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Combating Dengue: A US Military Perspective

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ABSTRACT

Throughout history, dengue virus infections have negatively impacted the mission capabilities of US Service Members. Currently, the expansion of dengue into new regions via the spread of the *Aedes* genus along with the global presence of the US Military, poses an increased risk for Service Members to contract the virus. Dengue virus infection would not only lead to significant medical costs and a lack of military readiness, but to mission impairment and failure. Therefore, it is important that the US Military explore the virulence, outbreaks, and treatments of dengue virus infection to help prevent its spread and determine solutions for its eradication. This review examines current dengue epidemiology by Combatant Commands, field detection, treatments, preventive measures, prophylactic capabilities, and directions of future research.

INTRODUCTION

Dengue virus (DENV) infections have been a cause of illness in military personnel throughout history with the erosion of mission capabilities and lost duty days being heavily underestimated. Additionally, the risk of dengue among US Service Members is increasing, consequently leading to mission impairment and significant costs. Corroborating this is a recent study involving 1,000 US Army personnel with a single deployment to a dengue-endemic region identified 15 Soldiers who developed antibodies to at least one DENV type [1]. This information demonstrates the need for increased research of DENV and a more comprehensive understanding of risk factors associated with vector virus exposure and infection. Dengue is a vector-borne disease spread through the bite of the mosquito genus *Aedes* and is endemic in more than 120 countries causing upwards of 390 million diagnosed infections a year [2,3]. There is a high likelihood that US Service Members (Army, Marines, Navy, Air force, and Coast Guard) will encounter the virus at some point in their careers. Therefore, as an issue of military readiness, it is important that the military explore the virulence, treatments, outbreaks, and other traits of dengue to determine the best future actions to prevent the spread of the disease as well as solutions to eradicate the disease altogether. There are four DENV serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) all of which cause a range of symptoms depending on the individual infected. The manifestations of dengue range from an asymptomatic infection or mild fever to hemorrhagic fever and dengue shock syndrome [3]. Further, if an individual is exposed to one of the

serotypes and recovers from an infection, but is subsequently re-exposed to another serotype, his/her chances for exacerbated illness increase. The US Military operates globally, and therefore, Service Members are deployed to many DENV endemic regions. Thus, analysis of the detection and treatment methods of a DENV infection is imperative to understand so that military medicine and medical policy reflects how best to address this viral disease. This review examines current epidemiology by Combatant Commands, field detection, and treatments for dengue in order to establish the military's baseline diagnostics, preventive measures, and prophylactic capabilities and to project the direction of future research.

MATERIALS AND METHODS

For the purpose of this review, the information presented about DENV and its infection was limited to reports conducted within the last ten years (2009-present) due to the dramatic growth in incidence of dengue worldwide in the recent decade [4]. Moreover, this review organizes DENV outbreaks and studies by US Military Combatant Commands (geographic and functional) to better facilitate military missions, functions, and planning. Examples of internet search words used for this review were "dengue virus," "military," and "outbreaks" on Google, Bing, and PubMed. Only peer-reviewed and fully accessible articles were reviewed to ensure that the discoveries and information presented are current and thorough.

RESULTS

Dengue and its Health Consequences

Dengue is caused by four antigenically distinct, single-stranded, positive-polarity RNA viruses, identified as dengue virus (DENV-1 through 4), denoting the various serotypes of the virus [5]. A serotype is a distinct variation of a virus which contains varying virulence factors, and the prevalence of serotypes varies by geographic region. Being infected by one serotype leads to lifelong protection from that serotype; however, a secondary infection with a different serotype increases the risk of severe dengue manifestation [2]. This phenomenon is known as an antibody-dependent enhancement (ADE) of infection. In ADE, preexisting antibodies from the first DENV infection bind to the second DENV serotype virion particles. Rather than neutralizing the viral infection threat, the antibody merely attaches to new viral particles produced after infection. The antibody within this antibody-virus complex then attaches to circulating receptors on monocytes and allows the virus to infect the monocytes. The resulting monocyte infection is associated with an increase in virus replication and a higher risk of severe dengue disease [4]. Therefore, the elicitation of broadly neutralizing antibodies present alluring targets for vaccine discovery [2]. Broadly neutralizing antibodies recognize all four DENV serotypes, thereby destroying the virus instead of promoting virus uptake into the cell. The cell and organ tropism of DENV can explain disease symptoms and impact infection severity. Although cell and organ tropisms have been best studied through animal models, to date, the murine and non-human primate models are not considered acceptable representations of viral replication and disease manifestation in humans [6]. Furthermore, clinical autopsies investigating viral tropism are limited despite the large number of confirmed infections, which is believed to be due to the remote locations of where many of these fatal cases occur [6]. Thus, the limited studies and clinical data collected identify three predominant organ systems associated with DENV infection: the immune system, the liver and endothelial cells. Within the immune system, infected dendritic cells responding to a mosquito bite site inadvertently transport DENV to lymph nodes where they amplify and spread infection to blood-derived monocytes, myeloid dendritic cells, and splenic and liver macrophages [6]. The liver is a commonly noted viral target in severe murine, non-human primates, and human DENV infections. DENV is present in high viral loads within human hepatocytes and Kupffer cells and is responsible for observed apoptosis, necrosis, and the eventual development of coagulopathy, or the absence of blood clotting [6]. The development of coagulopathy and plasma leakage is thought to be further amplified with the infection of endothelial cells [6]. However, the role of endothelial cells remains controversial with the limitations of current study models. DENV infections present a broad spectrum of symptoms, ranging from asymptomatic to undifferentiated fever, Dengue Fever, Dengue Hemorrhagic Fever (DHF), or Dengue Shock Syndrome (DSS) (Figure 1) [7]. Dengue fever is characterized by incapacitating symptoms of fever, severe headache, myalgia, arthralgia, and gastrointestinal discomfort [5,6]. Symptoms manifest in two phases with the first phase of illness occurring 3-8 days after

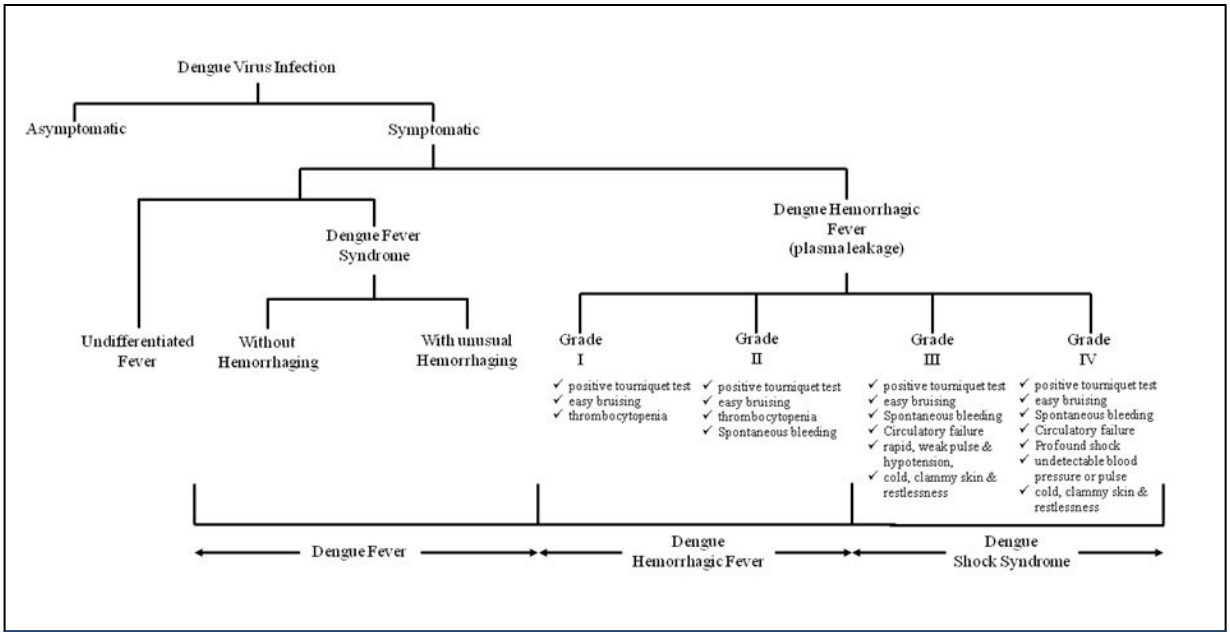

Figure 1: Symptoms of dengue virus infection. Dengue is difficult to clinically identify given the variability in the manifestations associated with infection. However, the World Health Organization (WHO) has outlined a general classification system with respect to dengue virus infection. This figure is adapted from dengue hemorrhagic fever: diagnosis, treatment, prevention and control. 2nd edition. Geneva: World Health Organization.

the mosquito bite, and the second phase of illness occurring after a period of improvement. The second phase can be followed by reappearance of fever and development of measles-like-rash, generalized lymphadenopathy, and minor hemorrhagic phenomena [5]. The categorically more severe cases of DHF present all symptoms of dengue fever, but also express hemorrhagic manifestations and plasma leakage. Hemorrhagic manifestations encompass bleeding in the skin and gastrointestinal tract. The gravest DENV symptom classification, DSS, is reached once shock sets in, and without treatment intervention, becomes fatal within 12 to 36 hours [5]. Annually, there are 100 million cases of dengue fever and half a million cases of DHF. While DENV serotype has no correlation on disease classification, greater severity cases trend in children and the elderly.

Dengue Outbreaks within Combatant Commands

Since the U.S. Military operates within the construct of Combatant Commands, it is necessary to understand how and where DENV infection is in relation to operations to appropriately assess and control risk (Figure 2). The following sections are divided into DENV infection outbreaks specifically within geographic commands and one functional command to provide clarity of the threat posed. It should be noted that within each of the Combatant Commands addressed below, the estimates of DENV infection cases are underreported signifying that the military must be wary of the severity of the occurring outbreaks. Overall, identifying and learning from these outbreaks will help the US Military face the threat and take preventive measure to address DENV infection globally.

Indo-pacific command (INDOPACOM): Dengue fever outbreaks have been occurring intermittently in the Pacific region, and in 2012 for the first time, the Pacific region registered a high prevalence of all four serotypes of DENV [8]. In 2014, Taiwan experienced its largest dengue fever outbreak, with 15,372 cases reported [9]. The heterogeneity in DENV seroprevalence within a single metropolis during this outbreak was a source of concern due to current vaccines against DENV having low efficacy and to the potential to enhance the disease with the mixing of different serotypes [9]. Also, in 2014, 131 DENV infection cases were reported in Yoyogi Park located in Japan [10]. It should also be noted that during the past 10 years there appears to be little dengue incidence in Korea and China, both of geopolitical interest, perhaps due to some environment and climate factors related to the *Aedes* genus (Figure 2).

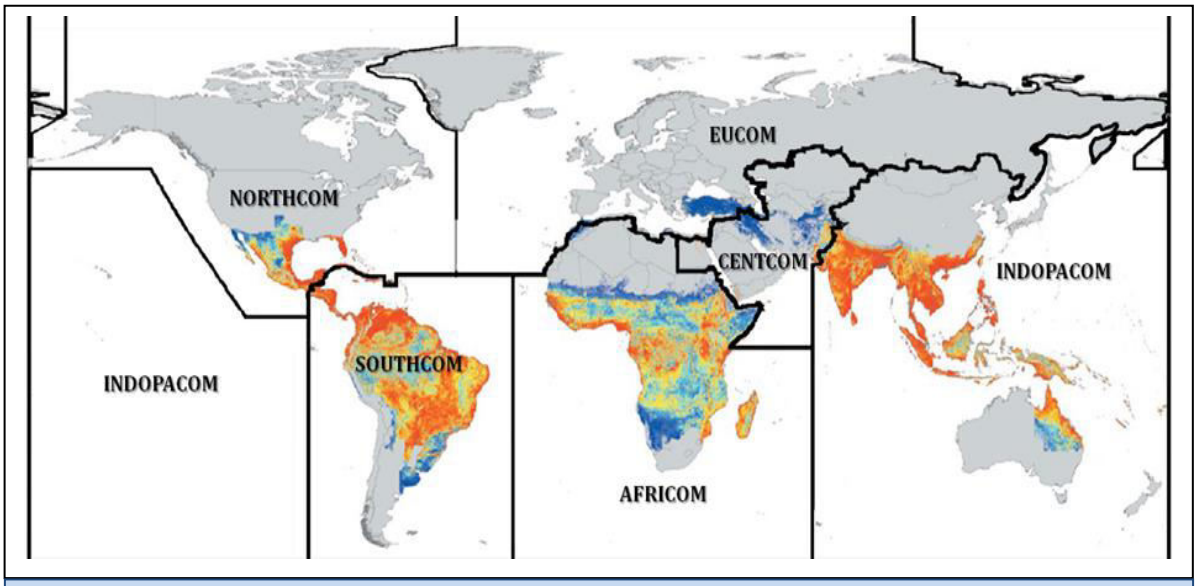

Figure 2: Global dengue risk assessed by geographic Combatant Command. Special Operations Command (SOCOM) operations occur in all five geographic Combatant Commands. The underlying map was adapted from Figure 1 of Simmons CP, Farrar JJ, van Vinh Chau N, Wills B. Dengue. New England Journal of Medicine. 2012; 366(15):1423-1432. The colors signify suitability for dengue transmission with red being high suitability all the way down to blue for low suitability. The grey signifies non-endemic regions/unsuitable for dengue transmission.

Southern command (SOUTHCOM): In Central America, multiple countries have experienced DENV infection epidemics. Dengue is known to be persistent in the jungle areas, with epidemics frequent in the wettest months, and subsequently imported into the coastal regions, especially during times when environmental conditions sustained year-round mosquito breeding [11]. This phenomenon was seen in Peru in 2001, with 137 confirmed cases in the province of Trujillo.

Brazil has experienced dengue outbreaks since 1845, but the current situation is alarming. Due to the tropical climate of Brazil, the country is susceptible to the circulation of arboviruses such as DENV across its states [12]. In 2013, there were a total of 1,468,873 reported DENV infection cases, of which 6,969 were severe (DHF and DSS) cases with 545 deaths [12]. However in 2014, there was a 39% decrease in mortality rates. In 2015, the Brazilian Ministry of Health registered 1,254,907 cases, which was an increase compared to that of 2014. Not only is DENV circulating on the continent, there has been circulation of all four serotypes reported in the Hispanic Caribbean Islands. Additionally, while not formally part of Southern Command, DENV-2 has circulated continuously in Puerto Rico for 25 years, including an outbreak in 2007 and a prolonged epidemic in 2010. During this epidemic, there were 26,766 cases and 148 fatalities, the greatest number ever recorded [11]. Among these infections, DENV-1 was the most prevalent. Adults consistently represent roughly one-half of the reported cases [13]. The Passive Dengue Surveillance System (PDSS) currently monitors DENV infection in Puerto Rico, to include the early detection of the virus to enable timely intervention and evaluate programs to prevent and control dengue [13]. Although the information gathered through PDSS is helpful towards accumulating data, it does not help control this virus. Studies show that for each case of dengue reported to the PDSS, an additional 10-27 cases were not reported [13].

Africa command (AFRICOM): Studies show that a better surveillance and diagnosis system for dengue is necessary due to the prevalence of DENV infection in Africa, yet these countries are still struggling to implement an effective system despite the rising number of cases. Sero-epidemiological surveys have demonstrated that DENV infection is widespread in Nigeria, yet there is little information on dengue fever and little attention paid to DENV infection overall because it presents as a classical fever with the main misdiagnosis of the disease being malaria [14]. This highlights the need for African countries to enhance the capacity of laboratories to diagnose

DENV infection correctly with modern equipment and new infrastructure. Another study conducted in various urban centers of the rainforest region of Nigeria found an overall DENV infection prevalence of 23.4% with the highest monthly prevalence of 40% in April and August [15]. The monthly differences indicate efficient virus transmission from mosquitoes to humans during these times and show that routine diagnosis for DENV infection is necessary [15]. In Djibouti, the first epidemic of DENV-3 was reported in a study by the French Armed Forces Health Service between 2011 and 2014 [16]. Of the 354 suspected cases of DENV infection, 128 confirmed cases were discovered and demonstrated the circulation of DENV-1, -2, and -3 in all hospitalized patients in the study [16]. At the Malindi District Hospital in Kenya, DENV infection outbreaks in 2017 were associated with the DENV-2 serotype. However, the strains isolated from 10 different patients were all quite different [17]. Thus, as potential new serotypes start to emerge in African countries, it raises the concern for the prevalence and severity of dengue outbreaks due to ADE. Additionally, it highlights the need for an easily identifiable way to detect not just one DENV serotype, but the creation of a diagnostic and treatment plan that covers all four serotypes.

Central command (CENTCOM): Within CENTCOM, in 2016 there were dengue outbreaks in Yemen and Saudi Arabia. Dengue is not known to be endemic in the western and southern regions of Saudi Arabia, so this outbreak caused great concern [18]. Although there is lower burden of DENV infection in the Middle East and North Africa than Asia, it cannot be discounted within this Combatant Command because antibodies to DENV were detected in nationals from countries within CENTCOM, supporting the need to better understand the regional epidemiology of dengue [19].

European command (eucom) and northern command (NORTHCOM): DENV is not known to be endemic within EUCOM. Thus far, there have only been singular and isolated DENV infection cases in EUCOM, with no disease spread or outbreaks [8,20,21]. However, there is a clear presence of dengue in Mexico and the Caribbean Islands leading to cases of DENV infection in the US, specifically in Florida due to its close proximity to the Caribbean [22]. An influx of dengue infected migrant workers and immigrants into susceptible *Aedes* populations are a way dengue could travel to infect populations in regions where it was not previously endemic. This, coupled with climate change leading to vector migration of mosquitoes northward into the US, could spark a potential outbreak in the near future. Since DENV can travel within infected mosquitoes from one continent to the next, the US Military must be wary and account for possible outbreaks of DENV infection in regions not normally endemic to the virus.

Special operations command (SOCOM): Although not a geographic Combatant Command, Service Members within SOCOM are at increased risk of exposure to DENV infection based on their multiple deployments to endemic regions of the world. These repeated deployments increase their risk not only of contracting dengue fever, but of developing more serious manifestations of the disease to include DHF and DSS. To highlight the seriousness of DENV exposure to SOCOM forces, a two-part study was conducted 2012-2014 examining baseline exposure to DENV in 500 de-identified SOCOM Service Members as well as the exposure rate of 414 de-identified SOCOM Service Members based on pre- and post-deployment sera. The first part of the study concluded that 48 out of 500 (9.6%) specimens exhibited a baseline DENV exposure rate from all the missions preformed throughout endemic regions [23]. The second part of the study showed that 55 of 414 (13.2%) specimens were seropositive to DENV only after deployment to AFRICOM, INDOPACOM, SOUTHCOM, or Central America [23]. Since SOCOM Service Members operate in small teams, an exposure rate as high as 10% can cause mission failure.

Difficulties Diagnosing DENV Infection in the Field

Four roles of medical care exist in regard to the US Military field environment [24]. (Table 1) summarizes the functions and location of each particular role as it pertains specifically to the Army (main ground force). As depicted, Role I is the first responder with unit level care, and it resides far-forward on the battlefield with the Soldier and at battalion aid stations. Role II is forward resuscitative care residing at medical companies within forward operating bases and has the function of treating trauma after the Role I immediate measures. Role III is theater hospitalization and resides at the established combat support hospital(s) and medical treatment centers in the country of operation. Finally, Role IV is definitive care residing at permanent base hospitals located around the world. To combat

Table 1: Roles of medical care within the US Army.

Role I	Role II	Role III	Role IV
MAIN ROLE: • Unit-level medical care • Immediate life saving measures WHO: • Combat medics • Self-aid and buddy aid • Combat Lifesaver • Special Forces Medical Sergeant (I8D) • Medical technicians CAPABILITIES: • Disease /non-battle injury prevention and care • Combat operational stress prevention • Primary healthcare, routine sick call • First aid and triage • Resuscitation and stabilization • Casualty collection and evacuation preparation WHERE: • Within the unit /on battlefield LIMITATIONS: • Holding patients • Healing serious injury or illness	MAIN ROLE: • Advanced trauma and Management emergency medical treatment WHO: • Role 2 Light Maneuver (2LM): • Mobile medical units • Conduct advanced resuscitation up to damage control surgery • Prepare for Role 3 and 4 • Role 2 Enhanced (2EN): • Basic secondary healthcare • Stabilize post-surgical cases for evacuation straight to Role 4 CAPABILITIES: • Carry packed blood products • Limited x-rays • Laboratory access • Dental support • Combat/operational stress control • PVTMED WHERE: • Within the unit/FOB LIMITATIONS: • Holding many patients • Holding for more than 72 hours	MAIN ROLE: • Providing support to units without organic medical assets • Caring for patients in medical treatment facility (MTF) with proper staff and equipment WHO: • Physicians/physician assistant • Medical personnel in MTF CAPABILITIES: • Resuscitation • Initial wound surgery • Specialty surgery • Post-op treatment • Evacuating patients from supported units • Physical therapy • Holding 200+ patients • Pharmacy access • Nutrition consultation WHERE: • Medical treatment facility	MAIN ROLE: • Most definitive medical care in military health system WHO: • Physicians/physician assistants • Medical personnel in hospital • Department of Veterans Affair-, CAPABILITIES: • Post-op rehabilitation • Significant/life-threatening surgeries • Advanced equipment access • Usage of CONUS and civilian Hospitals WHERE: • CONUS in base hospitals or robust overseas facilities

This table was extracted from Cubano, M. A., & Butler, F. K. (2018). Emergency War Surgery. (pp. 19-22). Fort Sam Houston, TX: Borden Institute, US Army Medical Department Center and School, Health Readiness Centre of Excellence, Fort Sam Houston, Texas, Office of The Surgeon General, United States Army.

Figure 3: The Next Generation Diagnostics System Increment 1 (NGDS-1), also known as the Bio Fire Film Array® 2.0. This figure was extracted from Next Generation Diagnostics System, Increment 1 Fact Sheet, 7/27/2017. Point of Contact: Chemical, Biological, Radiological, & Nuclear Information Resource Center (CBRN IRC), CBRN.IRC@mail.mil, Toll Free: 18008314408, Commercial: (309) 7827349, DSN: 7937349, Fax: (309) 7821919.

disease symptoms associated with DENV infection in the field, timely identification of US Service Members infected with DENV provides for "rapid and appropriate patient management decisions" such as supportive care therapies and medical evacuation [25]. Identification and diagnosis of DENV infection within Service Members has been done in the last 20 years with the use Polymerase Chain Reaction (PCR) technology. The "Ruggedized" Advanced Pathogen Identification Device (RAPID) PCR platform

was used to identify DENV early on. When a dry-format PCR assay was performed on this device, the assay demonstrated 100% analytical specificity for detecting DENV. Both human and mosquito samples were tested in austere field conditions where the RAPID system was set up in six hours in open air locations and operated using a generator or a car battery [25]. Results of this system, including the set up mentioned above, took anywhere from 2-4 days to accurately identify the agent of interest. The RAPID system was replaced by BioFire's Joint Biological Agent Identification and Diagnostic System (JBAIDS), which was designed to improve upon the RAPID system by increasing the accuracy and speed of biological warfare agent detection and identification [26]. The JBAIDS was able to fit into a rucksack and perform agent detection/identification on location with electricity in about 40 minutes with 85-90% accuracy [27]. The Joint Program Executive Office – Chemical, Biological, Radiological, and Nuclear Defense (JPEO-CBRND) Medical Countermeasure Systems (MCS) fielded approximately 350 JBAIDS to the Joint Services over its lifecycle. A DENV assay was available for use on the JBAIDS PCR platform; however, BioFire is no longer making systems or assays, and by 2020 the JBAIDS will no longer be operational [28]. The Next Generation Diagnostics System Increment 1 (NGDS-1), also known as the BioFire FilmArray® 2.0 (Figure 3), will replace the JBAIDS one-for-one, and offer a simple, portable PCR platform to aid the diagnosis of both clinical and environmental samples [29]. JPEO-CBDND MCS has completed fielding of NDGS-1 to the Air Force environmental and diagnostics users and is planning to field to the Army (Role 3) and Navy users as well [29]. The NGDS-1 Infectious Disease Panel (also called the Global Fever Panel) does detect dengue. This is still in advanced development awaiting FDA approval [30]. Currently, there are no Food and Drug Administration (FDA) compliant diagnostics for DENV infection at Roles 1, 2, or 3. Accordingly, a 2016 study on assessing the diagnosis capability gap of DENV in the military health system concluded that any diagnostic tests used at far-forward locations (Roles 1 and 2) need to be rugged, simple to operate, and used primarily to inform medical evacuation decisions [31]. Antigen-antibody based detection assays similar to those used for vector pathogen detection (discussed later in this article) were recommended as potential far-forward diagnostics. This same study suggests that diagnostics in fixed hospitals (Role 3) do not need to be as portable as those far-forward, but must be very accurate to inform patient care decisions [31]. Hence, the NGDS-1 will fill this capability gap as soon as the Global Fever Panel is available for use on it. However, clear guidelines for dealing with a positive or negative result are currently being formulated to establish clear courses of action for identified DENV infection [31]. At Role 4, DENV infection diagnosis is performed usually with host nation assistance, by commercial diagnostic companies, or by other facilities using PCR assays on various platforms [31]. This Role 4 diagnosis process is concerning in that this has reduced reliability both in speed and in receiving the results, which is why having diagnostic capabilities at Role 3 is imperative. Within the US Military, medical evacuation decisions are executed prominently on symptom-based medicine at Roles 1 to 3 when diagnostics are unavailable. For DENV infection, identification of it can be quite challenging compared to other febrile illnesses meaning that some cases of DENV infection that lead to DHF are missed [31]. Furthermore, antibiotics are sometimes inappropriately prescribed when the diagnosis is incorrectly assigned as bacterial instead of virus [31]. Therefore, it is important for the military to consider the effects of DENV infection and integrate new equipment and measures to provide the most efficient and greatest amount of care while minimizing the cost and mission capability losses.

Preventive Measures - Vector Control and Surveillance

Due to the absence of vaccine treatments and licensed prophylactics for DENV infection, the US Military must utilize preventive measures such as vector control and surveillance to limit personnel risk. Thus, vector control is the main way the military is combating dengue. A key tenet of military vector control operations with respect to dengue is not only diminishing the number of potential mosquito vectors, but decreasing transmission of DENV to military personnel [32]. The vector control technique of reducing contact is primarily used when in endemic areas. In line with this, Service Members should be required to sleep with a mosquito net within dengue endemic regions. Second, units should choose field positions away from standing water to avoid potential mosquito breeding centers.

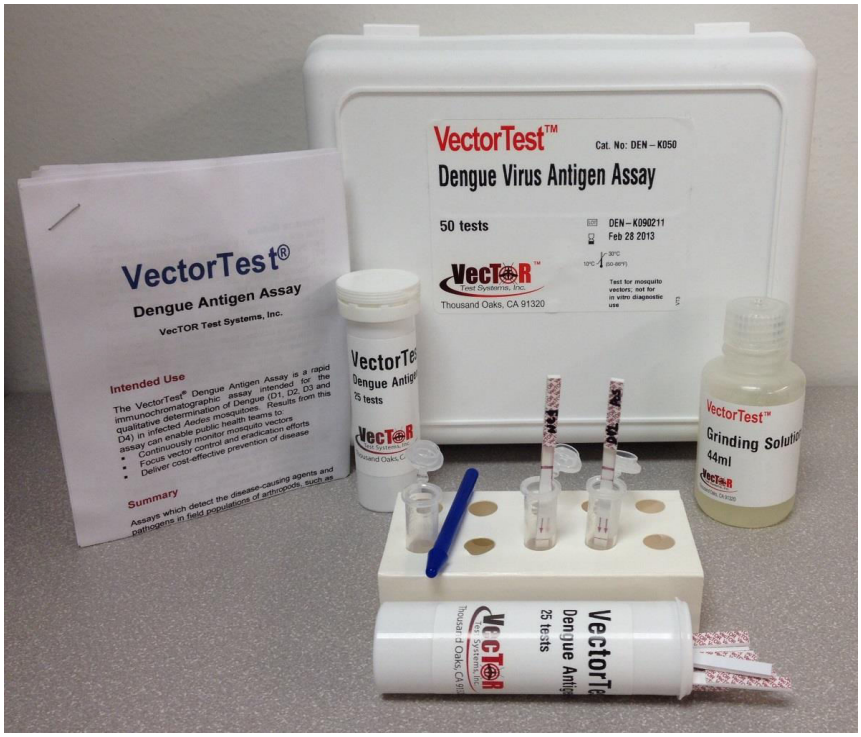

Figure 4: Arthropod Vector – Rapid Detection Devices (AV-RDDs), better known as "dipstick" tests, come in kits that contain sample holding tubes, grinding tools and the buffer solutions necessary to run the test. The AV-RDD targets arthropod-borne diseases, a primary readiness threat to warfighters. Photo taken by LTC Vanessa R. Melanson and published in Reference 34.

These two techniques used in combination with chemical control methods such as insecticides and larvicides can prevent DENV infection. With the addition of permethrin-treated uniforms and chemical insect repellants, a DENV infection is preemptively combated to protect military personnel [25]. Along with the vector control methods of reducing contact and chemical controls, biological control methods have been focused on effectively reducing DENV transmission in areas where the risk to deployed military forces is greatest. Interventions are being developed to include reducing the mosquito population by manipulation of female mosquito behavior as well as replacing endogenous DENV infected mosquitoes with ones that are unable to transmit the virus [33]. Some courses of action to address the mosquito population include introducing male mosquitoes with Wolbachia bacteria, which renders them reproductively incompatible or genetically engineering mosquitoes that need a drug added to their larval diet to survive [33]. It is important to note that vector-borne pathogens such as DENV are not uniformly or randomly distributed throughout the environment and are very focal [32]. The distribution of vector-borne pathogens such as dengue in a given environment reflects many factors to include the mosquito's ability to effectively transmit DENV as well as the presence of appropriate environmental conditions. However, it must be understood that the presence of a potential vector such as the *Aedes* mosquito alone is not always indicative of risk of disease; therefore, surveillance is key to monitoring the disease threat to military personnel. A wide variety of methods are currently used for vector surveillance. The different methods vary greatly in the amount of training required, logistical support, sample throughput, and ability to be conducted in an operational setting. The selection of the method whether baseline, operational, or specific surveys depends on the specific objective of the surveillance [32]. One method in use, aligning with specific surveys and done for approximately the last 5 years for dengue surveillance, is mosquito vector testing through Vector Pathogen Detection (VPD). VPD provides military medical professionals with the necessary information to

implement very specific disease and vector control measures [32,34]. VPD can minimize dengue disease transmission by sampling mosquitoes before human transmission occurs, thereby decreasing the health impacts on military operations. Military VPD is done in different ways, depending on the battlefield location. To detect potential dengue infected mosquitoes in a specific area of operation, two different types of biochemical assays can be performed. One type is that of an immunological or "dip-stick" assay, which is based on antigen-antibody interactions. The assay specific for DENV detection in mosquitoes is known as the Dengue Virus Arthropod Vector Rapid Detection Device (AV-RDD). It is relatively easy to perform and to interpret because it relies on visual assessment for determination of positive or negative results (Figure 4) [32-35]. This assay can be done with the use of minimal equipment. The second type of assay for VPD is PCR based. DENV specific PCR assays have been developed by the services for application on the JBAIDS, and conversely, with JBAIDS DENV assay available for use on mosquitoes [36,37].

Prophylactics

The current standard of treatment for DENV infection is supportive care. The infected individual should get plenty of rest, drink fluids, and take pain relievers such as acetaminophen. Even when this treatment regiment is followed, it takes approximately a week to recover if the dengue fever does not progress into DHF or DSS. Thus, alternate supportive care treatments as well as vaccines and drugs are in development and testing to address treating DENV infection symptoms.

Alternative supportive care: Two recent studies have examined natural ways to treat DENV infection. A Malaysian study conducted with 306 patients noted that 85.3% used complementary alternative medicines [38]. The top alternative treatments were all various diet changes including isotonic drinks, crab soup, papaya leaf extract, coconut juice, and watermelon [38]. Since this study aimed to understand the types, reasons, and prevalence of alternative treatments for DF, a recommendation of which treatment(s) works best was not proposed. However, another Malaysian study tested the effects of the papaya leaf extract [39]. During the study, the intervention group of 111 patients who received the papaya extract in the form of juice had a significantly higher mean platelet count at both 40 and 48 hours after treatment than the control group of 117 patients. Ultimately, this study found that the arachidonate 12-lipoxygenase and Platelet Activating Factor Receptor genes were expressed by the patients who had been taking the juice for three days indicating that the *Carica papaya* juice did help raise the platelet counts [39]. Both of these studies suggest that including papaya into a supportive care regiment for DENV infected patients will help them recover faster.

Vaccine development: Vaccine development is crucial to prevent the contraction of DENV from mosquitoes and to protect the US Service Members abroad. Developing a vaccine to prevent DENV infection is challenging because of the many serotypes. Vaccines, therefore, must be able to induce serotype-specific responses for each of the four. Consequently, to be truly protected against DENV infection, an individual either needs to receive a vaccine adequate to address all serotypes or multiple vaccines each aimed at a specific serotype. A vaccine that only addresses one of the serotypes can have disastrous effects as the disease could increase the severity of disease via ADE if infected by a different serotype [40]. Thus, several vaccines that have been developed, while others are in beginning research phases, all aiming to address the complex nature of DENV infection. To create a successful DENV vaccine, researchers have tried to use vaccines previously developed to viruses similar to it. For example, the chimeric yellow fever dengue tetravalent vaccine was one of the first vaccines developed, which is also called Dengvaxia [41]. This live attenuated vaccine was shown to elicit neutralizing antibodies after three doses, yet these antibodies had low effectiveness against DENV-1 and 2 [42].

Other potential live attenuated chimeric vaccines created to address DENV are the TV003/TV005 vaccine and the Tetravalent Dengue Vaccine (TDV) [41]. The TV003 vaccine developed by the National Institute of Allergy and Infectious Diseases and the National Institute of Health was promising at first, but it too had a weak seroconversion against DENV-2 [41]. Perhaps the most promising vaccine is the TDV vaccine. Phase 2 of the clinical trials of the TDV vaccine showed inducement of antibodies against all four serotypes and is currently in Phase 3 where it will enroll 20,000 children to determine the efficacy of the vaccine [41]. While showing

potential to induce immune protection against many of the DENV serotypes, attenuated tetravalent vaccines have difficulty protecting against all of them. This may be due to the unbalanced replication of vaccine viral strains when using an attenuated virus [40]. Another possibility for developing an efficient DENV vaccine is to use the envelope protein in a protein-subunit vaccine [40]. Using the envelope protein as the viral antigen paired with polylactic-co-glycolic acid nanoparticles has been shown to induce enhanced immune responses in both monovalent and tetravalent forms. Also, the technique of nanoparticle attachments has helped create balanced serotype specific immune antibody responses to each DENV serotype [40]. This nanoparticle vaccine platform can be a promising alternative in discovering a vaccine for not only DENV but also West Nile, yellow fever, and Zika viruses [40].

Drug development: Currently, there is no available drug to stop the pathogenicity of DENV. However, a few possible drug compounds have been tested in clinical trials. Examples of these compounds include chloroquine, balapiravir, and celgosivir [42]. None of these drugs successfully reduced or prevented symptoms of spread of the virus [42]. Corticosteroids and lovastatin are two additional drugs that were used at one time to treat dengue fever. Both were tested in clinical trials, and while corticosteroids showed no efficacy against DENV infection, lovastatin did inhibit replication in vitro [42]. Like the development of the DENV vaccine, more treatment is needed to evaluate the true efficacy of these potential drugs.

Future drug development: Because there is no vaccine or drug currently used to prevent or treat DENV infection, research is still fervently ongoing in these areas. With respect to future drug development, research into specific biochemical inhibitors should ensue. A recent study examined the interaction between DENV and its host, showing DENV-2 entry into antibody absent myeloid cells was inhibited by early treatment of the cells with ammonium chloride, chlorpromazine and dynasore, but not affected by methyl-B-cyclodextrin [3]. This result indicated that DENV-2 utilizes a low pH-dependent, clathrin- and dynamin-mediated endocytic infectious pathway for direct entry into human myeloid cells [3]. Furthermore, with antibody-mediated entry of DENV, the experimental conditions for ADE of infection were established with the formation of immune complexes, and showed the internalization of these complexes into myeloid cells was also dependent on pH and dynamin; however, the requirement of clathrin-mediated endocytic route depended on the receptors involved in the complex uptake [3]. Therefore, DENV entry into myeloid cells in the absence or presence of antibody can be blocked by diverse biochemical inhibitors affecting the cellular factors involved in endocytosis [3]. For this reason, specific biochemical inhibitors may be the key to creating a drug to block endocytosis of the virus.

CONCLUSION

It is estimated that more than 3 billion people living in more than 120 countries are at risk of DENV infection and approximately 390 million DENV infections occur annually [9]. Dengue is highly prevalent in tropical countries due to climate, population growth, unplanned rapid urbanization and increased travel and trade [43]. As such, prevention of DENV infection and other vector-borne diseases should be a health priority for US Military Commanders deployed to INDOPACOM and SOUTHCOM. All subordinate leaders should also understand the risk to their personnel's health and ensure that the appropriate vector control and surveillance measures are enacted to best achieve mission accomplishment especially since, there are no currently approved dengue diagnostics available far-forward on the battlefield.

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REFERENCES

1. Hesse EM, Martinez LJ, Jarman RG, Lyons GA, Eckels HK, et al. (2017). Dengue Virus Exposures Among Deployed U.S. Military Personnel. The American Journal of Tropical Medicine and Hygiene. 96: 1222-1226.
2. Frei JC, Wirchnianski AS, Govero J, Vergnolle O, Dowd AK, et al. (2018). Engineered Dengue Virus Domain III Proteins Elicit Cross-Neutralizing Antibody Responses in Mice. J Virol. 92.
3. Carro AC, Piccini LE, Damonte EB. (2018). Blockade of dengue virus entry into myeloid cells by endocytic

inhibitors in the presence or absence of antibodies. PLoS Negl Trop Dis. 12.

4. Questions and Answers on Dengue Vaccines. World Health Organization.
5. Zulfiqar S, Malik MF. (2017). Epidemiology, Management and Control of Dengue Virus Infections: A Review. Bio Science Research Bulletin-Biological Sciences. 33: 23-36.
6. Martina BE, Koraka P, Osterhaus AD. (2009). Dengue Virus Pathogenesis: an Integrated View. Clin Microbiol Rev. 22: 564-581.
7. Yousseu FBS, Nemg FBS, Ngouanet SA, Mekanda FMO, Demanou M. (2018). Detection and serotyping of dengue viruses in febrile patients consulting at the New-Bell District Hospital in Douala, Cameroon. PLOS ONE. 13.
8. Cucunawangsih, Lugito NPH. (2017). Trends of Dengue Disease Epidemiology. Virology (Auckl). 8.
9. Tsai JJ, Liu CK, Tsai WY, Liu TL, Tyson J, et al. (2018). Seroprevalence of dengue virus in two districts of Kaohsiung City after the largest dengue outbreak in Taiwan since World War II. PLoS Negl Trop Dis. 12.
10. Arima Y, Matsui T, Shimada T, Sunagawa T, Kinoshita H, et al. (2014). Ongoing local transmission of dengue in Japan, August to September 2014. Western Pac Surveill Response J. 5: 27-29.
11. Torres JR, Orduna T, Piña-Pozas M,Vazquez-Vega D, Sarti E. (2017). Epidemiological Characteristics of Dengue Disease in Latin America and in the Caribbean: A Systematic Review of the Literature. J Trop Med.
12. Fares RCG, Souza KPR, Anez G, Rios M. (2015). Epidemiological Scenario of Dengue in Brazil. Biomed Res Int.
13. Noyd DH, Shar TM. (2015). Recent Advances in Dengue: Relevance to Puerto Rico. P R Health Sci J. 34: 65-70.
14. Fagbami AH, Onoja AB. (2018). Dengue haemorrhagic fever: An emerging disease in Nigeria, West Africa. J Infect Public Health. 11: 757-762.
15. Onoja AB, Adeniji JA, Olaleye OD. (2016). High rate of unrecognized dengue virus infection in parts of the rainforest region of Nigeria. Acta Trop. 160: 39-43.
16. Le Gonidec E, Maquart M, Duron S, Savini H, Cazajous G, et al. (2016). Clinical Survey of Dengue Virus Circulation in the Republic of Djibouti between 2011 and 2014 Identifies Serotype 3 Epidemic and Recommends Clinical Diagnosis Guidelines for Resource Limited Settings. PLoS Negl Trop Dis. 10.
17. Gathii K, Nyataya JN, Mutai BK, Awinda G, Waitumbi JN. (2018). Complete Coding Sequences of Dengue Virus Type 2 Strains from Febrile Patients Seen in Malindi District Hospital, Kenya, during the 2017 Dengue Fever Outbreak. Genome Announc. 6.
18. Hotez PJ, Savioli L, Fenwick A. (2012). Neglected tropical diseases of the Middle East and North Africa: review of their prevalence, distribution, and opportunities for control. PLoS Negl Trop Dis. 6: e1475.
19. Humphrey JM, Al-Absi ES, Hamdan MM, Okasha SS, Al-Trmanini DM, et al. (2019). Dengue and chikungunya seroprevalence among Qatari nationals and immigrants residing in Qatar. PLoS One. 14: e0211574.
20. Eckerle I, Kapaun A, Junghanss T, Schnitzler P, Drosten C, et al. (2015). Dengue virus serotype 3 infection in traveler returning from West Africa to Germany. Emerg Infect Dis. 21: 175-177.
21. Zakham F, Al-Habal M, Taher R, Alaoui A, El Mzibri M. (2017). Viral hemorrhagic fevers in the Tihamah region of the western Arabian Peninsula. PLoSNegl Trop Dis. 11: e0005322.
22. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, et al. (2012). Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoSNegl Trop Dis. 6: e1760.
23. Caci JB, Blaylock JM, De La Barrera R, Griggs AN, Lin L, et al. (2014). Seroprevalence of Dengue Fever in US Army Special Operations Forces: Initial Results and the Way Ahead. J Spec Oper Med. 14: 111-115.
24. (2012). Joint Publication 4-02, Incorporating Change 1: Joint Health Services.
25. Pal S, Richardson JH, Murphy JR, Krairojananan P, Kongtak P et al. (2015). Detection of Dengue Virus in Mosquito Extracts and Human Clinical Samples Using a Field Expedient Molecular Platform. Mil Med. 180: 937-942.
26. (2005). Joint Tactics, Techniques, and Procedures for the Joint Biological Agent Identification and Diagnostic System (JBAIDS). JBAIDS JTTP. 31-32.

27. (2005). JBAIDS: a Step Forward for Bioweapon Detection, by Defense Industry Daily staff.
28. (2014). NGDS and JBAIDS Systems. BioFire Defense.
29. (2018). FY 2018 Annual Report. Director, Operational Test and Evaluation. 53-54.
30. Clinical Evaluation of the FilmArray® Global Fever (GF) Panel. National Institute of Health, U.S. National Library of Medicine. ClinicalTrials.gov.
31. Pal S, Jasper LE, Lawrence KL, Walter M, Gilliland T, et al. (2016). Assessing the Dengue Diagnosis Capability Gap in the Military Health System. Military Medicine. 181: 756-766.
32. (2013). Technical Guide 48 – Contingency Pest and Vector Surveillance. Silver Spring, MD: Armed Forces Pest Management Board, Office of the Under Secretary of Defense.
33. Achee NL, Gould F, Perkins TA, Reiner RC Jr, Morrison AC, et al. (2015). A Critical Assessment of Vector Control for Dengue Prevention. PLoS Negl Trop Dis. 9.
34. Melanson VR. (2014). Rapid detection devices for pathogen vectors are case study in leveraging small business innovation funding. Army AL&T Magazine. 118-123.
35. Wanja E, Parker ZF, Odusami O, Davé K, Davé S, et al. (2014). Immuno-Chromatographic Wicking Assay for the Rapid Detection of Dengue Viral Antigens in Mosquitoes (Diptera: Culicidae). Journal of Medical Entomology. 51: 220-225.
36. Melanson VR, Scheirer JL, Van de Wyngaerde MT, Bourzac K, Wu SJ, et al. (2014). Leveraging Arthropod-Borne Disease Surveillance Assays for Clinical Diagnostic Use. Mil Med. 179: 1207-1211.
37. Harrison GF, Scheirer JL, Melanson VR. (2015). Development and validation of an arthropod maceration protocol for zoonotic pathogen detection in mosquitoes and fleas. J Vector Ecol. 40: 83-89.
38. Ching S, Ramachandran V, Gew LT, Lim SMS, Sulaiman WAW, et al. (2016). Complementary alternative medicine use among patients with dengue fever in the hospital setting: a cross-sectional study in Malaysia. BMC Complement Altern Med. 16: 37.
39. Subenthiran S, Choon TC, Cheong KC, et al. (2013). Carica papaya Leaves Juice Significantly Accelerates the Rate of Increase in Platelet Count among Patients with Dengue Fever and Dengue Haemorrhagic Fever. Evid Based Complement Alternat Med. 7.
40. Metz SW, Thomas A, Brackbill A, Xianwen Y, Stone M, et al. (2018). Nanoparticle delivery of a tetravalent E protein subunit vaccine induces balanced, type-specific neutralizing antibodies to each dengue virus serotype. PLoS Negl Trop Dis. 12: e0006793.
41. Pang EL, Loh HS. (2017). Towards development of a universal dengue vaccine – How close are we? Asian Pac J Trop Med. 10: 220-228.
42. Simmons CP, McPherson K, Vinh Chau NV, Hoai Tam DT, Young P, et al. (2015). Recent advances in dengue pathogenesis and clinical management. Vaccine. 33: 7061-7068.
43. Jiménez-Silva CL, Carreño MF, Ortiz-Baez AS, Rey LA, Villabona-Arenas CJ, et al. (2018). Evolutionary history and spatio-temporal dynamics of dengue virus serotypes in an endemic region of Colombia. PLoS One. 13: e0203090.