.391)	0.004 (0.001, 0.008)
Iraq[i] (2003–present)	NA	63	2,854	0.02:1 (0.011, 0.033)	NA

Notes: Statistical methods: Disease-to-combat mortality ratios were computed. Simultaneous confidence intervals (CIs) around the ratios were computed by normal approximation of the binomial distribution, application of Fieller's theorem to the ratio of those approximations, and Bonferroni-adjusted confidence levels. If the CIs for ratios from two wars do not overlap, then those two wars are considered to have statistically significantly different ratios. Simultaneous CIs for the mortality rates were computed similarly. CIs were computed for each ratio and rate. Since none of the CIs for the Disease Era overlaps any of the CIs from the Trauma Era, the two eras are statistically significantly different to the alpha level of <0.01. [a]Estimate includes an undetermined number of deaths by accident, drowning, homicide, suicide, and execution. [b]Union forces only; most of the Confederacy's official records were destroyed during the conflagration of Richmond on April 3, 1865. [c]U.S. Army only. [d]Of the 107,973 volunteers in the national assembly camps who never saw combat, 1,832 died from disease, a mortality rate of 1.6%. Typhoid fever accounted for 86.8% of the total disease deaths (Cirillo 2004b, p. 71). [e]The Army accounted for 2,452 (75.5%) of the 3,249 non-battle deaths that occurred among U.S. forces in the Korean theater. [f]Includes 312 deaths from heart disease and stroke. [g]Includes the mobilization phase (Operation Desert Shield, Aug. 1990–Jan. 1991) and the combat phase (Operation Desert Storm, Jan.–Mar. 1991). [h]Includes 17 deaths from cardiovascular diseases, and only one from infectious disease. [i]As of June 2, 2007.

Figure 1-1. Ratios of Disease Deaths to Combat Deaths (Killed in Action and Died of Wounds) and Disease Mortality Rates among U.S. Armed Forces in America's Principal Wars, 1775-2008 (99.98% Confidence Intervals (CIs) in Parentheses) [5]

Given the risk, the U.S. military has invested in and put numerous countermeasures in place. These countermeasures encompass both prevention – for example, vaccines, disease vector control, chemoprophylaxis – as well as response, including treatment measures such as mobile hospitals, rapid diagnostics, and post-exposure prophylaxis (PEP).

1.2 Problem Statement

Despite the existence of such countermeasures to fight infectious disease, these maladies remain a serious risk to military members and must be understood and taken into consideration when performing contingency planning at all levels of war. For some diseases, effective countermeasures remain in development, with personnel at continuing risk in their absence. For others, existing countermeasures have proven effective when used correctly, but operational constraints and logistical challenges have made their adequate implementation difficult.

While great progress has been made since conflicts such as the Civil War, in which more combatants died from disease than wounds, infectious agents remain a substantial threat to the combat readiness of U.S. military forces. Many of the factors that are known to be precursors to the spread of disease are inherent in military operations; operating in close quarters in spartan conditions, deployments to remote areas and exposure to unknown pathogens, the rigors and wounds associated with combat, and the potential for encountering weaponized agents are but a few of the many unavoidable conditions inherent in the military, both in garrison and in operations.

This report will explore the impact of infectious disease on military personnel, providing both an historical and ongoing risk profile of the various infectious diseases that put the warfighter at risk. It will start with a look at the historical impact of infectious diseases on past conflicts before going on to detail current and future infectious disease risks, their impact on the warfighter, challenges in prevention or treatment, and both state of the art developments and recommended countermeasures. A full summary of the latter two pieces – state of the art developments and recommended countermeasures – can be found at the end of this report.

While exposure to infectious diseases is a “fact of life” associated with military operations, progress has been and must continue to be made. While it will not be possible to plan for every contingency, it is possible to understand the infectious disease threat, plan to mitigate it, and employ countermeasures to reduce its impact on combat readiness. This report provides a history to aid in understanding, ongoing developments to aid in planning, and countermeasures to support the commander in accomplishing his or her mission.

2

Historical Degradation of Warfighter Capabilities due to Infectious Diseases and Countermeasures Against Ongoing Threats

2.1 Introduction and Historical Overview

In 2002, a report entitled "Protecting our Forces" was published by the National Academy of Sciences [7]. This review by a panel of infectious disease experts concluded that infectious agents have been and remain a substantial threat to the operational capacity of U.S. military forces for three distinct reasons: 1) recruits continue to train in groups under crowded conditions, increasing the risk of spread of infectious agents; 2) deployed warfighters, whether on combat or peacekeeping missions, continue to come into contact with pathogens with which they have no prior experience and for which they have no immunity; and 3) warfighters, along with others, face an increasing risk of the intentional use of weaponized infectious agents.

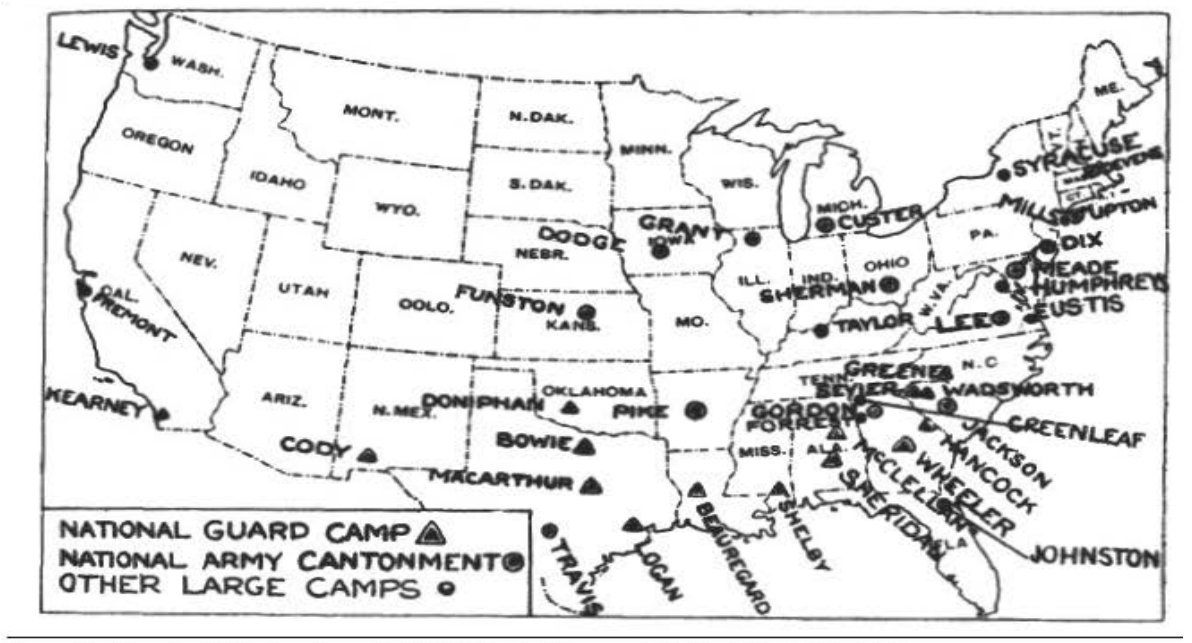
Infectious diseases as a cause of disease nonbattle injuries (DNBI) have historically and continue to be a crucial factor in determining the success or failure of military deployments and combat operations. Napoleon marched his Army to Moscow in June of 1812, seemingly assured of a great conquest. Epidemic typhus caused by a bacteria named *Rickettsia prowazekii*, spread by infected lice, quickly spread through Napoleon's forces, resulting in deaths and DNBI in four-fifths of his Army, stopping their advance and forcing their retreat back to France [8]. General George Washington, Commander of the Continental Army and himself a survivor of smallpox, understood the importance of protecting his soldiers from DNBI, and in particular from smallpox. On February 14, 1777, he issued a command directive that all soldiers undergo variolation consisting of inoculation of smallpox into the skin as a vaccination and protection against severe infection [9]. This action was largely responsible

for maintaining combat effectiveness of his soldiers and their ultimate defeat of British forces. In a more recent example, a longitudinal cohort analysis of DNBI was performed in 4,122 soldiers of a U.S. Army brigade combat team during Operation Iraqi Freedom [10]. During their deployment, the DNBI casualty rate was 257.0/1,000 soldiers, the vast majority due to musculoskeletal injuries and psychiatric disorders with approximately 16% from infectious causes.

In the following sections, infectious DNBI will be presented historically and during contemporary warfighter deployments as an illustration and "lessons learned" of how susceptible and preventable infectious DNBI are to our soldiers.

2.2 WW1 and the 1918 Influenza Pandemic

In 1918, then President of the U.S. Woodrow Wilson declared World War I as "a war to end all wars." The U.S. was training and sending over a million soldiers to fight in France, and numerous training camps across the U.S. were established and expanded (Figure 2-1).



Source: War Department (US). Annual report, 1919. Washington: Government Printing Office; 1920. p. 1519.

Figure 2-1. Locations of Army Training Camps in 1918 [11].

Fort Riley and an expanded cantonment named Camp Funston, both located in Kansas, were the training grounds of the 89th Infantry Division and Camp Devens in Massachusetts was the training grounds of the 12th Infantry Division. Each consisted of 45,000 men living and training in an encampment designed to hold half that number [11]. Figure 2-2 demonstrates the tight quarters for soldiers at the Camp Funston hospital. Overcrowding is a known risk factor for the transmission of a number of diseases, including respiratory pathogens and those transmitted by a fecal-oral route. Aerosolization from a cough or sneeze for example can transmit viruses up to 6 feet, and some pathogens may stay viable on surfaces for 3 days or more. Poorly washed hands and contaminated bathrooms in overcrowded conditions can remain infective for several weeks without adequate decontamination.

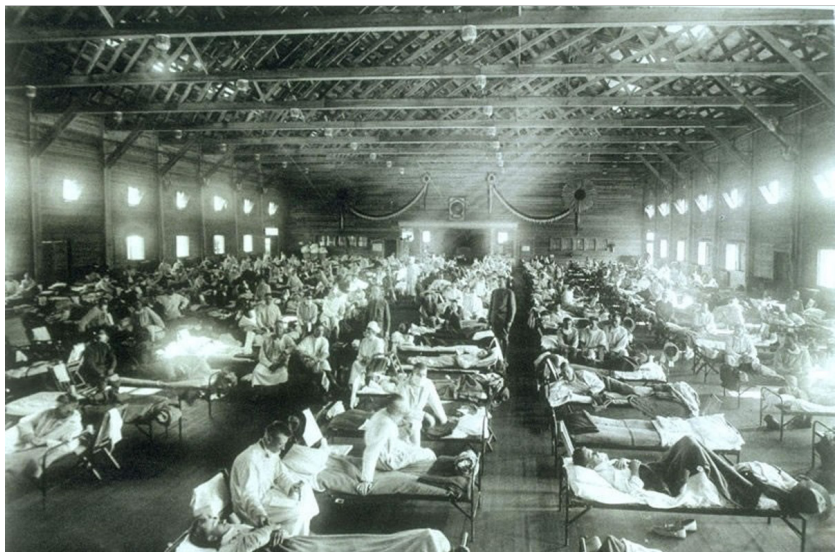


Figure 2-2. Soldiers suffering from influenza at the hospital in Camp Funston, Kansas in 1918. Camp Funston was where the influenza epidemic, which would kill more than 50 million people worldwide, including 675,000 Americans, first made a major appearance. (Photo Credit: U.S. Army)

Influenza is a respiratory virus that is transmitted in waves through a population either locally as an epidemic or globally as a pandemic. Each year, the influenza strains change, creating another transmission pathway through a susceptible population. Figure 2-3 demonstrates the large variety of influenza viruses that exist in our environment. With genetic mixing and adaptation through the swine population or direct transmission from birds to humans, a new influenza virus circulates each year.

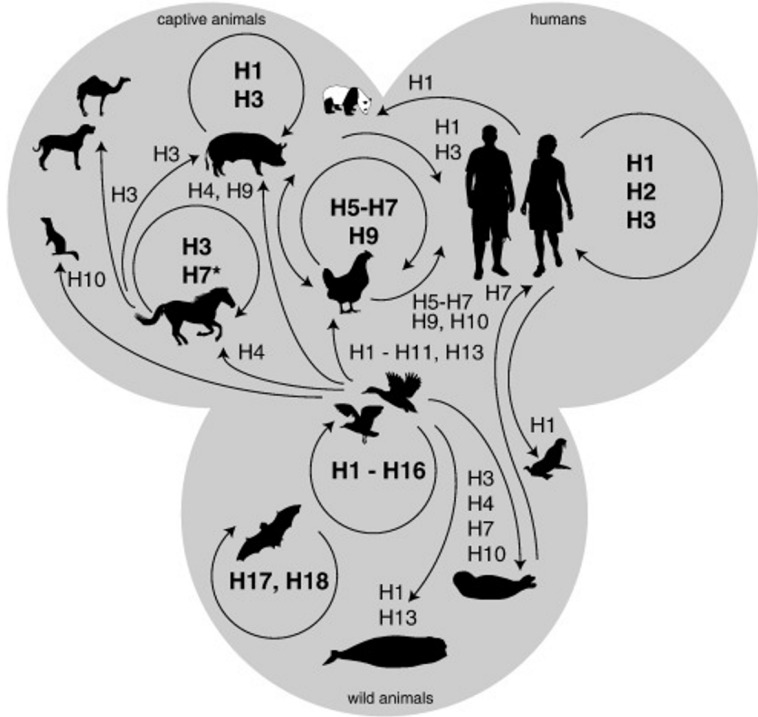


Figure 2-3. Interspecies mixing, reassortment, and transmission of Influenza virus. https://www.sciencedirect.com/science/article/pii/S2352771415000026

The illness from influenza is characterized by fever, muscle aches, respiratory symptoms, and, in the majority of cases, an illness that results in several days of sickness followed by recovery. In the very young and very old, influenza can cause a serious infection that results in death. Deaths from influenza can occur in two ways: the first by the virus itself producing damage to the lungs, causing fluid accumulation and respiratory failure by a process known as acute respiratory distress syndrome (ARDS), in which the body dies due to lack of oxygen; the second way is by depressing the host's immune system and creating an environment in the lungs in which bacteria can propagate, causing a secondary bacterial pneumonia, e.g. from *Streptococcus pneumonia*, then also causing ARDS and death by lack of oxygenation. The influenza strain of 1918, known as the H1NI strain, is unlike other strains of influenza viruses as it produced a very severe disease in the healthy young adult population. It is unclear where the 1918 strain originated, though current thoughts are that it emerged from China in 1917 and then spread globally. The first U.S. cases of 1918 influenza, or "Spanish flu", were reported at Camp Funston and Ft Riley as well as Camp Devens in March of 1918. The disease quickly spread throughout the U.S. and its impact on the warfighter was especially dramatic as forces were transported to fight in France. Among American soldiers during the months of September, October, and November, there were over 90,000 influenza admissions resulting in 9,000 deaths among forces fighting in France [12]. Figure 2-4 demonstrates the total deaths in the U.S. Army from 1917 to 1919 and the proportion of deaths from the influenza virus (50%) [11]. By the end of the epidemic it was estimated that 500 million people, or one-third of the world's population, were infected with this strain of influenza, resulting in 50 million deaths. Of them, 675,000 deaths occurred in the U.S.

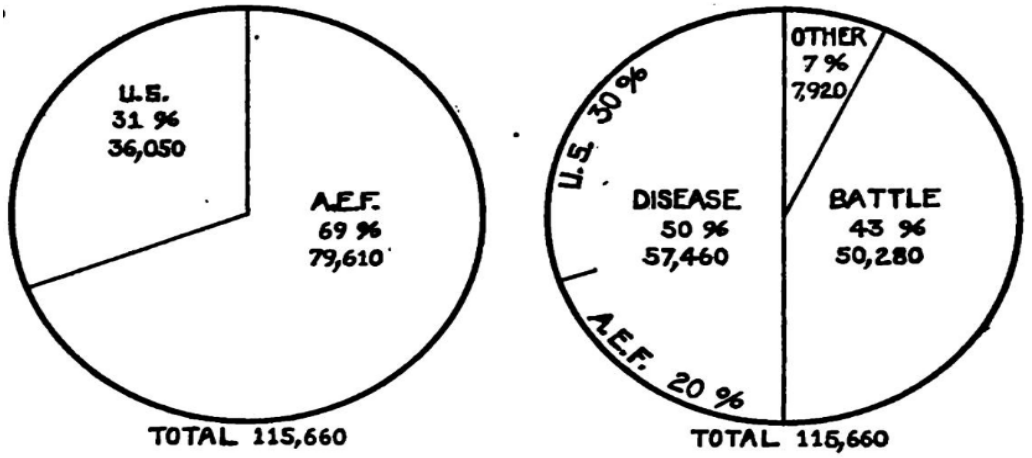


Figure 2-4. Total deaths and by cause in the U.S. Army from April 6, 1917 to July 1, 1919 [11].

2.3 Dengue and U.S. Marine Forces in Saipan WWII

Saipan is the largest island of the Mariana Islands located in the Pacific. In 1944, it was a stronghold for Japanese forces and a key area in U.S. plans to take back control of the Pacific and defeat Japan. The U.S. allied invasion fleet left Pearl Harbor on 5 June 1944, consisting of the 2nd and 4th Marine Divisions and the Army's 27th Infantry Division. Together, there were 60,000 U.S. Marines and Soldiers who were to land and invade Saipan.

Dengue is a mosquito-borne viral infection that occurs as four different types. The mosquitoes that transmit dengue to humans are females of the tropical species *Aedes aegypti* or *Aedes albopictus*. These are human-loving, urban mosquitoes that breed in discarded bottles, tires, canisters, and in anything that can hold standing water. Infection from dengue can cause severe fever, headache, and muscle and bone pain lasting from three to six days followed by several weeks of recovery. The U.S. Army Medical Corps was very familiar with dengue, having dealt with a similar outbreak in U.S. forces 30 years previously while deployed to the Philippines during the Spanish-American War. U.S. forces invading Saipan faced a perfect environment for the mosquito-vector for dengue and for obtaining the infection. Discarded vehicles, armaments, and standing water led to a large increase in the mosquito population. Soldiers and Marines were deployed on the line and exposed to numerous mosquito bites. Dengue cases quickly appeared, and by September 14, 1944 there were 393 cases in one day and 426 the next. For the entire month of September, over 3,000 Soldiers were incapacitated with acute dengue [13]. The number of DNBI during the Saipan conflict was considerable and was a factor in the duration of the conflict.

2.4 Dengue and U.S. Forces in Somalia and Haiti

From December 1992 to May 1993, U.S. forces deployed as a unified task force in support of Operation Restore Hope in Somalia, in order to provide political stability in a country torn by civil war. Dengue, among other pathogens, was known to circulate in the local population. A prospective study was conducted in hospitalized soldiers with fever as well as a seroepidemiologic survey of 520 troops [14]; among the 289 febrile hospitalized soldiers, 59 soldiers were found to have acute dengue fever (20%). Among the 520 soldiers involved in the serosurvey, 7.7% were found to have contracted dengue infection.

In September 1994, U.S. forces began Operation Uphold Democracy in Haiti to restore the elected government back to power and create political stability. U.S. forces consisted of elements of the Joint Special Operations Command and the U.S. Army's 10th Mountain Division. Haiti was known to be endemic for dengue and other tropical diseases. Of all hospital admissions for U.S. soldiers, 103 (25%) of them presented with fever of which 30 (29%) were diagnosed with dengue fever. The mosquito vector for dengue preferentially feeds on humans, requiring a blood meal to form eggs and propagate. They are daytime feeders and thus the risk of infection is greatest during the day. Combat operations for U.S. forces in Haiti were primarily focused on night operations with soldiers sleeping in the local environment during the day. These combined factors – a population and environment endemic for dengue and downtime during the mosquito vector's feeding time – made this operation particularly conducive to the transmission of dengue.

2.5 Leishmaniasis in U.S. Forces during Persian Gulf War II

Beginning in 2003, U.S. forces were deployed to three countries, Afghanistan, Iraq, and Kuwait, as part of Operation Enduring Freedom and Operation Iraqi Freedom. Leishmaniasis is a parasitic infection transmitted by the female blood feeding sand fly (genus *Phlebotomus* in Southwest/Central Asia). It has a global distribution with endemic areas in South America, Africa and the Middle East (Figure 2-5).

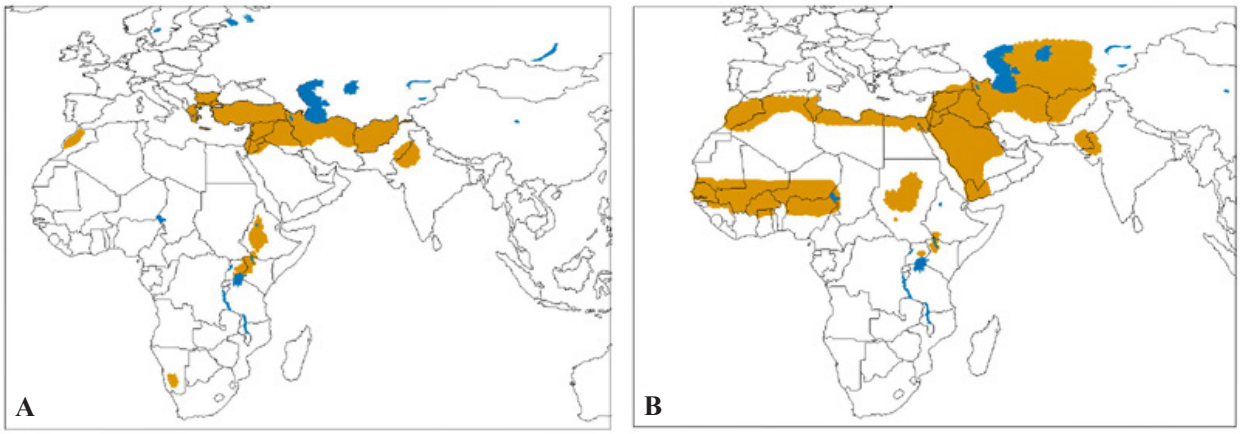


Figure 2-5. (A) Cutaneous Leishmaniasis due to L. Tropica and (B) from L. Major. https://www.who.int/leishmaniasis/leishmaniasis_maps/en/index1.html

The infected sand fly will feed on exposed skin areas and transmit the parasite which can cause a chronic infection leading to a breakdown and ulceration of the skin and in severe cases infection of the vital organs known as visceral leishmaniasis. The life cycle (Figure 2-6) involves an animal reservoir, primarily infected dogs, where sandflies feed for a blood meal, become infected by the parasite, and then infect humans.

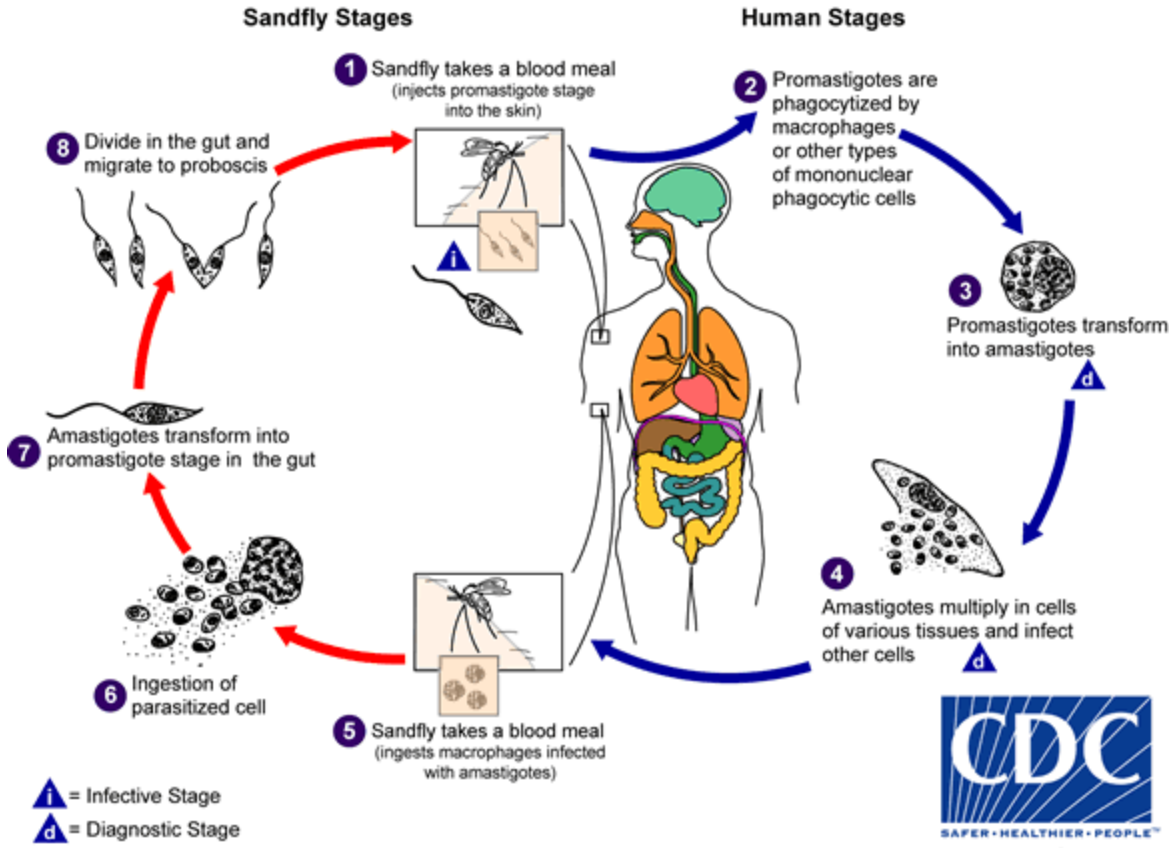


Figure 2-6. Life Cycle of Leishmaniasis and Human Infection. https://www.cdc.gov/dpdx/leishmaniasis/index.html

Figure 2-7 is an example of a cutaneous lesion from a soldier infected in Iraq and treated at the Walter Reed Army Medical Center.

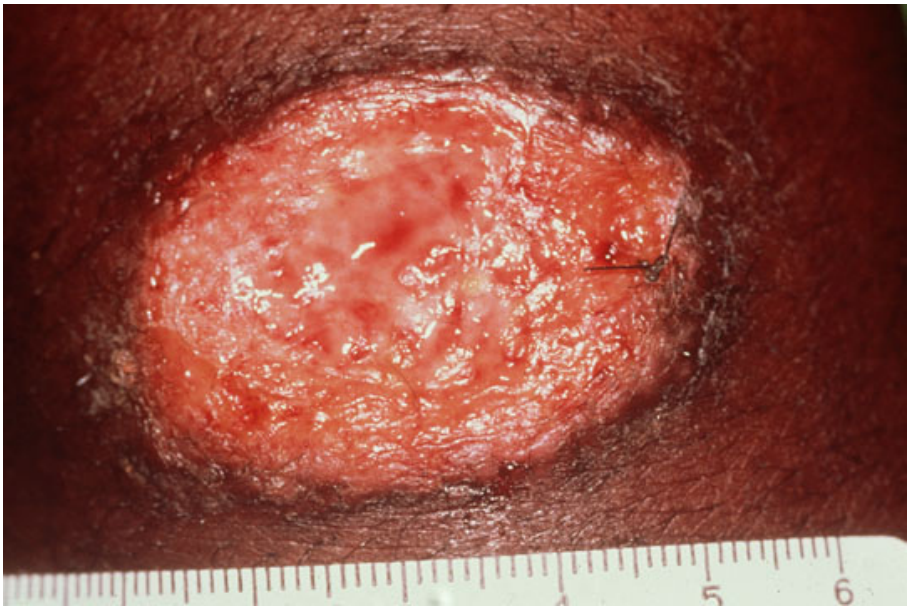


Figure 2-7. Cutaneous Leishmaniasis Skin Ulcer

Leishmaniasis has been well described in other forces deployed to Southwest Asia including British forces as early as the 1800s. The threat to U.S. forces wasn't appreciated until cases of severe cutaneous ulcers were being diagnosed but were not responding to antibiotics. Ulcers occurred above the boot line, arms, and face, and were frequently secondarily infected with bacteria, causing pain and limiting combat effectiveness. Evaluation by specialized testing revealed the skin form of leishmaniasis called cutaneous leishmaniasis (CL). From August 2002 to February 2004, 522 confirmed cases of CL were diagnosed in U.S. forces. These soldiers were removed from the theater of operations and medically evacuated back to Walter Reed Army Medical Center for treatment. Interventions to prevent further infections were instituted to include improved living conditions, use of personal protective measures such as permethrin-treated clothing, impregnated bed nets, DEET-containing insect repellant lotions, and vector-control activities.

2.6 State of the Art

For influenza A, the state of the art involves research and development of new antivirals that act against the virus as well as the development of a universal vaccine that will prevent infection from all strains of flu virus, eliminating the need for a new flu vaccine every year. Two promising antivirals are baloxavir and favipiravir [15, 16]. Baloxavir inhibits the cap-dependent endonuclease of the influenza virus, preventing the virus from transcribing its RNA for replication. Favipiravir is licensed in Japan as an anti-influenza drug and acts as a chain terminator preventing the influenza virus from replicating. Both drugs show promise both as a prophylactic to prevent infection and as a therapeutic to diminish severe complications from infection. As previously noted, the challenges of creating a vaccine against influenza A virus is its ability to change its genetic structure every year. This creates the need every year of predicting which strains will be circulating in the human population and creating a

new corresponding influenza vaccine. A universal vaccine for influenza, one that creates immunity to the common genetic structures of the virus regardless of its overall genetic changes, will simplify the approach of vaccinating large populations and obviate the need for an annual vaccine. This a topic of intense research and there are several candidate vaccines that are in human clinical trials [17-19].

For leishmaniasis, the state of the art involves rapid diagnostic testing and new treatment modalities. Polymerase chain reaction (PCR) entails the amplification of the genetic markers of the parasite and can have a result within a day [20]. New markers and improved test kits for serologic diagnosis are also being developed, allowing a simple blood test to diagnose leishmaniasis [21]. Standard therapy for all forms of leishmaniasis used to be with a drug called pentostam, known as sodium stibogluconate, which is an antimonial that required 21 days of intravenous therapy with many side-effects. New drugs have shown efficacy against leishmaniasis, in particular miltefosine, which can be taken orally and is well tolerated [22-24]. Other new treatments for the cutaneous form of leishmaniasis, perfected at Walter Reed Army Medical Center, use a thermal device applied to the cutaneous lesion, killing the parasites [23].

The state of the art for dengue will be discussed in Section 5.

2.7 Recommended Countermeasures

For all diseases discussed in Section 2, the recommended countermeasures involve: 1) understanding the risk of locally acquired diseases to the susceptible deployed warfighter; 2) employing effective countermeasures such as social distancing, avoiding overcrowding, and disinfection of common sources for contamination; 3) personal protective measures to avoid infection whether by aerosol, fecal-oral, or an insect vector; 4) quick diagnostics to diagnose infection; 5) prophylactic drugs or vaccines if available to prevent infection; and 6) prompt treatment returning the warfighter back to combat effectiveness.

2.8 Summary

To recapitulate the National Academy of Sciences report on protecting U.S. forces, each of the examples presented in Section 2 are of infectious DNBIs due to troops living in crowded conditions allowing the spread of respiratory pathogens, with deployed soldiers being highly susceptible to infections that are endemic in the country where they are deployed. Key to the lessons learned to prevent infectious DNBI are the following: 1) an accurate understanding and intelligence on the disease pathogens that are endemic in the countries where U.S. forces are deployed; 2) targeted interventions through the use of vaccines or prophylactic drugs if available and protective measures available to soldiers such as bed nets, insect repellant, and permethrin-impregnated clothing; 3) education of the command to the individual level on recognizing and preventing infections; and 4) vector control measures in the areas of deployment.

3

Protecting the Warfighter from Malaria

3.1 Malaria Disease Overview

Malaria has long been a problem among deployed military personnel in endemic areas. Malaria is a vector-borne parasite spread by the bite of an infected female *Anopheles* mosquitoes [25]. The world-wide burden of malaria is large with 91 countries reporting active transmission (Figure 3-1). There are five species that can primarily infect humans and cause illness. These include: *Plasmodium falciparum*, *P. vivax, P. ovale, P. malariae,* and *P. knowlesi*. Cases have been recorded of monkey species infecting humans, but these are uncommon. Species vary by geographic region; thus, understanding the epidemiology of each species in specific regions of the world is essential in guiding appropriate malaria prophylaxis and treatment.

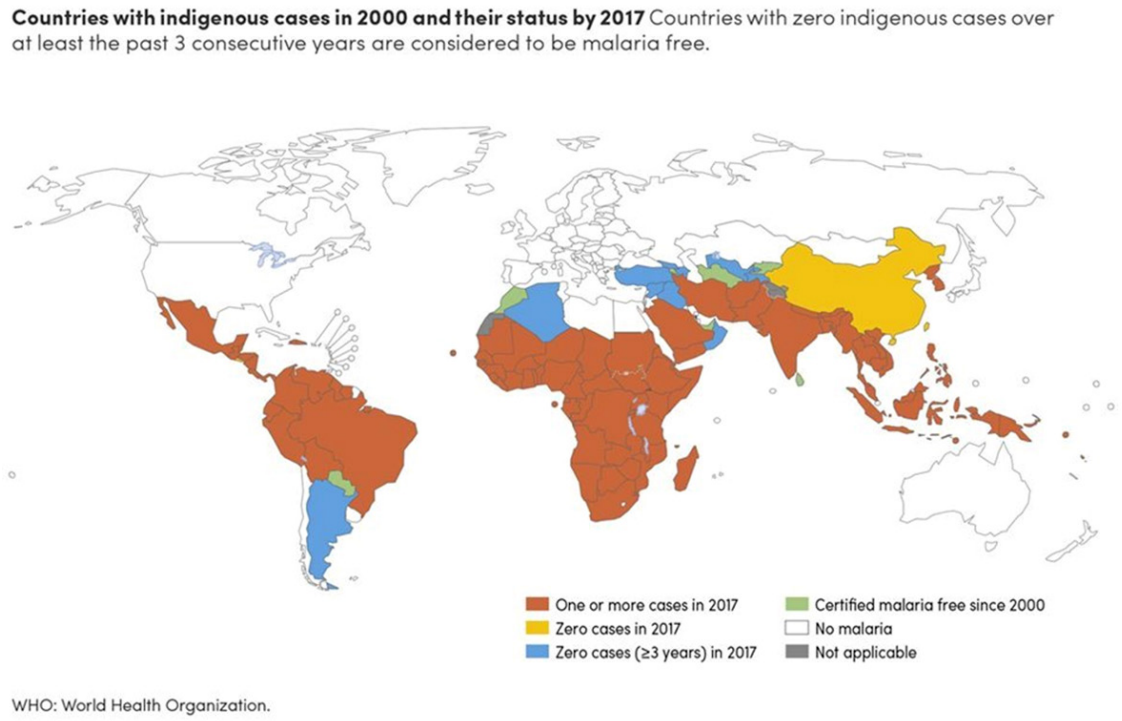


Figure 3-1. Geographic Distribution of all Malaria Globally. https://www.who.int/gho/malaria/malaria_003.png?ua=1

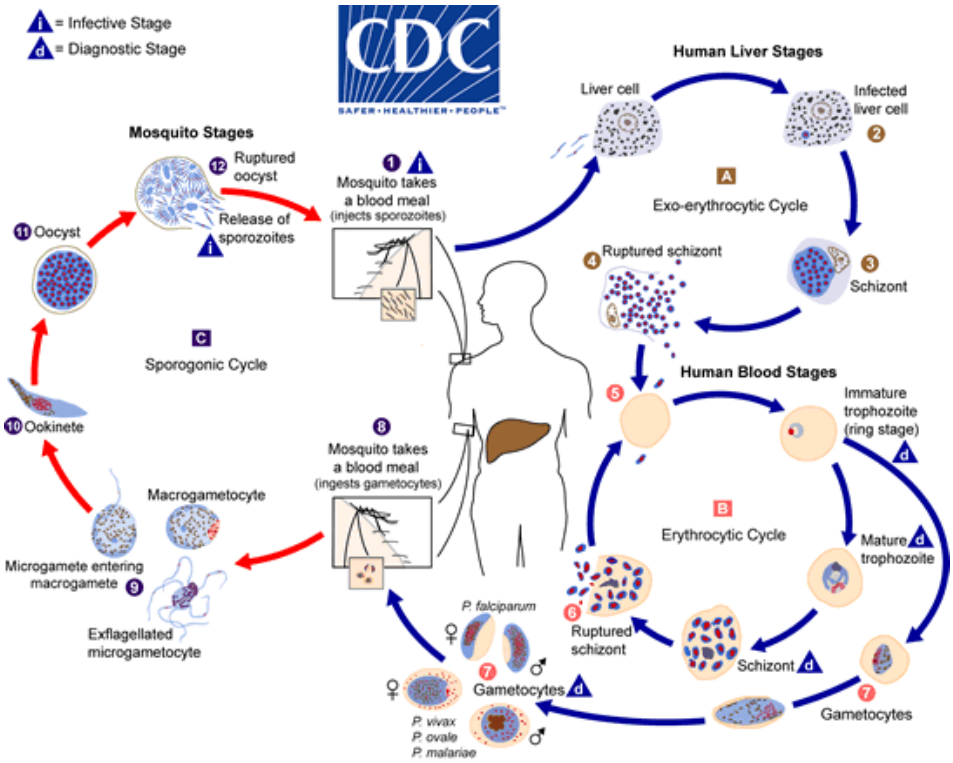


Figure 3-2. Life Cycle of Malaria from Human to Mosquitoes. https://www.cdc.gov/malaria/about/biology/index.html

The life cycle of malaria is complex and requires transmission from an infected female mosquito to a human and back to an uninfected mosquito taking a blood meal (Figure 3-2). The now newly infected mosquito can go on and infect other humans when it takes a blood meal. Once a person is infected with malaria from a mosquito, there is an incubation period of 10 to 14 days for *P. falciparum* and *P. knowlesi* and two to three weeks for the other species, though an incubation as long as three to six months after infection has been observed for *P. vivax* [25]. Clinical symptoms start after the incubation period and include fevers, headache, body ache, and diarrhea. Symptoms are severe and, in general, patients are unable to work or, in the case of the warfighter, are rendered combat ineffective. The most serious form of malaria is by *P. falciparum* and clinically it is considered to be a medical emergency if not treated quickly. P. falciparum can double quickly in the bloodstream, causing not only a drop in the blood count but also producing ARDS, which as mentioned in Section 2, consists of severe congestion in the lungs. ARDS requires mechanical ventilation and, in some cases, can cross into the brain, causing a severe infection known as cerebral malaria.

3.2 Case Study: Failure of Command Oversight for Prophylaxis

In 2003, Liberia was in a state of political and humanitarian crisis caused by the Second Liberian Civil War. As rebel forces closed on Monrovia, the U.S. Ambassador to Liberia requested military assistance. In response, Joint Task Force Liberia was formed. In August 2003, 225 Marines with the 26th Marine

Expeditionary Unit were deployed to Roberts International Airport outside of Monrovia [26]. Liberia is a tropical African country and endemic for a number of vector-borne diseases and tropical pathogens. Monrovia, due to the disruption and destruction of infrastructure from the civil war and lack of a vector control program, was endemic for malaria in its most serious form, known as *Plasmodium falciparum.* Marines worked and slept in an abandoned warehouse that was noted to have a high burden of mosquitoes. Malaria prophylaxis was given prior to their landing in Monrovia and consisted of mefloquine taken weekly and self administered by the individual Marine. The Marine unit spent 10 days and returned to their ships when a febrile illness developed in 80 Marines. Of the 80 Marines, 36 were treated on board ship with presumptive malaria, 39 were medically evacuated to the former National Naval Medical Center in Bethesda, MD for treatment, and five were medical evacuated to Landstuhl Medical Center in Germany for severe complicated malaria (two with cerebral malaria and three with acute respiratory distress syndrome) [26].

As part of the outbreak investigation, mefloquine levels were determined in a subset of ill Marines, tablets were tested for drug viability, and parasites were tested for resistance. Blood levels determined that drug levels were below protective levels, tablets were viable, and malaria parasites were not resistant to mefloquine. The conclusion was that malaria prophylaxis was inadequate and there was a failure of command to ensure adequate prophylaxis [26].

3.3 Malaria Prophylaxis and Treatment

Prevention measures for malaria include antimalaria drug prophylaxis and personal protective measures. Drug prophylaxes include the use of atovaquone-proguanil, doxycycline, mefloquine or primaquine. Personal protective measures include the use of insect repellants such as DEET-containing solutions, permethrin-impregnated combat fatigues, and the use of bednets at night.

There are several highly effective treatments for all forms of malaria. Treatments consist of a combination of drugs known as artemisin-based combination treatments and include, for example, artemether-lumefantrine or artesunate-amodiaquine, as well as atovaquone-proguanil, which has been highly effective. For severe malaria, an intravenous formulation of artesunate is available from the U.S. Centers for Disease Control (CDC). *P. vivax* and *ovale* can reoccur after a treatment course and require a medication called primaquine to kill the parasites that live in the liver. Preventing acute malaria infection is key in protecting the fighting capacity of the warfighter.

3.4 Status of Malaria Vaccine Development

There are several malaria vaccines that are in development with the RTS,S/AS01 demonstrating some effectiveness in children living in endemic areas [25]. There is currently no licensed vaccine against malaria for travelers or military personnel traveling to endemic areas.

3.5 State of the Art

The state of the art for malaria involves point of care diagnostic devices, new antimalarial treatments and, as noted previously, a vaccine against malaria. For diagnostics, the traditional method involves taking a blood sample, making a glass smear, staining the sample, and looking for parasites under a microscope. This is time consuming, requires a skill set, and is unavailable to most combat treatment

units on the front lines. Rapid diagnostic tests (RDTs) offer the opportunity for the rapid diagnosis of malaria with a fingerstick of blood, a positive or negative indicator on a test strip without the need for a microscope, and a result within several minutes. An RDT that has been used for malaria diagnosis for specifically *Plasmodium falciparum* is sensitive and specific and can be forward deployed to combat units [27]. New antimalarial drugs are being developed as resistance to the major drugs by the malaria parasite is an emerging problem. A new drug developed by the U.S. Army has recently been approved by the Food and Drug Administration (FDA) to prevent all types of malaria called tafenoquine [28, 29]. Tafenoqune is an 8-aminoquinoline, oral drug for the prophylaxis and treatment of *Plasmodium vivax* and has been shown to be highly effective in preventing infection and for treating the liver phase responsible for relapses in infection. A number of malaria vaccines are in clinical development and undergoing human clinical trials though none are currently approved for use in adult travelers or for soldiers deployed to malaria endemic areas.

3.6 Recommended Countermeasures

The recommended countermeasures against malaria involve: 1) understanding the risk of locally acquired diseases to the susceptible deployed warfighter; 2) employing effective countermeasures such as vector control and personal protective measures to avoid bites from the mosquito vector such as bednets, DEET insect repellant, and permethrin-impregnated combat uniforms; 3) rapid diagnostics to diagnose infection; 4) prophylactic drugs or vaccines if available to prevent infection; and 5) prompt treatment returning the individual back to combat effectiveness.

3.7 Summary

In summary, malaria historically has been and currently is a major threat to U.S. servicemembers deployed to areas of the world endemic for malaria and is a major cause of infectious DNBIs. As the case study illustrates, malaria can eliminate the combat effectiveness of an entire deployed force. It is preventable through drug prophylaxis and personal protective measures and requires command emphasis with direct observed prophylaxis to maintain adequate malaria protection.

4

Protecting the Warfighter from Rabies

4.1 Rabies Disease Overview

Rabies virus is an RNA virus, serotype 1 of 7 serotypes of the genus *Lyssavirus*, family *Rhabdovirus* [30]. It is a zoonotic disease meaning it is transmitted to humans from infected animals through saliva contact, most commonly from the bite of an infected animal. Rabies is a global health problem with 55,000 deaths occurring every year and is highly endemic in most areas of the world [31]. Rabies virus is endemic throughout the world due to high rates of both wild and domestic animal rabies, and the risk to deployed military in endemic areas is considerable (Figure 4-1).

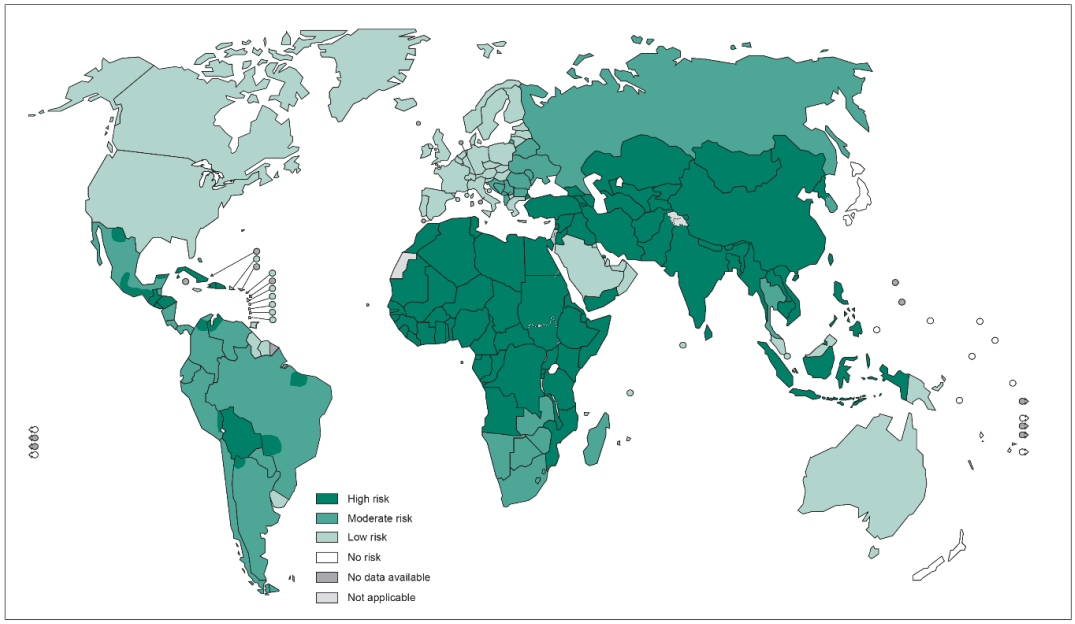


Figure 4-1. Global Distribution of Rabies, 2013 (https://www.who.int/rabies/Global_distribution_risk_humans_contracting_rabies_2013.png?ua=1)

In the U.S. for example, because of mandatory vaccination of domestic pets, i.e. cats and dogs, rates of human rabies have declined to several cases per year (36 cases in the last 20 years). However, the animal population in the U.S. continues to have high rates of rabies, particularly in the skunk, raccoon, fox, coyote, and bat populations. Of the cases in the U.S., 71% were transmitted by bats and the rest by rabid coyotes and skunks. Globally, in countries that do not have an active domestic pet vaccination program, dogs remain the primary animal that transmits the virus to humans. Once a human is infected with rabies, the incubation period – time from infection to clinical disease – can vary from days to as long as 19 years. Clinical illness starts with chills, fever, headache, eye pain to light, loss of appetite, nausea, vomiting, diarrhea, and sore throat. Patients can develop abnormal sensations around the bite site to include itching, burning, numbness, or tingling sensations. The virus travels up the nervous system and crosses into the brain, producing an inflammation termed encephalitis. This is characterized by delirium, agitation, paralysis, coma, and death. There is a disruption in the autonomic nervous system, which creates an exaggerated respiratory tract protective reflex elicited by water or other stimuli. Once rabies encephalitis occurs, mortality is near 100%.

4.2 Vulnerability of Military Personnel to Rabies

Rabies has long been recognized as a threat to military operations with deaths in soldiers reported as early as 1926 [32]. Rabies is still a threat to the deployed warfighter as most countries where soldiers are deployed are highly endemic for rabies. According to a report by Cooper and Debboun, the Armed Forces Health Surveillance Center (AFHSC) reported – from 2001 to 2020 – approximately 643 animal bites (50% dog bites) to U.S. personnel deployed to Southwest Asia and the Middle East and over 20,000 in U.S. Armed Forces worldwide during that same time period [32]. Less than 50% of those with animal bites reported having received rabies vaccine.

For example, in August 2011 a U.S. Soldier of the 10th Mountain Division previously stationed at a forward operating base in Afghanistan was returning to Ft Drum, New York [33]. During his travels back to the U.S., he experienced pain, tingling, and numbness from his right hand radiating up his arm. On his return he presented to a local emergency room and was thought to have a tendinitis. He then experienced fever, nausea, vomiting, and difficulty walking and presented to the emergency room where he demonstrated hydrophobia (painful salivation on seeing water) and aerophobia (muscle spasms on feeling air ventilation). The patient was transferred to a higher level of care due to concerns for rabies encephalitis where he was subsequently diagnosed with a rabies infection and later died from complications of this disease. On evaluation of his history, it had been revealed by the patient that, while in Afghanistan, he was bitten in the right hand by a dog 6 months prior to clinical symptoms and did not receive post-exposure prophylaxis (PEP) for rabies consisting of rabies immune serum globulin and rabies vaccine.

The lack of PEP in the preceding case study left the solider at substantial risk, as currently, pre-exposure rabies vaccination is not standard practice for deploying soldiers to endemic areas, relying instead on self-reporting of animal bites and prompt medical attention for PEP with immune globulin and vaccine. As a report by Moe and Keiser illustrates, this scenario can be problematic [34]. They describe a Forward Operating Base in eastern Afghanistan that had enough rabies vaccine to vaccinate soldiers for pre-exposure prophylaxis and enough vaccine for post-exposure vaccination but the rabies immune globulin (RIG) had expired. A soldier presented with a dog bite to the battalion aid station; due to the lack of RIG he was taken off the line and medically evacuated to a facility at Jalalabad Airfield, but RIG was not available at this facility either. He was eventually flown to Bagram Airfield and received

RIG 32 hours after exposure. While the 32-hour delay likely did not affect treatment and prevention of infection in this case, appropriate RIG treatment should be given as soon as possible.

4.3 Rabies Pre-Exposure and Post-Exposure Prophylaxis

The rabies vaccine that is currently available in the U.S. is derived from cell cultures, either as a human diploid cell vaccine (HDCV) or as a purified chick embryo cell vaccine (PCECV) [30]. Imovax Rabies (HDCV for pre- or post-exposure) was developed by Sanofi Pasteur and licensed in 1980. RabAvert (PCECV for pre- or post-exposure) was developed by Novartis and licensed in 1997. The current rabies vaccines produce antibody response in 100% of individuals with durable immunity for years [30]. In a prospective study over 10 years of follow-up, an antibody titer of 130 IU following vaccination indicated prolonged seropositivity with titers of 0.5 IU or greater considered protective [35]. Pre-exposure vaccination is recommended for people who will be exposed to rabies virus either in the laboratory, environment, or by occupation. It is recommended for travelers or global health workers who may have potential exposure [30]. Reactions to the current rabies vaccines are mild with local pain, erythema, and swelling at the injection site. Systemic reactions with headache and malaise are less frequent [30]. The current licensed delivery of the rabies vaccine is by the intramuscular (IM) route. Currently pre-exposure prophylaxis regimen for rabies, in the U.S., is comprised of three 1.0 ml intramuscular (IM) injections of the human diploid cell vaccine (HDCV) or purified chick embryo cell (PCEC) rabies vaccine on days 0, 7, and 21 or 28. Modified, two and three dose schedules of intradermal (ID) injections of 0.1 ml of HDCV and PCEC are the standard outside the U.S.. These two and three dose intradermal schedules share a similar safety and immunogenicity profile to intramuscular vaccinations and are easily boosted at one year after vaccination.

4.4 Rabies Vaccine and Anti-Malarial Drug Interactions

Soldiers are often deployed to areas that are both malaria and rabies endemic, requiring malaria prophylaxis and, if an animal bite occurs, also requiring rabies vaccine. Chloroquine has previously been shown to impair the immune response to ID rabies vaccination. A potential interaction of chloroquine and rabies vaccination was first suggested in 1983 when a 23-year-old Peace Corps worker died of rabies after a dog bite despite receiving pre-exposure prophylaxis (PrEP) with a three-dose series of ID HDCV [36]. Subsequently, the United States Centers for Disease Control and Prevention (CDC) conducted a serosurvey in 333 Peace Corps volunteers (PCVs) to assess the effectiveness of rabies PrEP [37]. All PCVs received the three-dose regimen of 0.1 ml ID HDCV. Results revealed that 32% of PCVs in Kenya and 43% outside of Kenya did not achieve a protective titer. Several factors were hypothesized as contributing to the inadequate antibody response including the effects of multiple vaccinations, vaccine potency, or interactions with malaria chemoprophylaxis. A survey was performed in PCVs in Thailand who received three ID doses of HDCV while receiving chloroquine and fansidar malaria chemoprophylaxis [38]. PCVs who were on chloroquine for more than seven weeks had significantly lower antibody titers than those who did not take chloroquine (.65 IU/ml vs 2.18 IU/ml respectively). The authors concluded that continuous antimalarial chemoprophylaxis with chloroquine during primary rabies immunization is associated with a poor antibody response to ID HDCV rabies vaccine. The only randomized controlled trial (RCT) to study the interaction of chloroquine and rabies vaccination was performed in veterinary students using ID HDCV for PrEP administered with and without chloroquine [39]. Of 51 students not previously vaccinated against rabies, 26 received

300mg chloroquine base per week and 25 did not. All received 0.1 mL of HDCV ID on days 0, 7, and 28. Chloroquine was given weekly to the treatment group starting nine days before the first dose of vaccine and continued until day 48. Rabies-neutralizing antibody titers (log geometric mean titer (GMT)) for the chloroquine group were significantly lower than for the control group on days 28 (1.7 vs 2.0), 49 (2.3 vs 2.6), and 105 (1.8 vs 2.2) post-vaccination, indicating that chloroquine taken in doses used for malaria prophylaxis reduces the antibody response to primary immunization with ID HDCV. Current Advisory Committee on Immunization Practices (ACIP) guidelines recommend that previously unvaccinated persons requiring rabies PEP who are taking immunosuppressive agents, antimalarials, or have immunosuppressive illnesses receive a fifth dose of rabies vaccine on day 28 [40]. The guidelines do not differentiate between malaria prophylaxis regimens; therefore, the fifth dose is indicated in persons taking any malaria prophylaxis. Recently, a randomized, open-label, single site study in 103 previously unvaccinated healthy adults age ≥ 18 to ≤ 60 years old was performed to evaluate the effects of chloroquine, atovaquone/proguanil, and doxycycline on the antibody response to a PCECV, given on a PEP schedule [41]. All treatment groups received antimalarials 14 days prior to and during vaccination. All subjects achieved protective neutralizing antibody titers of >0.5 IU/mL following the second rabies vaccination dose and maintained this protection through the duration of the study. There was a reduction in rabies antibody GMT in the chloroquine versus control groups 28 days after vaccination: 2.3 vs 6.87 IU/mL respectively ($p < 0.001$, t-test). A significant difference was not observed for those taking atovaquone/proguanil or doxycycline. The conclusions of the study were that there is no reduction of rabies antibody response in subjects taking atovaquone/proguanil or doxycycline and these do not result in a diminished immune response to rabies vaccine.

4.5 State of the Art

Rabies currently has very effective vaccines to prevent infection, rabies immune globulin for bridging protection until the body responds to the vaccine to develop protective antibody, and diagnostics to measure antibody and diagnose infection.

4.6 Recommended Countermeasures

The recommended countermeasures against rabies involve: 1) understanding the risk of locally acquired diseases to the susceptible deployed warfighter and prompt reporting and care for animal bites; 2) employing effective countermeasures such as animal control, personal protective measures to avoid bites from potential rabid infected animals, and vaccination prior to entering endemic areas; 3) the use of pre-exposure rabies vaccination for deploying servicemembers; and 4) the use and availability of post-exposure vaccines and immune globulin to prevent infection.

4.7 Summary

Rabies is highly endemic in areas where soldiers are deployed. Both prophylactic and post-exposure treatment are highly effective in preventing rabies infection which has a near 100% mortality. Self-reporting of animal bites and prompt treatment is an accepted form of prevention of rabies; however, in soldiers deployed to remote areas of the world, both vaccine and rabies immune globulin may not be available, requiring resources dedicated to transporting the soldier to the proper medical treatment facility, in turn delaying treatment and resulting in a distraction to the mission, removal of a valuable line soldier, and diminished unit combat effectiveness.

5

Protecting the Warfighter from Dengue

5.1 Dengue Overview

Dengue is the most important arboviral disease afflicting the world today. Hundreds of millions of infections occur each year, of which more than 90 million are clinically apparent [42]. Mortality is reportedly low compared to other vector borne diseases, but the brunt of severe dengue disease and death in many regions occurs disproportionately in children [43]. Dengue has a considerable impact at the personal, community, and regional level and is a leading cause of febrile, systemic illness in travelers [44, 45]. Dengue has placed deploying military personnel at risk for over a century [46-51].

Conditions favoring the close juxtaposition of virus, vector, and susceptible host, a requirement for sustained transmission, are numerous. Ecological conditions favoring vector expansion, population growth and increasing urbanization, and the ease of air travel have all contributed to the concentration, in time and space, of susceptible hosts, competent vectors, and dengue viruses (DENVs) [52].

Dengue is a significant infectious disease threat among travelers to endemic areas (Figure 5-1). Between 2000-2010, dengue was the third most commonly diagnosed illness among returning travelers, behind malaria and infectious diarrhea [53].

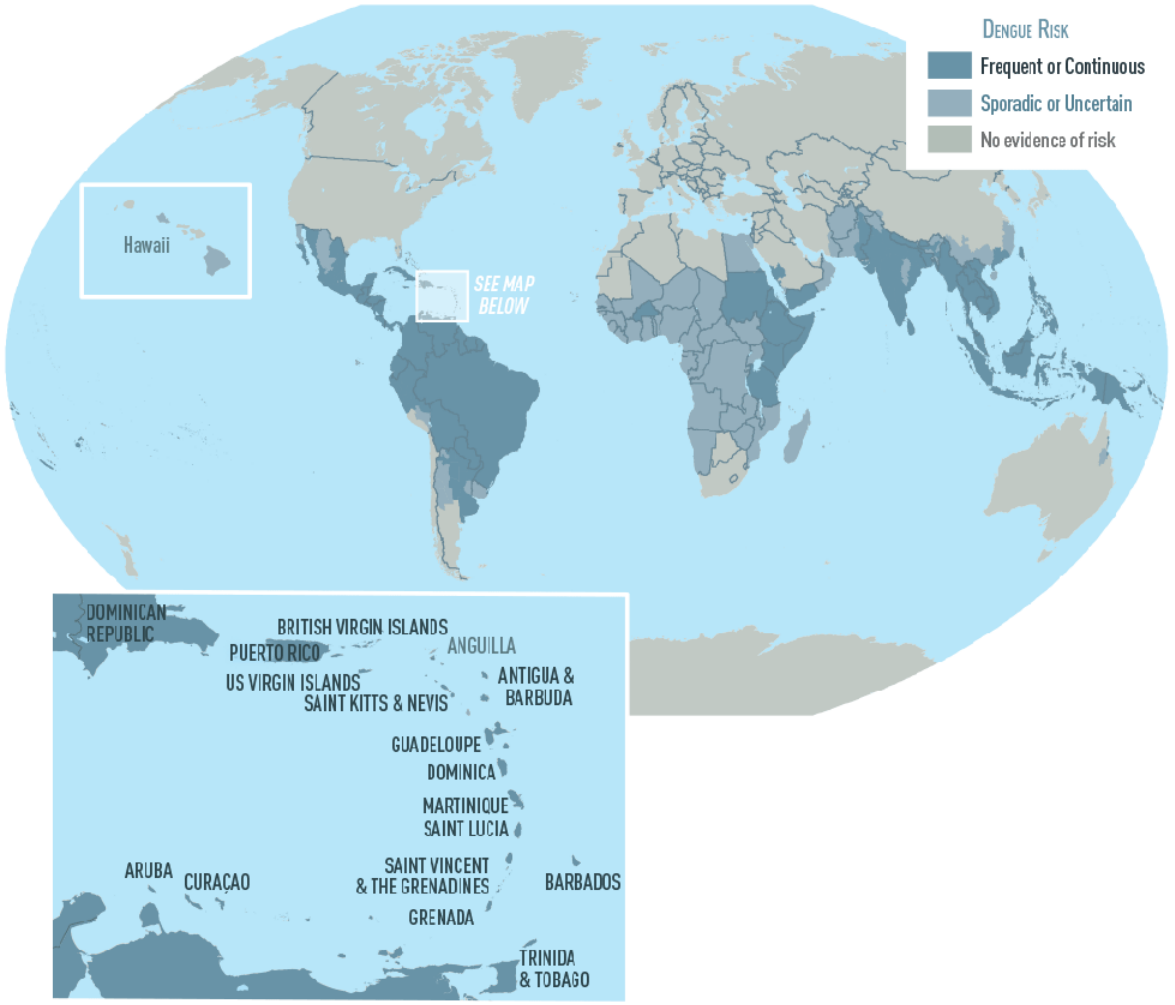


Figure 5-1. World Distribution of Dengue 2019 https://www.cdc.gov/dengue/areaswithrisk/around-the-world.html

A review of four prospective studies of travelers to dengue-endemic regions demonstrated an incidence of dengue that ranged from 10.2–30 infections per 1000 person-months, depending on the region of travel and duration of travel [54]. As of January 2020, there were 1,203 dengue cases reported in the U.S., 56 in U.S. Territories with local transmission reported in Florida and Puerto Rico (Figure 5-2). The majority of cases were traveler associated infections (98%).

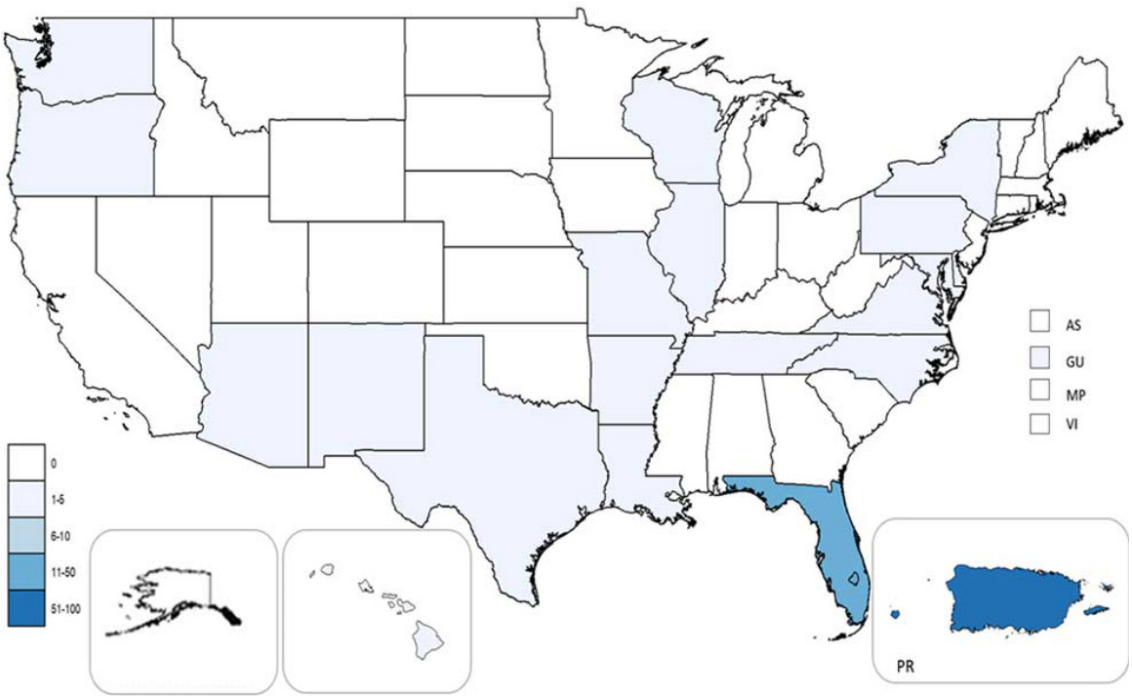


Figure 5-2. States and territories reporting dengue cases – U.S., 2019 (as of January 2, 2020) https://www.cdc.gov/dengue/statistics-maps/index.html

5.1.1 Clinical Manifestations of Dengue

Dengue is a febrile illness caused by infection with one of four DENV. As discussed briefly in Section 2, transmission to humans occurs when *Aedes aegypti* or *Aedes albopictus* mosquitoes take a blood meal from a susceptible host (Figure 5-3) [55, 56].

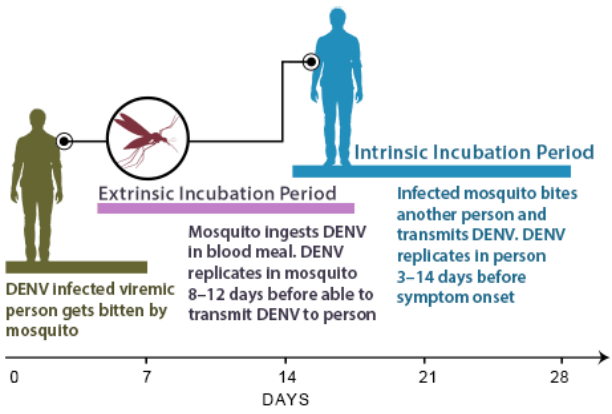


Figure 5-3. Dengue transmission. https://www.cdc.gov/dengue/training/cme/ccm/page46974.html

Infection may be asymptomatic or present with a broad range of clinical manifestations including a mild febrile illness to a life-threatening shock syndrome. Numerous viral, host, and vector factors are thought to impact risk of infection, disease, and disease severity.

There are four closely related but serologically distinct DENV types, called DENV-1, DENV-2, DENV-3, and DENV-4. Once infected with one DENV type, there is transient cross-protection against the other types, which weakens and disappears over the months following infection. A primary DENV infection is the first wild type infection an individual sustains while a secondary infection is the second wild type infection caused by a different DENV type.

The incubation period for infection and dengue disease ranges from three to 14 days [57]. Dengue disease may follow three phases; a febrile phase, a critical phase, and a recovery phase. The critical phase occurs in the minority of infections [58]. The febrile phase includes sudden, high-grade fever of 38.5°C (101.3°F) or higher, accompanied by headache, vomiting, myalgia, arthralgia, and a transient macular rash in some cases. Additional manifestations may include gastrointestinal symptoms (including anorexia, nausea, vomiting, abdominal pain, and diarrhea) and respiratory tract symptoms (cough, sore throat, and nasal congestion). The febrile phase lasts for three to seven days, after which most patients recover without complications.

Hemorrhagic manifestations may be observed in the febrile phase and/or critical phase. The range and severity of hemorrhagic manifestations are variable. Major skin and/or mucosal bleeding (gastrointestinal or vaginal) may occur in adults. In children, clinically significant bleeding occurs rarely, usually in association with profound and prolonged shock. Other less frequent manifestations include hematemesis (15 to 30 percent), menorrhagia (40 percent of women), melena (5 to 10 percent), and epistaxis (10 percent). Comorbid or pre-existing medical conditions (such as peptic ulcer disease) may increase the risk for hemorrhage. Although significant thrombocytopenia increases the risk of hemorrhage, it is not always present when hemorrhagic manifestations occur [59, 60].

Leukopenia and thrombocytopenia (≤100,000 cells/mm^3) are common. Serum aspartate transaminase (AST) levels are frequently elevated; the elevations are usually modest (2 to 5 times the upper limit of normal values), but marked elevations (5 to 15 times the upper limit of normal) occasionally occur. Although liver associated enzyme elevations are frequently elevated in the febrile phase, synthetic liver dysfunction (i.e. elevated activated partial-thromboplastin time) and decreases in fibrinogen are not frequently identified [61].

Between days three and seven of the illness, the clinician must watch for signs of vascular leakage and the clinical manifestations of the same. Significant vascular leakage reduces intravascular volume and decreases organ perfusion. Clinical and lab evidence of this occurring may include persistent vomiting, increasingly severe abdominal pain, tender hepatomegaly, a high or increasing hematocrit level (≥20 percent from baseline) concurrent with a rapid decrease in the platelet count, development of pleural effusions and/or ascites, mucosal bleeding, and lethargy or restlessness [62].

Around the time of defervescence (typically days 3 to 7 of infection), a small proportion of patients have the potential to develop a systemic vascular leak syndrome characterized by plasma leakage, bleeding, shock, and organ impairment. The greatest risk of patients entering into this critical phase and developing severe disease occurs with a secondary infection [63, 64]. The critical phase lasts for 24 to 48 hours.

During the recovery phase, plasma leakage and hemorrhage resolve, vital signs stabilize, and accumulated fluids are resorbed. An additional rash (a confluent, erythematous eruption with small islands of unaffected skin that is often pruritic) may appear during the convalescent phase. The recovery phase typically lasts two to four days and adults may have profound fatigue for days to weeks after recovery.

5.1.2 Dengue Pathogenesis

A number of different risk factors have been proposed to predispose someone to severe dengue disease such as medical co-morbidities, age, sex, nutritional status, and the infecting viral serotype and genotype. The most compelling data, however, supports that immune mediated mechanisms following a secondary infection drive disease pathogenesis.

Both innate and adaptive immune responses induced by DENV infection are likely to play a role in the clearance of infection [65]. Infection of human cells *in vitro* induces antiviral responses (interferons) and has been measured in children with dengue [66]. In response, DENV proteins appear able to inhibit both the production of interferons and their antiviral function in infected cells [67]. It also appears the expression of genes associated with type I interferon signaling is lower in patients with dengue shock syndrome (DSS) than in patients without DSS [68].

The antibody response to DENV infection is primarily directed at serotype-specific determinants, but there is a substantial level of serotype–cross-reactive antibodies. In vitro, Envelope (E) protein–specific antibodies can mediate neutralization of infection, direct complement-mediated lysis or antibody-dependent cellular cytotoxicity of dengue virus–infected cells, and block virus attachment to cell receptors [69]. Non-structural protein 1 (NS1) is not found in the virion; NS1-specific antibodies are therefore incapable of neutralization of virus infection but can direct complement-mediated lysis of infected cells [70].

Virus neutralization clearly requires a threshold level of antibodies; when the concentration of antibodies is below this threshold, the uptake of antibody-bound virus by cells that express immunoglobulin (Ig) receptors may paradoxically increase, a process known as antibody-dependent enhancement (ADE) [71]. In rhesus monkeys, passive transfer of low levels of dengue-immune human sera or a humanized chimpanzee DENV–specific monoclonal antibody resulted in a 2- to 100-fold increase in dengue-2 or dengue-4 viremia titers as compared with controls [72]. Dengue virus entry via ADE has also been found to suppress innate immune responses in infected monocytes in vitro [73].

The T lymphocyte response to DENV infection also includes both serotype-specific and serotype–cross-reactive responses [74]. Dengue virus–specific CD4+ and CD8+ T cells can lyse DENV–infected cells *in vitro* and produce cytokines such as IFN-gamma, tumor necrosis factor (TNF)-alpha, and lymphotoxin. In vitro, IFN-gamma can inhibit DENV infection of monocytes. However, IFN-gamma can also increase the expression of Ig receptors potentially augmenting ADE [75].

Primary infection provides long-lasting immunity to infection with a virus of the same serotype but immunity to the other dengue serotypes is transient, allowing for secondary infections. Studies have reported that higher peak plasma DENV titers occur in secondary dengue infections and are associated with more severe illness [76]. Other studies have failed to demonstrate this phenomenon [77].

The kinetics of DENV–specific antibodies in secondary dengue infections differ from those of primary dengue infections in several ways (Figure 5-4).

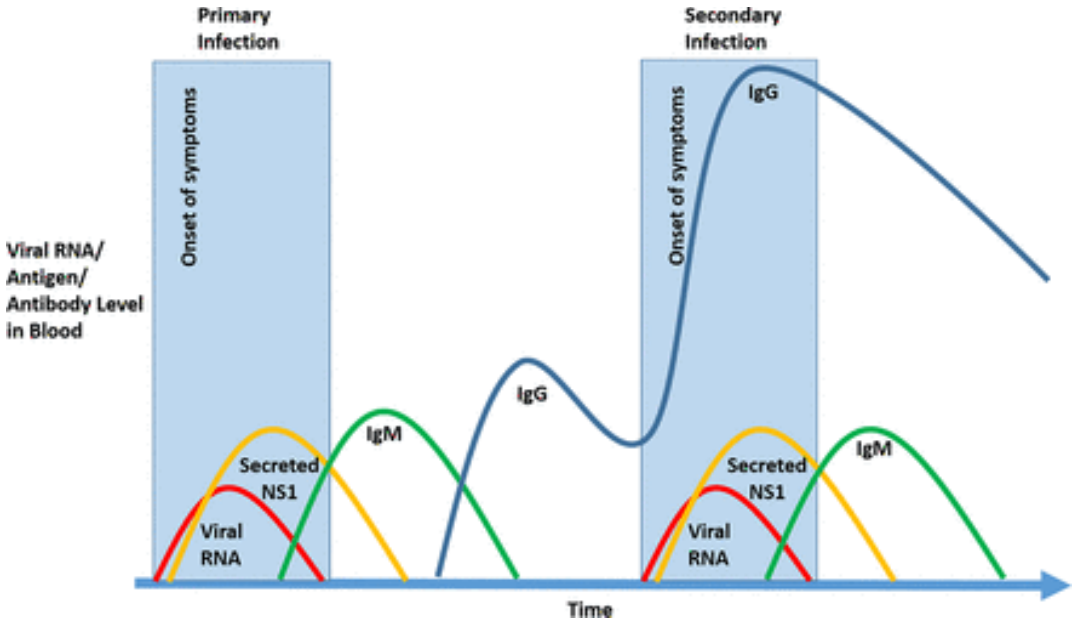


Figure 5-4. Viral and antibody kinetics during primary and secondary dengue infection. (Progress and Challenges towards Point-of-Care Diagnostic Development for Dengue. Junxiong Pang, Po Ying Chia, David C. Lye, Yee Sin Leo. Journal of Clinical Microbiology Nov 2017, 55 (12) 3339-3349; DOI: 10.1128/JCM.00707-17)

Low concentrations of anti-DENV antibodies to the DENV causing the secondary infection are present before exposure to the virus; as a result, ADE could occur [78, 79]. DENV–specific antibody titers increase earlier in secondary infection, reach higher peak titers, and have a lower IgM:IgG ratio, suggestive of an anamnestic response. As such, DENV–specific antibody titers are much higher during the late stage of viremia in secondary infections, with greater potential for forming immune complexes and activating complement.

The kinetics of the T lymphocyte response in secondary infections would include an earlier onset and higher level of DENV–specific T lymphocyte proliferation and cytokine production [80]. Interestingly, the severity of dengue disease and its correlation with the level and quality of the DENV–specific T lymphocyte responses has been inconsistent [81].

5.2 Dengue Impact on the Warfighter

As with recreational travelers, dengue threatens deploying U.S. military personnel. It has been a cause of febrile illness in troops deployed in tropical areas since the Spanish-American War [46], including the Pacific Theater of World War II [82], Vietnam (1969) [83], Somalia (1992-1993) [48], and Haiti (1997) [47]. Dengue affected French forces in New Caledonia (1989) [84], French Polynesia, and the West Indies (1997) [85]; and Australian forces and Italian troops in East Timor (1999-2000) [86]. The modern-day burden of dengue infections among recently and currently globally deployed troops is still largely unknown. A serologic survey of troops hospitalized with acute febrile illness during Operation Restore Hope in Somalia from 1992 to 1993 revealed of 96 patients with unspecified febrile illness, 43% had positive serological evidence of dengue infection [48]. This study did not capture the total incidence of dengue infections but analyzed sera only from febrile patients. In order to appreciate the infection, versus clinical disease risk, an anti-dengue virus antibody seroprevalence study was completed in 500

U.S. Special Forces soldiers who spent 30 days or greater in South America between 2006-2008. Testing of post-deployment serum found an 11% dengue seroprevalence rate among this population [87]. In another study testing pre- and post-deployment serum samples from 1,000 U.S. military personnel, a total of 76 (7.6%) post-deployment samples were positive; of these, 15 of the pre-deployment samples were negative. These figures represent an infection incidence of 1.5% and total of 17.6 seroconversions per 10,000 deployment months [50].

5.2.1 Operational Case Study

On September 19, 1994, 20,000 U.S. soldiers were deployed to Haiti as part of Operation Uphold Democracy [47]. The purpose of the mission was to provide security to the Haitian government, return the democratically elected President to office, and create a stable and secure environment in which democratic institutions could take hold. Living conditions for U.S. troops, especially the 10th Mountain Division, were described as spartan [88]. Latrines and fresh water were in short supply. Tropical rains from Tropical Storm Gordon and poor drainage meant that soldiers were living and working in areas ankle-deep in water. As forces extended their operations to provide security, troop movement and operations occurred during the night, with day hours designated as the time for rest and sleeping. From an infectious disease perspective, it was known that both malaria and dengue were endemic in the Haitian population, and U.S. personnel were given malaria prophylaxis and also insect repellant. The combination of a wet environment with deployed U.S. forces and accompanying equipment and trash build-up meant a rapid increase in the breeding environment for the *Aedes* mosquito vector. As discussed in Section 2, the nature of the deployment, with soldiers resting during the day in the shade and at peak biting times for the *Aedes* mosquito, translated into a situation ideal for DENV transmission and infection of a susceptible population. Between September 27, 1994 and November 5, 1994, 112 U.S. soldiers (0.1% of the total U.S. deployed forces in Haiti), were evaluated for fever, of which 30 soldiers were confirmed as dengue infected. Common symptoms included malaise, headache, chills, backache, loss of appetite, rigors, joint and muscle pains, and nausea [47]. Several required evacuations back to medical treatment facilities in the U.S. In a survey of the patients with dengue, 16.7% always used DEET and 13.3% had treated their uniforms with permethrin [47].

5.3 Challenges in Dengue Vaccine Development

There is no licensed prophylactic or therapeutic dengue drug (i.e. antiviral or anti-inflammatory). Vector control, even when successful from an entomologic perspective, does not always translate into a reduction in human infection or disease [89]. Personal protective measures such as wearing long sleeves and pants, use of bed nets, use of insecticides (e.g. *N*,*N*-Diethyl-*meta*-toluamide, DEET), avoidance of vectors during prime feeding times, and reducing the number of man-made vector breeding sites (e.g. standing water) are inconsistently applied [90]. A safe and efficacious dengue vaccine capable of protecting the recipient from disease caused by any of the DENV serotypes is considered the best option to reduce the global dengue burden.

However, numerous challenges plague the dengue vaccine development field. The existence of four DENV serotypes all capable of causing disease and death requires a dengue vaccine capable of preventing clinical disease caused by infection with any of the DENV [91-93]. Therefore, developers need to make tetravalent vaccines with components of each DENV serotype in the final formulation.

The coordination of innate and adaptive immune responses which confer protection or contribute

to pathogenesis following a DENV infection is incompletely understood [74]. As a result, there is concern an imperfect vaccine could induce ADE. Gaps in the understanding of dengue immunology are complicating the process of defining an immune correlate of protection.

Adversely impacting the exploration for a correlate of protection is the absence of an animal dengue disease model. Humanized small animal models are being aggressively studied but they do not appear to offer a comprehensive view of in vivo human dengue disease pathology at this time [94, 95]. There is also no validated dengue human infection model. Dengue human infection models expose healthy volunteers to mildly attenuated DENVs to produce an uncomplicated and mild dengue disease. These models can then be used to support drug and vaccine development allowing for early looks into clinical benefit before large field trials. There is active progress on developing a dengue human infection model, but it remains niche and not mainstream [96-99].

Another issue impacting the dengue vaccine field is the portfolio of assays used to measure vaccine immunogenicity during pre-clinical and clinical development activities. Neutralizing antibodies have the greatest likelihood of being identified as a correlate of protection [100]. Unfortunately, the classic assay platform designed to measure neutralizing antibodies (Plaque Reduction Neutralization Test, PRNT) has significant inter-assay and inter-lab variability and is not robust [101-104]. Assay platforms measuring cellular mediated immunity (CMI) have also been applied to dengue vaccine development programs but remain experimental [103].

5.3.1 Dengue Vaccine Candidates

There are numerous dengue vaccine candidates in pre-clinical and clinical development with three in efficacy trials [105]. Dengvaxia® is a chimeric live virus vaccine with the pre-membrane and envelope (preM and E) genes for each of the DENV serotypes replacing the analogous proteins in the yellow fever 17D virus [106, 107]. Takeda Pharmaceuticals has initiated a phase 3 clinical program using a DENV-DENV chimeric live virus vaccine where the preM and E genes from DENV-1, -3, and -4 are inserted into an attenuated DENV-2 backbone [108]. The U.S. National Institutes of Health (NIH) and Butantan are conducting an efficacy study using a live virus vaccine attenuated through directed mutagenesis of the DENV-1, -3, and -4 types and chimerizing DENV-2 preM and E genes into the attenuated DENV-4 backbone [109]. Merck &Co., Inc. have entered into agreements with both organizations to further develop the candidate. Dengvaxia® is the only vaccine which has been licensed and has been registered in over 20 countries. A safety signal observed in the youngest recipients and those who were dengue seronegative at baseline has shaped an indication for people 9-45 years of age who have been previously infected with dengue [110]. Takeda vaccines has published data out to 12 months of follow-up following vaccination [111]. The overall vaccine efficacy was 80.9%. Approximately 28% of the per-protocol population was seronegative prior to vaccination, in this group vaccine efficacy was 74.9%. Efficacy trends varied according to serotype with DENV-3 having no efficacy and there were not enough DENV-4 cases to make an efficacy determination [111]. Butantan is developing an NIH vaccine construct and is currently executing a phase 3 trial in Brazil with intent to enroll approximately 17,000 people.

5.3.2 Dengvaxia® Review

Dengvaxia® has been studied in 26 clinical trials including more than 41,000 volunteers. At least one injection of final tetravalent formulation has been administered to more than 28,500 individuals from 9 months through 60 years of age and 20,974 individuals aged 9 years through 45 years. Clinical end-point

studies were performed in Thailand (phase 2b, CYD23) and Asia (CYD14) and Latin America (CYD15) [106, 107, 112].

Numerous phase 1 studies together with three phase 2 studies provided data on safety and immune responses induced by several different vaccine formulations and immunization schedules. The results of these studies supported the selection of the final vaccine formulation and schedule: ~5 log10 CCID50 of each live, attenuated, DENV type 1, 2, 3, 4 given as 3 injections 6 months apart [113-116]. Additional phase 2 trials testing Dengvaxia® were performed in multiple endemic and non-endemic countries in Asia (India, Philippines, Singapore, Vietnam), Latin America (Brazil, Colombia, Honduras, Mexico, Peru), Australia and the U.S, addressing questions related to dose, schedule, priming by other flaviviruses or flavivirus vaccines, and the safety of co-administration with other vaccines[117-125]. Safety and immunogenicity were assessed in Indian populations and a co-administration phase 2 study was also conducted together with measles/mumps/rubella (MMR) vaccine. An indication for traveler/non-endemic populations was explored (shorter schedule) in a phase 2 adult study in the U.S. A booster dose (five years after dose three of the primary series) has been evaluated in two phase 2 studies and alternate vaccination schedules and booster dose study in individuals 9 to 50 years of age was conducted in the Philippines and Colombia with results pending. A prime boost with Japanese encephalitis (JE) vaccine before or during Dengvaxia® vaccination along with a shortened schedule was also explored showing equivalent neutralizing antibody titers with a shortened inoculation schedule of 0, 2 and 6 months [126]. Giving JE vaccine as a prime or concurrently didn't boost or negate neutralizing antibody titers.

Four phase 3 clinical studies were performed in dengue naïve adults in Australia up to 60 years of age and provided data to support phase 2 to phase 3 bridging required due to new manufacturing processes. A phase 3 trial was conducted in Malaysian children (2-11 years of age) assessing Dengvaxia's® safety and immunogenicity. Studies in Peru and Colombia assessed Dengvaxia® co-administration with yellow fever vaccine in infants and toddlers less than two years of age, while a study in Mexico assessed co-administration of DTacP-IPV (diphtheria and tetanus toxoids and acellular pertussis adsorbed and inactivated poliovirus vaccine) as a booster administered with the second injection of Dengvaxia®. Three co-administration studies with human papilloma virus (HPV) vaccine were completed in individuals 9 to 13 years of age in Australia and 9 to 14 years in Mexico. The third study (Philippines) assessed co-administration of a tetanus/diphtheria/pertussis vaccine in individuals 9 to 60 years [127-129]. Dengvaxia's® acute safety profile was found to be similar to licensed yellow fever vaccine (YF-VAX®, Sanofi Pasteur, Swiftwater, PA) and not affected by pre-existing yellow fever immunity. Most volunteers seroconverted in the monovalent DENV-2 trial and pre-existing yellow fever immunity contributed to a more cross-reactive and enduring anti-DENV antibody response [113]. Second and third doses of vaccine were also safe and demonstrated sequential increases in immune responses [114]. Studies in Mexico, the Philippines, and Australia continued to confirm acute safety in children and adults with varied pre-existing flavivirus immunity. The benefit of this pre-existing immunity towards developing rapidly increasing, broad, and potent immune responses after Dengvaxia® administration was also reinforced [115-117].

Three clinical endpoint studies have been conducted with Dengvaxia®; a phase 2b trial in Thailand and two phase 3 trials in Asia Pacific and Latin America [106, 107, 112]. Vaccine or control/placebo was administered at study months 0, 6, and 12. The primary efficacy endpoint was protection against dengue disease of any severity caused by any DENV type. The active follow-up phase of the study assessing for all symptomatic dengue was completed between study months 0 and 25 while hospital-based surveillance was originally planned from month 25 thru year 6; mid-way thru year 4, the surveillance expansion phase (SEP) was instituted marking a return to active surveillance. Acute safety

and reactogenicity in 9-17-year-olds revealed the frequency of grade 3 (severe) reactions was low. Most reactions were mild and resolved within a few days and the frequency of reactions lessened with each subsequent injection. For those with available baseline dengue serostatus (determined by PRNT50), there was no difference in the frequency or severity of acute adverse events as a function of serostatus. Finally, there were no safety concerns related to vaccine viremia, co-administration of other vaccines, or the inadvertent vaccination of pregnant women.

A phase 2b proof of concept study in 4 to 11-year-old children residing in Thailand included 2,452 vaccine and 1,221 placebo recipients. From 28 days following the last dose of vaccine (injections at time 0, 6 months, 12 months) to the end of the active surveillance phase (study months 0-25), 78 virologically confirmed dengue cases occurred in 77 subjects. The study did not meet the primary efficacy endpoint with an overall efficacy of 30.2% [95% CI: -13.4; 56.6]. Important observations from this study included: 1) tetravalent dengue vaccines may have variable DENV type-specific efficacy; 2) neutralizing antibody titers may not predict efficacy; and 3) powering a study to assess for DENV type-specific efficacy or efficacy against preventing severe disease would require extremely large sample sizes.

Two clinical end-point efficacy studies were conducted in five Asia Pacific countries (Philippines, Thailand, Indonesia, Malaysia, and Vietnam) and five Latin American countries (Brazil, Colombia, Honduras, Mexico, and U.S.) [130]. Subjects 2-14 years (N = 10,275) and 9-16 years of age (N = 20,869) were enrolled and were randomized 2:1 (vaccine: placebo). Both studies met the primary efficacy endpoint (2-16 years old, after 3 injections, during active surveillance months 13-25) with an efficacy in Asia of 56.5% (43.8-66.4) and Latin America of 60.8% (52.0-68.0); the combined study efficacy endpoint was 59.2% (52.3-65.0).

Vaccine efficacy in the same population from the first injection (months 0-25) was very similar. Combining studies, DENV type-specific efficacy was greatest for DENV-4 [76.9% (69.5-82.6), followed by DENV-3 [71.6% (63.0-78.3) and DENV-1 [54.7 (45.4-62.3)], with the lowest efficacy against DENV-2 [43.0% (29.4-53.9%)]. Efficacy against hospitalized dengue due to any of the DENV types after the first injection (months 0-25) was 67.4% (50.6-78.7) for Asia and 80.3% (64.7-89.5) for Latin America, with a combined efficacy of 72.7% (62.3-80.3). Combined efficacy against severe dengue was 79.1% (60.0-89.0). The relative risk (RR) of hospitalized dengue due to any DENV type in Asia favored Dengvaxia® during the active study phase (years 1 and 2) and for the entire study period, but was inconclusive for years 3 and 4 as the upper limit of the RR confidence intervals crossed 1. The results were somewhat different in Latin America with more convincing RR's for the active phase [0.197 (0.11-0.35)] and entire study period [0.323 (0.22-0.47)]. Years 3, 4, and 5 all had RR's below 1, but the upper limit of the CIs crossed 1. The RRs of experiencing severe disease conclusively favored Dengvaxia® in Asia only for the active phase [0.300 (0.13-0.64)]. In year 3, the data strongly favored the control with a RR of severe disease of 5.497 (0.80-236.60). In Latin America, the data favored the vaccine during the active phase and the entire study period; while the year 3 safety signal was not observed.

In summary, in three clinical endpoint studies, Dengvaxia® maintained the positive acute safety and reactogenicity profile established in early clinical studies. DENV type-specific and mean tetravalent neutralizing antibody responses were superior to placebo/control, moderate in titer, and relatively balanced across the different DENV types, but were not directly associated with DENV type-specific efficacy (i.e., immunogenicity by PRNT for a certain type did not predict type-specific efficacy). Vaccine efficacy against any dengue, of any severity, caused by any DENV type was low to moderate, with DENV-4 and -3 efficacy superior to DENV-1 and -2. Efficacy against hospitalized and severe dengue was superior when compared to prevention of any dengue. Efficacy as a function of time from injection demonstrated a positive trend towards the vaccine in years 0-2 and overall (0-5 years), but there was

a safety signal in year 3 among some vaccine recipients. There is a clear beneficial effect of DENV seropositivity on vaccine efficacy.

5.4 State of the Art

The state of the art for dengue infection is focused on improved diagnostics including point of care rapid diagnostic tests (RDTs), antiviral for prophylaxis and treatment of severe disease, and a tetravalent vaccine that produces durable protection. Several RDTs are available commercially and include tests to detect IgM and IgG dengue specific antibody as well as tests to detect the NS1 protein. All utilize a wicking fiber paper with embedded dengue specific proteins or antibody that wick a drop of blood and gives a color band indicating a positive test. These are sensitive and specific, can give results within an hour, and are deployable to the warfighter medical unit as a point of care device. Several antiviral drugs specific to DENV are in clinical development both to prevent infection and to decrease severity of dengue illness. As discussed, there are several dengue vaccines in clinical development that show promise in providing protection against all four DENV serotypes and potentially appropriate to protect the warfighter.

5.5 Recommended Countermeasures

Countermeasures against DENV infection currently include: 1) understanding the risk of locally acquired diseases to the susceptible deployed warfighter; 2) employing effective countermeasures such as vector control, use of insect repellants like DEET, and permethrin impregnated battle uniforms; 3) quick diagnostics to diagnose infection; and 4) prompt recognition of severe dengue illness with supportive treatment.

5.6 Summary

Dengue is a growing public health problem with the global disease burden growing annually. Infection may not manifest clinically or cause severe plasma leakage and/or hemorrhage and death. Numerous risk factors for severe disease have been proposed but the most convincing data points to sequential infections with different DENV serotypes as a primary culprit. Treatment is supportive as there are no licensed anti-DENV antivirals or immuno-therapeutics. A single dengue vaccine has been licensed and there are two others in advanced clinical development. Dengvaxia® has been licensed in more than 20 countries but use has been minimal as a result of safety signals in the youngest recipients and those who were dengue naïve at the time of vaccination. The world still requires a safe and efficacious tetravalent dengue vaccine capable of protecting seropositive and seronegative recipients across a broad age range.

6

Protecting the Warfighter Against Tick-Borne Illnesses

6.1 Tick-borne Illness Overview

Since Lyme disease was first described in 1977 as a debilitating cause of arthritis in children [131], there has been a steady expansion of the disease across the Northeast and Upper Midwest portion of the U.S., as well as other areas [132].

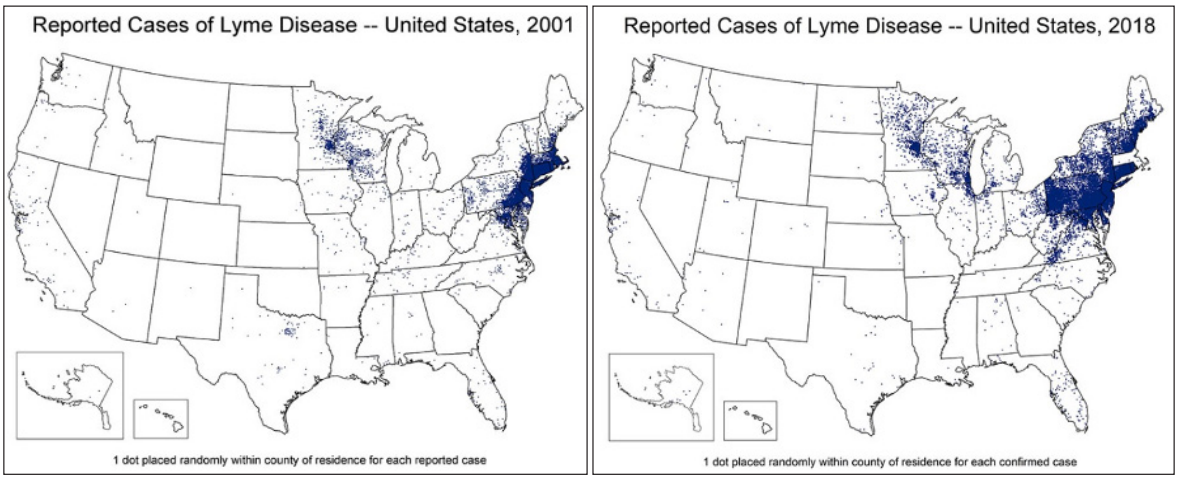


Figure 6-1. Comparison of Reported Cases of Lyme Disease in the U.S., 2001-2018 (https://www.cdc.gov/lyme/datasurveillance/index.html)

Estimates indicate that more than 300,000 cases of Lyme disease occur annually [133] as the vector spreads across the region with climate change, bringing Lyme and other co-infections with it. Tick-borne diseases can vary depending on region, tick species, and clinical presentation, ranging from debilitating to potentially fatal as was unfortunately observed in one deployed servicemember in Afghanistan who contracted Crimean Congo hemorrhagic fever (CCHF) in 2009, likely from an infected *Hyalomma* species tick [134]. The costs associated with these diseases are extensive, with Lyme disease alone potentially costing several hundred million dollars annually [135].

6.1.1 Lyme Disease

Lyme disease has a diverse presentation of disease manifestations caused by the *Borrelia burgdorferi* spirochete and spread by the *Ixodes scapularis*, otherwise known as the blacklegged tick or deer tick [136]. Early localized infection can result in the potential for a rash at the bite site, or perhaps multiple rashes associated with the dissemination of the infection. The classic erythema migrans rash, or "bull's eye" rash (Figure 6-2) is particularly characteristic and is considered to be pathognomonic in areas endemic for Lyme disease. This particular presentation is not seen in all cases, and patients can have atypical presentation of the rash, or even absence of a rash in general. A flu-like illness may accompany this rash. Facial paralysis, otherwise known as Bell's palsy, may also be a sign that the rash is associated with an acute Lyme infection. A similar rash can be seen after the bite of the *Amblyomma americanum,* or lone star tick, found primarily in the Southeast U.S., resulting in a syndrome referred to as Southern Tick-Associated Rash Illness [137]. This is thought to be caused by a different species of spirochete known as *Borrelia lonestari*, and is generally treated in a similar manner to Lyme disease, but without any long-term sequelae. For years, the geographic distribution of the lone star tick and blacklegged tick appeared separate; but with climate change providing more hospitable environments further north, these ticks may be found more consistently in the endemic regions known to harbor the Lyme disease spreading vectors [138].

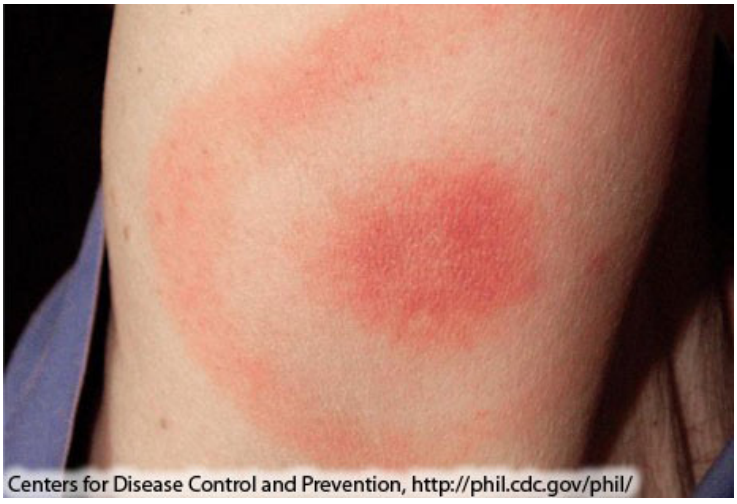


Figure 6-2. Classic erythema migrans rash, or "bull's eye" rash (https://www.cdc.gov/lyme/signs_symptoms/rashes.html)

After the organism disseminates from the skin, there are several common clinical presentations [139]. Early disseminated Lyme disease can often result in neurologic or cardiac manifestations.

Neurologic Lyme, or neuroborreliosis, can manifest as Lyme meningitis or encephalitis, or potentially peripheral nerve involvement to include the previously mentioned seventh cranial nerve neuropathy associated with Bell's palsy [140]. Cardiac manifestations are predominantly associated with conduction delays, resulting in the possibility of complete heart block. Less common manifestations include myocarditis, pericarditis, and heart failure, with sudden death rarely reported [141]. Late dissemination of the infection often results in Lyme arthritis, which can be quite debilitating, often affecting the knees (Figure 6-3) but potentially affecting any joint [142].

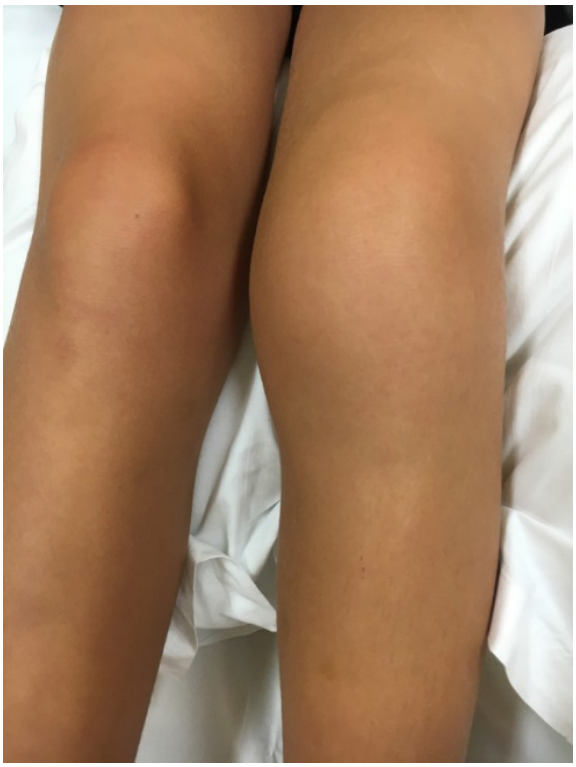


Figure 6-3. Swollen left knee from Lyme arthritis (Photo source: author)

Arthritis can be persistent after successful treatment and may not be associated with recurrent or persistent infection. This antibiotic refractory Lyme arthritis often requires treatment with anti-inflammatory agents or disease modifying anti-rheumatic drugs often reserved for autoimmune disorders such as rheumatoid arthritis [143]. In 15-20% of cases, patients can go on to develop chronic debilitating symptoms characterized by fatigue, joint and muscle pains, headaches, and cognitive disturbance. These symptoms can be very difficult to treat and do not appear to respond to long courses of antibiotics [144].

Diagnosis of Lyme disease is generally based on clinical or laboratory findings. Early in the course of the disease, if a patient has evidence of the classic erythema migrans rash and resides in an endemic area, the patient is considered to have Lyme disease and is treated without further delay [139]. This is primarily due to the fact that the Lyme diagnostics currently available and U.S. Food and Drug Administration (FDA)-approved may only be positive in 20-40% of cases at this stage due to the lack of time to develop a measurable immune response [145]. Testing sensitivity generally improves the longer someone is infected, but sensitivity is still not 100%, which is where much of the criticism is directed at testing algorithms promoted by the U.S. Centers for Disease Control (CDC) and Infectious Disease

Society of America (IDSA). Unfortunately, no other testing is reliable. Many assays are being advertised as being the most sensitive tests available but lack the rigorous validation procedures required for FDA licensure, and many have been found to have a very high false positive rate in otherwise healthy individuals [146]. This lack of sensitivity early in the disease can lead to misdiagnosis and prolonged infection prior to treatment, which may predispose patients to many of the long-term sequelae.

6.1.2 Tick-borne Encephalitis Viruses

Several tick-borne flaviviruses can result in potentially severe neurologic manifestations. Unlike many other tick-borne infections that require the tick to feed for at least a day if not longer to transmit the pathogen, tick-borne encephalitis viruses often require only minutes for transmission [147]. The two primary viruses of concern are the tick-borne encephalitis (TBE) virus, comprised of three related viruses found in Europe and Russia, and Powassan virus which includes the closely related deer tick virus, found in the Northeast and Upper Midwest U.S. in the same geographic distribution as Lyme disease.

The TBE virus is a potential threat to military service members stationed in endemic regions in Europe. The infection can be transmitted both by tick bite as well as potentially through ingestion of unpasteurized dairy products [148]. This, along with other organisms found routinely overseas, is reason enough to avoid unpasteurized dairy. Infection classically is described as having a biphasic illness, with flu-like symptoms with fever, headache, and fatigue, followed by neurologic manifestations that can result in severe disease and death [148]. Treatment is supportive, so prevention is key. In addition to personal protective measures such as insect repellants on the skin and permethrin treated clothing and uniforms, an effective vaccine is available outside of the U.S. [149]. More information on TBE virus vaccines can be found in Section 6.3.

Powassan virus is a concern in the U.S. in areas endemic to the blacklegged tick. Although this pathogen remains rare, the incidence is increasing, with 20-30 cases annually in most recent years [150]. There is no vaccine available for this infection, and treatment is supportive. Patients can present with severe meningoencephalitis. The infection generally carries a 10% case fatality rate, but an estimated 50% of survivors are felt to have long term neurologic sequelae [151]. This infection received notoriety when former Senator Kay Hagan was diagnosed in 2017 and had subsequent neurologic sequelae due to the resulting encephalitis [152].

6.1.3 New Threats

With the expansion of Lyme disease across the Northeast U.S. and elsewhere in the country along with the large number of patients infected, other tick-borne illnesses are often overlooked or underrecognized. Severe cases of anaplasmosis and babesiosis can occur in this same endemic region, while other ticks can transmit the equally dangerous ehrlichiosis and Rocky Mountain spotted fever. Yet we still do not know all of the possible tick-borne risks in our own country, as we are still identifying new pathogens in our own backyard. Lyme-like infections secondary to *Borrelia miyamotoi* appear to be more prevalent than previously thought [153] and may be under-recognized if providers testing for Lyme disregard standard two tier testing with positive Lyme enzyme immunoassays (EIAs) that have negative Lyme Western Blot results. New viruses have been identified in the Midwest and Southern U.S. with associated fatalities secondary to both Heartland [154] and Bourbon viruses [155]. Additionally, new vectors being introduced to the country is also a concern. *Haemaphysalis longicornis*, or Asian longhorned tick, has been identified in New Jersey, and appears to be spreading along the East Coast

[156]. This is an aggressive species capable of swarming livestock and capable of parthenogenetic reproduction, i.e. females can lay eggs without mating. This tick has been shown to potentially transmit several pathogens overseas, including, in China and Japan, severe fever with thrombocytopenia syndrome virus (SFTSV) resulting in hemorrhagic fever with ~15% case fatality rate. This virus is not found in the U.S., but is a phlebovirus similar to the Heartland virus noted previously, raising concerns of the ability to potentially transmit this virus as well.

6.2 Tick-Borne Illness Impact on the Warfighter

There is notable concern for both deployed servicemembers and those stationed here in the U.S. Many of the military bases within our borders fall within one or more geographic distribution maps of these vectors, putting the warfighter at risk here at home. One soldier deployed to the Middle East presented with fever, flu-like symptoms, and what appeared to be a classic erythema migrans rash, despite being in a location that was not endemic to Lyme disease. His case and picture were presented to the infectious disease military providers via telemedicine service. It turns out that during time back home mid-deployment, he spent most of his days camping in New England where he likely encountered a blacklegged tick, but did not present with his acute Lyme disease until he returned overseas. A study from West Point identified 63 cases of Lyme disease at their site from FY2016-2018 [157]. The soldier discussed in Section 6.1 who was killed by CCHF in 2009 was unfortunately not wearing a permethrin factory-treated Army Combat Uniform, but these were then issued in 2013 in an attempt to protect servicemembers against various bloodsucking insects [158].

6.3 Vaccines Against Tick-borne Illness

Prevention against many of the tick-borne illnesses is focused on personal protective measures. The use of topical insect repellants and permethrin treated clothing are necessary to attempt to decrease the risk of transmission as several viral pathogens require little time for transmission after a bite. Frequent tick checks are useful in preventing many other nonviral pathogens as it often takes much longer for transmission to occur after attachment and post-exposure prophylaxis can be utilized in an attempt to prevent Lyme disease. With a few exceptions, vaccines unfortunately are not a common method for prevention for the majority of tick-borne illnesses as many of these diseases either have a finite geographic reach, or limited numbers of infections annually.

TBE virus vaccines are available for use outside of the U.S. A systematic review that included 8,184 individuals who had received one of three different TBE vaccines indicated an overall seroconversion rate of 87% [159], with side effects noted to be common but not severe. Although the vaccine appears to be quite efficacious [160], there may be an increased risk of more severe presentation in those with vaccine breakthrough [161]. The annual estimated number of Lyme cases and the associated morbidity due to long term effects of the infection would indicate that an effective vaccine could be cost effective. Previous Lyme vaccines had been developed, and even licensed in the U.S. – LYMErix and ImuLyme were vaccines targeting the outer-surface protein A (OspA) on the surface of *Borrelia burgdorferi*. LYMErix underwent a large phase III clinical trial in over 10,000 subjects living in Lyme endemic areas in the U.S. Vaccine efficacy was 76% in preventing definite Lyme disease, and 100% effective in preventing asymptomatic infection by the second year, with only mild to moderate reactions lasting only a few days in most cases [162]. The vaccine was subsequently licensed by the U.S. FDA. Unfortunately, within the first year, there were reports of increased adverse reactions, primarily with

musculoskeletal problems mirroring those of the disease it was attempting to prevent [163]. Despite a review of the available data to include a post-licensure vaccine safety and efficacy case-control study indicating that the vaccine did not appear to have a higher risk of adverse effects, the vaccine was ultimately withdrawn from the market due to negative press coverage, ongoing litigation, and falling sales. Currently there are new Lyme vaccines in development, with VLA15 (Valneva) currently the furthest along in phase 2 clinical development [164].

6.4 Treatment for Lyme Disease

Therapeutic recommendations for Lyme disease have been in place for quite some time with few changes over the years to the IDSA guidelines [139]. These guidelines have focused intently on the available literature to help guide clinicians in the treatment of Lyme disease, while minimizing the adverse effects of antimicrobial use. Given the complexity of the disease itself and the frequency of persistent symptoms after presumably effective treatment regimens, alternatives to the IDSA guidelines have been created [165]. Many of the endorsements outlined in these alternative guidelines are based on observational or retrospective studies, often recommending long or intermittent courses of antibiotics, and create a stark contrast to the IDSA recommendations that are often perceived as rigid assessments of the available literature. This has created difficulty for patients seeking help, as there tends to be a wide disparity amongst providers in the approach to treatment for diagnosed Lyme disease. Additionally, approaches to treatment of Lyme disease, and in particular Post-Treatment Lyme Disease Syndrome (PTLDS) or chronic Lyme disease, have been few and far between, leaving many patients with chronic symptoms suffering, searching for a panacea.

Studies of post-exposure prophylaxis have been performed to assess the efficacy of antimicrobials when given after a bite from the blacklegged tick, in an attempt to stop the infection earlier in the course. Studies assessing amoxicillin at the time of a tick bite were small and flawed and did not demonstrate evidence that the drug could prevent Lyme disease [166]. Doxycycline, when given as a single, 200 mg dose within 72 hours of a tick bite that has been attached for at least 36 hours, appears to have the best data to support its use and incorporation into the IDSA guidelines [167]. Despite this, many providers will treat patients with 10-21 days of therapy while having no evidence of active infection, primarily using these prolonged treatment regimens as a preventive measure.

Treatment regimens have been relatively static over the years with a handful of regimens that are utilized by providers. First line oral agents for early disease include doxycycline, amoxicillin, or cefuroxime with treatment recommendations of 10 to 21 days [139]. Azithromycin has activity against the spirochete, but given the increased number of treatment failures [168] when compared to the other oral options, it should not be used as a first choice. Treatment of neurologic, cardiac, or joint involvement with arthritis can be treated with up to 28 days of therapy [139], with intravenous ceftriaxone used in the most severe cases or in instances of potential recurrent disease. Assessing for treatment success is primarily a clinical exercise, although there is some evidence that a down trending Lyme C6 assay titer may correlate with effective treatment with good clinical response [169].

Chronic symptoms are one of the feared outcomes from infection with Lyme disease. Unfortunately, in approximately 15% of patients who are infected, there is a development of chronic debilitating symptoms often referred to as PTLDS or chronic Lyme disease [144]. Although recurrent infection is a potential cause of the infection, alternative causes are more likely, as longer courses of antibiotic therapy have been largely unhelpful in several randomized clinical trials [170]. There have been even fewer studies assessing potential non-antibiotic treatment options for these chronically

suffering patients, making the treatment of these case extremely difficult as patients and providers wrestle with what to do next.

6.5 State of the Art

There are few state of the art advances in the world of tick-borne diseases. The diagnostic algorithms have largely stayed the same for years. The development of antibody tests against the C6 surface protein [171] and the modified two-tier test algorithm [172] have been more recent, albeit subtle advances. Additional diagnostics focused on earlier detection as well as direct detection of the organism are being pursued, but no candidates appear ready for clinical use. Treatment options have also not changed, and there are no clear nonantibiotic-based treatment options for patients that go on to develop chronic symptoms. Hope for a novel vaccine against Lyme disease is on the horizon [164], but the leading candidate is still only in phase 2 testing to date.

6.6 Recommended Countermeasures

The recommended countermeasures against tick borne diseases include: 1) targeting vector control methods to stop the spread of the ticks; 2) improving diagnostic capabilities to detect infection earlier in the course and showing confirmation of disease eradication; 3) educating patients, about the risks of ticks and their diseases, and providers, on how to recognize the clinical manifestations of tick-borne diseases; 4) identifying more effective treatment options to prevent the development of chronic manifestations of Lyme disease; and 5) developing effective and safe vaccines for prevention of infection for those at risk in endemic regions.

6.7 Summary

Tick-borne diseases are a concern worldwide, and appear to be expanding within the U.S. The infections that are transmitted can cause the potential for long term morbidity as well as mortality depending on the pathogen involved. Little is being done to combat the spread of these vectors, making prevention through avoidance, personal protective measures, and the development of vaccines that much more important. Lyme disease remains the most prevalent threat, and given the often-complex nature of the disease presentation, as well as chronic symptoms, it remains a challenge to treat effectively as a subpopulation of those infected may go on to have chronic and debilitating symptoms.

7

Infectious Complications of Warfighter Battle Injuries and Multi-drug Resistant (MDR) Bacteria

7.1 Overview of MDR Bacteria Impacting Military Personnel

7.1.1 Initial Evidence of Multi-drug Resistant Bacterial Infections during Operations in Iraq

Risk of death due to battlefield injuries has decreased over time, but subsequent wound infections continue to be an unfortunate reality in modern warfare. In the current age of drug resistance, this is compounded by pathogens proving to be more difficult to treat. Multi-drug resistant (MDR) bacterial infections have been a common complication of many traumatic wounds and burns suffered on the battlefield. A collaborative study between the Army and the Navy performed in Bethesda, MD from 2007-2008, showed that although the majority of wounds were not found to be infected or even colonized upon arrival from overseas, 31% of the wounds biopsied showed growth, with *Acinetobacter baumannii* being identified as the most prevalent isolate in over 22% of all biopsies taken (Figure 7-1) [173].

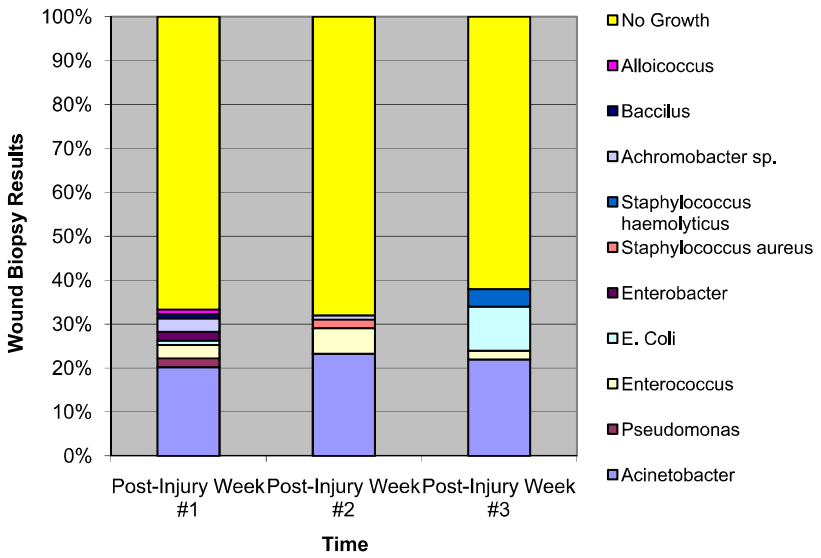


Figure 7-1. Frequency of isolates in 242 wound biopsies by time period [173]

There has been extensive review of the types of casualties in soldiers who have returned from the recent conflicts in the Middle East. Infectious complications have remained a constant problem, especially with many multi-drug resistant organisms (MDROs). One review article cited that approximately 15% of patients with traumatic injuries to the limbs have resultant underlying bone infection, with approximately 17% of these patients suffering from some form of recurrence of these infections following appropriate treatment [174].

Gram-negative rods

Any gram-negative rod that is resistant to all drugs tested in three or more of the following antimicrobial classes[†]

Class	Antimicrobials
Aminoglycosides	Amikacin
	Gentamicin
	Tobramycin
β-lactams	Ampicillin/sulbactam
	Piperacillin/tazobactam
	Ceftazidime
	Cefepime
Carbapenems	Imipenem/cilastatin
	Meropenem
Fluoroquinolones	Ciprofloxacin
	Levofloxacin

Any gram-negative rod that produces extended-spectrum β-lactamase

Stenotrophomonas spp., *Burkholderia cepacia*, and *Ralstonia* spp.

Gram-positive cocci

Methicillin-resistant *staphylococcus aureus*

Vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*

[†] Resistance or susceptibility to other antimicrobials tested not belonging in one of the four listed classes (e.g., colistin, polymyxin, minocycline, tigecycline, and trimethoprim/sulfamethoxazole) should not be considered in the classification of isolates as MDRO/MDR bacteria.

Figure 7-2. Definitions Used to Identify MDRO/MDR Bacteria [175]

One study out of Brooke Army Medical Center [176] assessed patients with open tibial fractures and found that 27 of 35 patients assessed had a least one organism present in deep tissue cultures, with *Acinetobacter*, *Enterococcus*, and *Pseudomonas* being the most commonly isolated organisms. Of these patients, 37% suffered nonunion of the affected bone lasting at least nine months. Four patients ultimately required amputation of the affected limb due to these infectious complications. The Burn Center at the same institution assessed the organisms associated with their admitted patients in the burn unit and found *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneuomoniae*, and *Staphylococcus aureus*, all of which are capable of carrying resistance genes [177]. *Acinetobacter* was the most common isolate identified in combat-injured burn patients and was the most common organism recovered in the first two weeks of hospitalization. A similar study in the same patient population looking at outcomes in patients with bloodstream infection reported the number of MDR isolates from these organisms, with at least 39.6% showing multidrug resistance amongst these organisms (Figure 7-3) [178]. Bloodstream infection with one of these organisms carried a relative risk of death of 2.6 in multivariate analysis.

Organism	n*	No. of isolates	Multidrug-resistant isolates	
			n	%
Pseudomonas aeruginosa	36	96	38	39.6
Klebsiella pneumoniae	34	83	59	71.1
Acinetobacter calcoaceticus-baumannii complex	44	67	45	67.2
Staphylococcus aureus	23	37	28	75.7

*Does not add up to 92, as numerous patients had more than one episode of bacteremia and a single patient might have had bacteremia with more than one of the top four pathogens during their admission.

Figure 7-3. Most Common Pathogens Recovered from Blood of 92 Burn Patients, January 2003 to May 2006 [178]

The patterns of MDRO colonization appear consistent across other major Medical Centers (MEDCENs), when several larger receiving military hospitals are evaluated together [175]. It appears that *Acinetobacter* colonization appears to decrease, only to be replaced with other MDROs.

7.1.2 Multi-drug resistant Acinetobacter and Fungal Infections during Operations in Afghanistan and the Impact on the Warfighter

Counter insurgency operations in Iraq and Afghanistan necessitated the performance of numerous foot patrols outside of vehicles in order to interact with the local populace as well as negotiate difficult terrain. In turn, this led to an increase of dismounted injuries to include massive trauma from IED blasts and resultant significant lower extremity injury. These wounds not only resulted in massive blood loss and an increased need for supportive blood products, but also an increase in debris embedded in these wounds.

The attempts to avoid the development of bacterial wound infections with broad spectrum prophylactic antibiotics, along with the resuscitative efforts with blood products may have resulted in a large increase in the number of invasive fungal wound infections [179-180]. A study focused on these cases coming out of Afghanistan from mid-2009 through 2010 [180] included 37 patients, of which 100% were injured due to a blast injury, with 92% on foot patrol suffering a dismounted injury. These infections in general result in significant myonecrosis and angioinvasion and require extensive surgical debridement as the primary intervention. Antifungal therapy alone is not sufficient. This often leads to further limb revision or amputation. Of the patients identified, there were five fatalities, with three cases directly attributable to the fungal infection. Figure 7-4 shows patients at Landstuhl Regional Medical Center who were wounded in Afghanistan and contracted an invasive fungal infection (IFI) during 2009-2010.

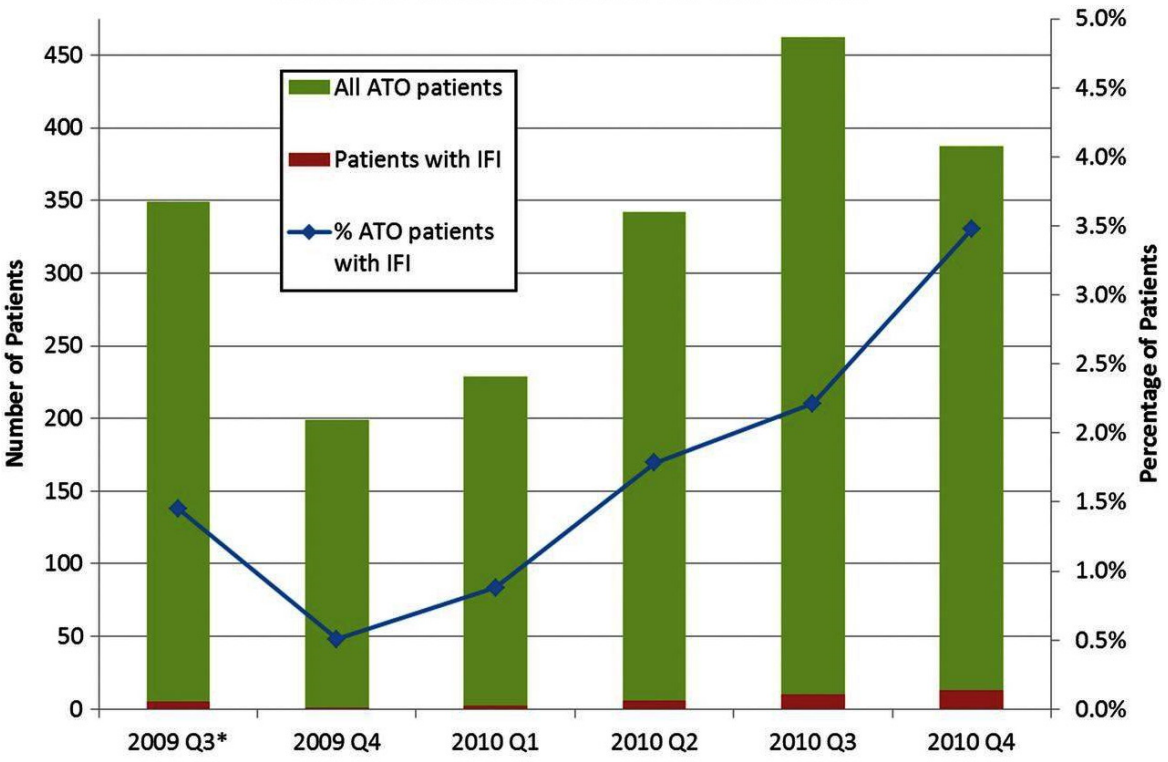


Figure 7-4. Landstuhl Regional Medical Center trauma admissions from the Afghanistan Theater of Operations (ATO), by Calendar Quarter [180]

7.2 Risk Factors of Acquired MDRO Infection

The risk factors associated with acquisition of these MDROs has been assessed at several levels. It is clear that deployed providers coming back from theater to the U.S. have an increased risk of MDRO colonization [181]. One study showed a notable jump of MDRO colonization rates jumping from 3% to 13% when comparing Landhstuhl Regional Medical Center to the three large receiving MEDCENS in the U.S., perhaps indicating an infection control risk in Germany or potentially en route to the U.S.

hospitals [175]. Another study, which focused on comparing U.S. patients to local national patients and host nation troops at Bagram Air Base in Afghanistan, assessed the numbers and rates of MDROs in each population, and clearly showed that there was a much higher rate of MDRO in Afghan inpatients compared to U.S. patients in the same hospital (Figure 7-5) [182]. This work appears to be consistent with a study done previously that indicated that the rate of *Acinetobacter* infections appeared to correlate with the mean census of host-nation patients admitted to the wards as well as the ICU (Figure 7-6) [183].

Studies of deployed servicemembers to Iraq prior to injury or hospitalization also indicated that prior colonization with these MDR pathogens, specifically *Acinetobacter*, is not a likely cause of these infections post-trauma, and that the organisms are likely acquired after the point of injury, supporting the nosocomial acquisition in the field hospitals [184].

	Afghan inpatients			
	Community acquired	Hospital acquired	Afghan outpatients	US patients
All bacteria	53/91 (58)	133/202 (66)	6/26 (23)	14/73 (19)
Gram negative	44/65 (68)	120/169 (71)	4/7	4/41 (10)
Escherichia coli	18/25 (72)	32/42 (76)	1/3	3/28 (11)
ESBL	12/25 (48)	24/42 (57)	1/3	2/28 (7)
Acinetobacter spp.	9/11 (82)	42/45 (93)	...	...
Klebsiella spp.	9/13 (69)	12/22 (54)	2/2	...
ESBL	9/13 (69)	8/22 (36)	2/2	...
Pseudomonas spp.	2/5	4/15 (27)	...	...
Enterobacter spp.	0/2	8/14 (57)	0/1	0/2
Citrobacter spp.	1/3	8/9	1/1	...
Proteus spp.	1/2	2/5	...	1/3
ESBL	1/2	1/5	...	1/3
Other gram-negative bacteria	4/4	12/18 (67)	...	0/5
Gram positive	9/26 (35)	13/33 (39)	2/19 (11)	10/32 (31)
Staphylococcus aureus	6/17 (35)	11/17 (65)	1/16 (6)	10/29 (34)
Enterococcus spp.	3/7	2/11 (18)	1/2	0/2
Other gram-positive bacteria	0/2	0/5	0/1	0/1

NOTE. Data are no. with multidrug resistance/total (%), unless the value of the denominator was less than 10, in which case the percentage is not included. "Community acquired" are isolates recovered within 48 hours after patient admission; "hospital acquired" are isolates recovered after 48 hours after patient admission. Data from 19 cultures that did not have associated patient nationality and hospitalization data were excluded. ESBL, potential extended-spectrum β-lactamase producer.

Figure 7-5. Multidrug Resistant Bacteria Recovered from 392 Cultures Performed at a U.S. Military Hospital in Afghanistan, September 2007 to August 2008 [182]

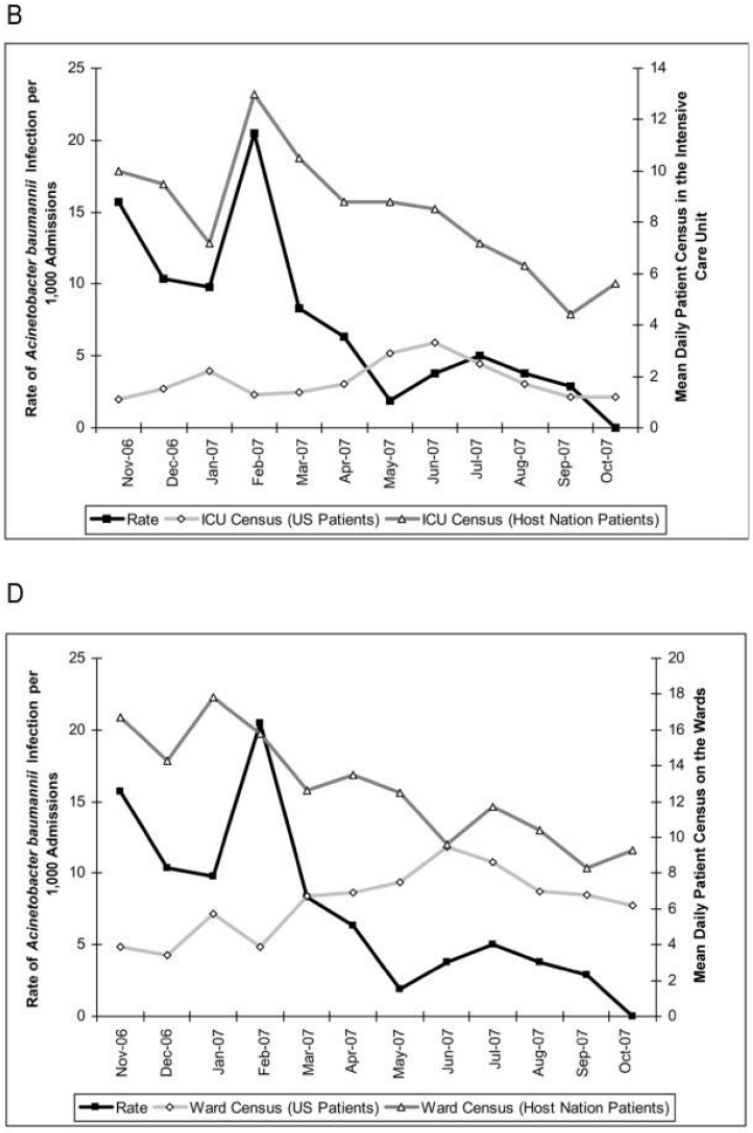


Figure 7-6. Comparisons of U.S. and Host Nation Patients' Rates of Acinetobacter baumannii Infection [183]

Acinetobacter is a gram-negative coccobacillus that tends to be opportunistic in its ability to cause human infections, with nosocomial infections becoming increasingly common [185]. Many of these organisms tend to carry multiple resistance genes, making the treatment more complicated than many of the other organisms frequently encountered in the hospital. This organism has the ability to cause nosocomial outbreaks. A study using pulsed-field gel electrophoresis to assess isolates found in seven field hospitals in Iraq and Kuwait and compare them to patient isolates identified five cluster groups that appeared to be related to the environmental findings [186]. The results indicated that the environmental contamination played a significant role in the outbreak, and that focus on strong infection control practices is a requirement in these settings. An outbreak of extremely drug resistant (XDR) Acinetobacter occurred that started in 2011 in a tertiary care hospital in the Northwest U.S. resulted in the death of all 6 patients [187]. The organism is also a potential threat to health care providers. One of the ICU nurses at former National Naval Medical Center (NNMC) in Bethesda, MD

acquired an infection from one of her patients with MDR *Acinetobacter* resulting in pneumonia and bloodstream infection, and a two month stay in the ICU herself that included time on the ventilator as well as blood pressure support for sepsis [188].

Attempts to prevent these infections have been evaluated. Many patients were prescribed a single dose of broad-spectrum antibiotics at the point of injury in the deployed setting. Studies evaluating this practice indicated that although there was no clear evidence of harm, there did not appear to be any benefit either as there was no effect on infection or colonization rates [189].

7.3 Ongoing Surveillance for MDR Bacteria in the DoD

Given the issues and concerns discussed previously over further development of MDR pathogens and increased servicemember morbidity and mortality, screening capabilities were created in 2009 as part of the Antimicrobial Resistance Monitoring and Research Program, also referred to as ARMoR (see Figure 7-7) [190].

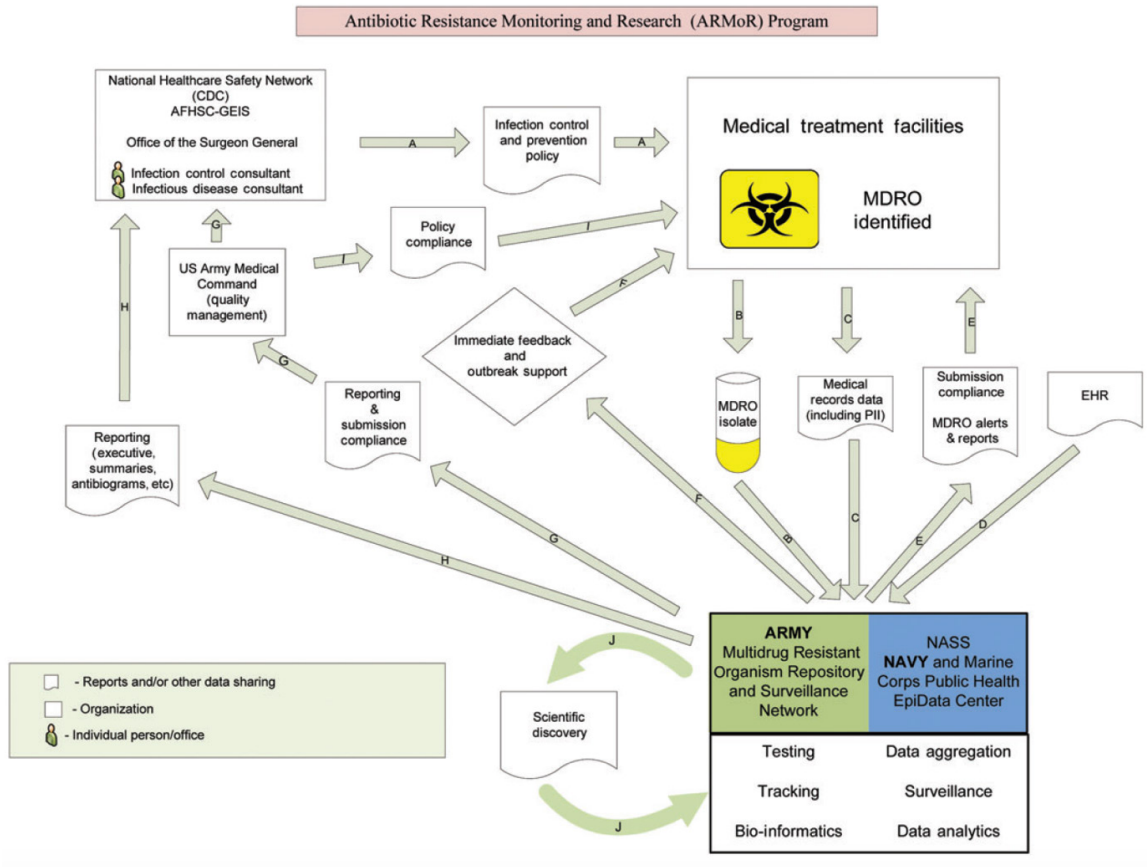


Figure 7-7. ARMoR Overview [190]

At the Walter Reed Army Institute of Research (WRAIR), the Bacterial Diseases Branch houses the Multidrug-Resistant Organism Repository and Surveillance Network, otherwise known as MRSN. This serves as the "primary surveillance organization for antibiotic-resistant bacteria across the Army, Navy, and Air Force" [191], with a focus on ESKAPE-E pathogens (*Enterococcus faecium*, *Staphylococcus*

aureus, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter species*, and *Escherichia coli*) which are the "most common bacteria in humans that are associated with antibiotic resistance". The repository contains over 60,000 clinical samples, and collaborates with both Federal and civilian entities across the world. The ability of this group to utilize a variety of technologies for surveillance and molecular assessment have assisted in identifying strains with resistance genes as well as identifying clusters of these strains which can help with the epidemiology of outbreaks and their prevention [192].

Part of the work performed by the MRSN includes whole genome sequencing. Using this technology, they have been able to provide rapid assessment of possible nosocomial outbreaks. Rapid turnaround time of just 48.5 hours was achievable in one study to help confirm likely nosocomial spread of a vancomycin-resistant *Enterococcus faecium* [193], which, as the study's authors note, is "an unprecedented level of resolution for outbreak investigations" [193]. The MRSN team has also utilized this approach to assist with outbreak investigations overseas to support deployed U.S. servicemembers [194].

7.4 Challenges in Treating MDRO Infection

Treatment options are limited for some of the more resistant forms of gram negative infections, with many hospitals resorting to use of invariably toxic medications such as aminoglycocides, tigecycline, or polymixins to treat some of these presentations [195]. There have been development and U.S. FDA approval of some new products in recent years to include drugs like ceftazidime-avibactam, ceftolozane-tazobactam, meropenem-vaborbactam, and imipenem-relebactam. Unfortunately, ceftazidime-avibactam alone does not have sufficient activity against *Acinetobacter*. The addition of either vaborbactam or relebactam does not appear to provide activity against carbapenem resistant *Acinetobacter baumannii* (CRAB) cases, while ceftolozane-tazobactam has variable activity. More recently approved drugs eravacycline [196] and cefiderocol [197] appear to show promise with activity against CRAB isolates.

7.5 State of the Art

The lack of substantial therapeutic options for the more resistant organisms has resulted in the increased interest in bacteriophage-based products. Both WRAIR and the Naval Medical Research Center (NMRC) have been working on bacteriophage products. In 2010, members of NMRC were involved in helping to develop a personalized bacteriophage cocktail for a 68-year-old male patient who developed complications associated with necrotizing pancreatitis [198]. He was found to have a pancreatic pseudocyst that became infected with an MDR strain of *Acinetobacter baumanni* with treatment failure on multiple antibiotics. He was given intravenous *Acinetobacter* bacteriophage, as well as intracavitary injections into the infected cysts as a compassionate use approach. He was eventually discharged home on day 245, despite an extremely grave prognosis prior to this treatment. Other members of the military research community also assisted in the treatment of a two-year old boy in a local Washington, DC children's hospital [199]. This child had multiple congenital anomalies as well as several severe antibiotic allergies and developed a recurrent form of *Pseudomonas aeruginosa* bloodstream infection that developed resistance to antibiotics while on therapy. Although the patient was unable to survive his dire situation, the clinicians noted that he was able to sterilize his blood after 4 weeks of persistent blood culture positivity when given a cocktail of two bacteriophages targeting his

Pseudomonas. Several other difficult cases have shown benefit and positive outcomes associated with the use of adjunctive bacteriophage therapy.

Components of bacteriophage therapy have also been evaluated. Bacteriophage lysins can potentially be isolated and recombinantly produced, acting directly on the bacterial cell wall causing the bacteria to undergo bacteriolysis. A leading bacteriophage lysin product against *Staphylococcus aureus* appears to be safe per company reports, with efficacy studies underway in phase 2 and 3 studies [200]. Additionally, these lysin products are being evaluated for other MDROs, to include *Acinetobacter* [201, 202]. The addition of these products to the armamentarium of antibacterial agents would also potentially help with treatment of these difficult to treat infections.

7.6 Recommended Countermeasures

The recommended countermeasures against MDRO infections, specifically gram-negative organisms to include *Acinetobacter,* will need to focus on the following: 1) continued strong antimicrobial stewardship practices in healthcare facilities, and potentially in the outpatient setting as well; 2) diagnostics focusing on culture results and specifically susceptibility reports that ensure that newer drugs are reported, as many of the newer products require a cumbersome and long send out process to reference laboratories; 3) continued education focusing on antibiotic stewardship for healthcare providers, to include ways to confront patients demanding antibiotics for unnecessary presentations (e.g. viral infections); 4) continued development of novel antimicrobial agents to combat the continued development of antimicrobial resistance mutations; and 5) the potential for vaccine development for commonly encountered infections with the potential for MDRO development (e.g. recurrent urinary tract infections).

7.7 Summary

MDR pathogens will continue to be a concern for patients. New drugs and novel therapeutics are desperately in need and war wounded individuals will continue to be at risk from these pathogens, so continued effort at developing countermeasures and other prevention techniques will continue to be needed, to include *Acinetobacter* as well as other MDROs.

8

Future Infectious Disease Threats and Need for Effective Countermeasures

8.1 Future Infectious Disease Threats

The infectious diseases discussed in the previous sections will continue to pose a threat to military personnel so long as they remain endemic in operating areas. However, the absence of existing endemic disease in any given location is not enough to guarantee a low-risk disease environment. To maintain force health and readiness, it is critical that the U.S. Department of Defense (DoD) also develop effective and timely countermeasures for future infectious disease threats.

Future infectious disease threats fall into one of two categories. The first category is new or previously unknown diseases [203, 204]. The 2019 Novel Coronavirus (COVID-19) falls into this group. At time of writing, global COVID-19 cases continue to rise, with the U.S. topping the list at over 544,000 confirmed cases [205]. While the latest publicly available data shows the DoD accounting for just a small fraction of those numbers (1,638 total, 953 of which are active-duty, as of 2 April 2020), cases within DoD are rising [206], indicating the potential for substantial future risk to personnel health and force readiness. As seen in the 1918 Spanish flu pandemic discussed in Section 2, a newly emerged pathogen can spread rapidly within the military, decimating numbers, overwhelming medical and logistical support resources, and exacerbating global transmission if forces are sent overseas or community transmission gains speed.

The second category is infectious diseases that are known, but are now spreading into new geographical areas or increasing in incidence [203, 204]. These diseases can pose significant risk to both the military and the homeland if the spread is not contained. However, doing so can be vastly

expensive. Responding to the 2014-16 Ebola outbreak in West Africa, for instance, cost at least $2.8 billion [207], $402.8 million of which came directly from DoD response activities including direct support, R&D, and cooperative threat reduction [208].

The threat from these types of diseases has grown over time as the pace at which they emerge has increased; the world has seen approximately 40 new infectious diseases since 1970, including Ebola, Zika, Severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), chikungunya, avian influenza (H5N1) [203] and most recently, COVID-19. There is no single cause for the heightened pace of emerging infectious diseases. Alongside natural adaptive emergence of new pathogens [209], a number of human-generated factors have likely combined to enable their spread: increases in international travel have brought humans into contact with pathogens they would not have encountered before, for which they do not have immunity and which they can carry back to their home countries; the warming climate has enabled disease-carrying mosquitos to expand their habitats into new geographic locations, widening their transmission radius; urban sprawl and resultant habitat crowding have brought humans into closer contact with animals, increasing the risk of zoonotic diseases; and distrust of the medical community – in particular, the anti-vaccination movement in the U.S. – has decreased vaccination coverage levels, threatening herd immunity [203]. These shifts combine to create a complicated landscape in which emerging infectious diseases remain a significant risk to the warfighter.

8.2 Preparing for Emergence of Previously Unknown Infectious Diseases

It is estimated that 1.67 million viruses that could affect humans are as yet undiscovered [209]. Given the sheer volume of the potential for new viruses, not to mention ongoing multi-drug resistant infections as previously discussed, maintaining the basics of infectious disease control should be a top priority. It is critical that basic ongoing countermeasures are followed, including maintaining clean food and water supply, encouraging self-reporting of illness, and following aerosol, fecal-oral, and insect vector control procedures. These measures can help minimize rapid transmission of emerging diseases as well as aid in early identification of an emergence event.

In addition, scenario planning for potential future diseases is key to maintaining response preparedness and minimizing outbreak impact, as even local outbreaks can have a significant security impact. The aforementioned 2014-16 West African Ebola outbreak illustrates this point, costing not only billions of dollars but also 11,000 lives [207]. Out of that event grew the now common outlook that global cooperative R&D and scenario planning are critical elements of emergency response to an emerging disease outbreak. In the case of Ebola, a former World Health Organization (WHO) assistant director-general credits global cooperation across the WHO, government, industry, public organizations, and scientists for the development of "…the first-ever fully effective vaccine against Ebola, developed and tested in 12 months as opposed to the five to 10 years such a process would normally take" [211]. In turn, the WHO added 'Disease X' to a new, prioritized list of future pandemic possibilities requiring urgent R&D, standing for an unknown but likely future global pathogen against which governments, industry, public organizations, and scientists globally could begin to plan and conduct R&D [211].

While creating countermeasures for 'Disease X' and other unknown future infectious diseases poses challenges, R&D efforts draw from existing knowledge about known diseases to build new approaches to prevention and treatment. One example of this is developing vaccines targeted at types of viruses

as opposed to the current approach of tailoring vaccines to individual viruses [210], for instance, developing a universal flu vaccine that would remain effective even as the virus mutates, something which is in development and showing promise [212], or using what is known about MERS, SARS, and now COVID-19 to try to develop a universal coronavirus vaccine in the future.

8.3 Department of Defense Role in State of the Art R&D for Known Emerging Infectious Diseases

Given the shifts in human interaction discussed previously, the risk of pandemic from emerging infectious diseases is notable. To encourage continued R&D into countermeasures, the WHO publishes a list of known emerging infectious diseases that it considers high-priority for R&D due to their pandemic potential. The list at time of writing includes: Crimean-Congo hemorrhagic fever (CCHF), Ebola and Marburg virus disease, Zika, MERS and SARS, Nipah and henipaviral diseases, Lassa fever, Rift Valley fever (RVF), and the recently added COVID-19 [213].

The DoD plays a critical role in pushing forward state of the art R&D into both preventive and therapeutic countermeasures for these high-priority diseases. While civilian research often focuses on treatment measures, proactive preservation of force readiness requires a heavy focus on prevention [4], positioning the DoD as a leading driver of vaccines and other preventive measures. The Walter Reed Army Institute of Research (WRAIR) Emerging Infectious Diseases Branch, for example, has dedicated research programs focused on developing vaccines and other preventive measures for Zika, Ebola, MERS, and Lassa fever. These programs have resulted in numerous R&D successes, including involvement in developing and advancing a Zika vaccine candidate to phase 1 clinical trials, several Ebola and filovirus vaccine candidate clinical trials, and the first in-human and only phase 1 trial to date of a MERS vaccine [2]. In response to the ongoing COVID-19 pandemic, WRAIR, alongside the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), is actively working to develop a vaccine for COVID-19, while simultaneously coordinating with the Department of Veterans Affairs (VA) to develop rapid diagnostic kits for the new disease [214]. As COVID-19 cases climb among military personnel, rapid testing in particular will be critical to identifying and isolating affected individuals, thereby slowing disease spread, improving protection of other servicemembers, and allowing mission-critical trainings to proceed with lowest possible risk.

In addition to researching and developing preventive measures, the DoD is also well-positioned to facilitate on-the-ground testing and clinical trials of not only preventive but also therapeutic measures during localized outbreaks of these diseases. The DoD uniquely has access to easily mobilized personnel with expertise in international stakeholder engagement, local population management, and clinical expertise, all of which are critical for an R&D endeavor of this nature [215]. The Joint Mobile Emerging Disease Intervention Clinical Capability (JMEDICC) presents a recent example. Established in the wake of failed clinical data collection during the 2013-2016 Ebola outbreak [215], JMEDICC is an ongoing effort, in collaboration with Ugandan researchers, to conduct clinical trials of preventive and therapeutic measures during ongoing filovirus outbreaks [216]. JMEDICC's unique access to patients with past exposure to a number of emerging infectious diseases however has also led to its suitability for research beyond filoviruses. This research includes an ongoing collaboration with both USAMRIID and the U.S. Food & Drug Administration (FDA) to collect serological samples of patients with previous exposure to not only Ebola or Marburg, but also RVF, CCHF, Chikungunya virus, and Zika, in an attempt to better understand microbial pathogenesis of these diseases and thereby develop more effective countermeasures [217].

8.4 Need for Effective Countermeasures

As reviewed throughout this report, infectious disease outbreaks can have a significant negative impact on force readiness and can put notable financial and logistical strain on military resources. Given the evolving landscape of infectious disease and the uncertain environments in which military personnel operate, DoD involvement in the development of effective countermeasures against emerging infectious diseases is paramount to maintaining force readiness and avoiding costly future health and logistical challenges posed by emerging infectious diseases.

9

Conclusion

Current and emerging infectious diseases pose significant risk to the warfighter. Deployment to areas of endemic disease, environmental conditions, shared living and training facilities, physical and mental stress, and risk of exposure to biological weapons combine to increase personnel risk of disease. While existing countermeasures against infectious disease are largely effective when applied correctly, operational realities can make effective implementation difficult. Furthermore, warfighters may be at risk of infectious diseases for which effective countermeasures are still in development. These factors combine to increase incidence of disease non-battle injuries (DNBI) across the military and in turn decrease force readiness, resulting in costly logistical and resourcing challenges. Recommended countermeasures (Table 9-1) should be prioritized for effective implementation across all military personnel and facilities. Meanwhile, evaluating and making use of state of the art solutions (Table 9-2) as they are developed and maintaining the DoD's role as a proponent of R&D against emerging infectious diseases will both be critical to continued future improvement of force health protection.

Table 9-1. Recommended Countermeasures to Protect the Warfighter from Infectious Disease

Recommended Countermeasures to Protect the Warfighter from Infectious Disease	
General	› Understanding the risk of locally acquired diseases to the susceptible deployed warfighter › Prompt recognition and treatment returning the warfighter back to combat effectiveness › Prioritizing clean food and water supply › Encouraging self-reporting of illness › Ensuring implementation of vector control measures › Scenario-planning against likely emergent diseases (e.g., 'Disease X')
Influenza	› Social distancing, avoiding overcrowding, disinfection of common sources for contamination › Personal protective measures to avoid infection › Quick diagnostics to diagnose infection › Prophylactic drugs or vaccines when available to prevent infection
Leishmaniasis	› Personal protective measures to avoid infection by an insect vector › Quick diagnostics to diagnose infection
Malaria	› Vector control and personal protective measures to avoid bites from the mosquito vector such as bednets, DEET insect repellant, and permethrin-impregnated combat uniforms › Rapid diagnostics to diagnose infection › Prophylactic drugs or vaccines (when licensed for use) available to prevent infection
Rabies	› Prompt reporting and care for animal bites › The use of pre-exposure rabies vaccination for deploying servicemembers › Animal control, personal protective measures to avoid bites from potential rabid infected animals, and vaccination prior to entering endemic areas › The use and availability of post-exposure vaccines and immune globulin to prevent infection
Dengue	› Vector control, use of insect repellants like DEET, and permethrin impregnated battle uniforms › Quick diagnostics to diagnose infection › Prompt recognition of severe dengue illness with supportive treatment
Tick-Borne Illness	› Targeting vector control methods to stop the spread of the ticks › Improving diagnostic capabilities to detect infection earlier in the course and showing confirmation of disease eradication › Educating patients, about the risks of ticks and their diseases, and providers, on how to recognize the clinical manifestations of tick-borne diseases › Identifying more effective treatment options to prevent the development of chronic manifestations of Lyme disease › Developing effective and safe vaccines for prevention of infection for those at risk in endemic regions
Acinetobacter and other gram-negative MDROs	› Continued strong antimicrobial stewardship practices in healthcare facilities, and potentially in the outpatient setting as well › Diagnostics focusing on culture results and specifically susceptibility reports will need to ensure that newer drugs are reported, as many of the newer products require a cumbersome and long send out process to reference laboratories › Continued education focusing on antibiotic stewardship for healthcare providers, to include ways to confront patients demanding antibiotics for unnecessary presentations (e.g. viral infections) › Continued development of novel antimicrobial agents to combat the continued development of antimicrobial resistance mutations › The potential for vaccine development for commonly encountered infections with the potential for MDRO development (e.g. recurrent urinary tract infections)

Table 9-2. State of the Art Developments in Infectious Disease Research and Development

State of the Art Developments in Infectious Disease Research and Development	
Influenza	› Research and development of new antivirals that act against the virus – notably, baloxavir and favipiravir, which show promise as a prophylactic to prevent infection and a therapeutic to diminish severe complications from infection › Universal vaccine that will prevent infection from all strains of flu, for which several vaccine candidates are in human clinical trials
Leishmaniasis	› Rapid diagnostic testing, including polymerase chain reaction (PCR) – which can have a result within a day – as well as new markers and improved test kits for serologic diagnosis › New treatment therapies including miltefosine, a well-tolerated oral medication, as well as, for cutaneous leishmaniasis, a thermal device which can be applied to the cutaneous lesion to kill the parasites
Malaria	› Point of care rapid diagnostic tests (RDTs) that eliminate the need for a microscope and provide a result within several minutes › An RDT for *Plasmodium falciparum* specifically that is sensitive, specific, and can be forward deployed to combat units › New antimalarial drugs, including tafenoquine, which is specifically used for prophylaxis and treatment of *Plasmodium vivax* › A number of malaria vaccines currently in clinical development and undergoing human clinical trials
Dengue	› Improved diagnostics including RDTs, some of which are available commercially and include tests to detect IgM and IgG dengue specific antibodies as well as tests to detect the NS1 protein; all are sensitive, specific, give results within an hour, and are deployable to the warfighter medical unit › Several antivirals specific to DENV which are in clinical development and show promise for both prophylaxis and treatment › Several dengue vaccines in clinical development which protect against all four DENV serotypes, including Dengvaxia®, which has been licensed and registered in over 20 countries
Tick-Borne Illness	› Development of antibody tests against the C6 surface protein and modified two-tier test algorithm › Novel vaccine against Lyme disease in phase 2 testing › Additional diagnostics focused on earlier detection as well as direct detection being pursued, but none ready for clinical use
Acinetobacter and other gram-negative MDROs	› Increased interest and testing of bacteriophage products and adjunctive bacteriophage therapies › Evaluation of components of bacteriophage therapies, such as isolated and recombinantly produced bacteriophage lysins, which are being evaluated against *Staphylcoccus aureaus* and other MDROs including *Acinetobacter*

**Rabies not explicitly included as current countermeasures – rabies vaccines, rabies immune globulin for post-exposure prophylaxis, and diagnostics – are highly effective and represent the state of the art.*

Abbreviations and Acronyms

ACIP ..... Advisory Committee on Immunization Practices
ADE ..... Antibody-dependent enhancement
AFHSC ..... Armed Forces Health Surveillance Center
ARDS ..... Acute respiratory disease syndrome
ARMoR ..... Antimicrobial Resistant Monitoring and Research Program
AST ..... Aspartate transaminase
ATO ..... Afghanistan Theater of Operations
CCHF ..... Crimean Congo hemorrhagic fever
CDC ..... Centers for Disease Control and Prevention
CL ..... Cutaneous leishmaniasis
CMI ..... Cellular mediated immunity
COVID-19 ..... 2019 Novel Coronavirus
CRAB ..... Carbapenem resistant Acinetobacter baumannii
CTC ..... Clinical Trials Center
DENVs ..... Dengue viruses
DHIM ..... Dengue Human Infection Model
DNBI ..... Disease nonbattle injuries
DoD ..... Department of Defense
DSS ..... Dengue Shock Syndrome
E ..... Envelope
EIAs ..... Lyme enzyme immunoassays
FDA ..... Food and Drug Administration
GMT ..... Geometric mean titer
H5N1 ..... Avian influenza
HDCV ..... Human diploid cell vaccine
HPV ..... Human papilloma virus
ID ..... Intradermal
IDSA ..... Infectious Disease Society of America
IFI ..... Invasive Fungal Infection
Ig ..... Immmunoglobulin
IGHTS ..... Institute for Global Health and Translational Science
IM ..... Intramuscular
JE ..... Japanese encephalitis
JMEDICC ..... Joint Mobile Emerging Disease Intervention Clinical Capability
MDR ..... Multi-drug resistant
MDROs ..... Multi-drug resistant organisms
MEDCENs ..... Medical Centers
MERS ..... Middle East respiratory syndrome

MMR..... Measles/mumps/rubella
MRSN..... Multidrug-Resistant Organism Repository and Surveillance Network
NIH..... National Institutes of Health
NMRC..... Naval Medical Research Center
NNMC..... National Naval Medical Center
NS1..... Non-structural protein 1
Osp1..... Outer-surface protein A
PCEC..... Purified chick embryo cell
PCECV..... Purified chick embryo cell vaccine
PCR..... Polymerase chain reaction
PCVs..... Peace Corps volunteers
PEP..... Post-exposure prophylaxis
preM..... Pre-membrane
PrEP..... Pre-exposure prophylaxis
PRNT..... Plaque Reduction Neutralization Test
PTLDS..... Post-Treatment Lyme Disease Syndrome
RCT..... Randomly controlled trial
RDTs..... Rapid diagnostic tests
RIG..... Rabies immune globulin
RR..... Relative risk
RVF..... Rift Valley fever
SARS..... Severe acute respiratory syndrome
SEP..... Surveillance expansion phase
SFTSV..... Severe fever with thrombocytopenia syndrome virus
SUNY..... The State University of New York
TBE..... Tick-borne encephalitis
TNF..... Tumor necrosis factor
USAMRIID..... U.S. Army Medical Research Institute of Infectious Diseases
VA..... Department of Veterans Affairs
WHO..... World Health Organization
WRAIR..... Walter Reed Army Institute of Research
XTR..... Extremely drug resistant

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