

**THE UNITED STATES ARMY  
MEDICAL DEPARTMENT**

# JOURNAL

**THE MULTIDISCIPLINARY ASPECTS OF PUBLIC HEALTH**

*January-June 2018*

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*The Army Medical Department Journal* [ISSN 1524-0436] is published quarterly for The Surgeon General by the Borden Institute, USAMEDDC&S, DOTAA 3630 Stanley RD Attn: AMEDD JRNL, JBSA Fort Sam Houston, TX 78234-6100.

Articles published in *The Army Medical Department Journal* are listed and indexed in MEDLINE, the National Library of Medicine's premier bibliographic database of life sciences and biomedical information. As such, the *Journal's* articles are readily accessible to researchers and scholars throughout the global scientific and academic communities.

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# First Record of *Aedes (Stegomyia) malayensis* Colless (Diptera: Culicidae) in the Lao People's Democratic Republic, Based on Morphological Diagnosis and Molecular Analysis

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## ABSTRACT

This is the first confirmed record of *Aedes (Stegomyia) malayensis* Colless from the Lao People's Democratic Republic. Its larvae were collected from rock pools and rock holes along the Nam Noy River in the Nakai Nam Theun National Protected Area, Khammuane Province. Larvae were reared in the laboratory and emerged adults were identified based on morphological characters and mitochondrial DNA analysis, using data from the mitochondrial cytochrome c oxidase subunit I. Detailed photographs of the morphological diagnostic characters and information on the bionomics of *Ae. malayensis* are included.

The Nakai Nam Theun National Protected Area (NNT NPA), known as the Watershed Management and Protection Authority area bordering Vietnam, is located in Nakai District, Khammuane Province, Lao People's Democratic Republic (PDR). It is an important Southeast Asian biodiversity area, containing mammals, birds, reptiles, amphibians,<sup>1</sup> and insects, including mosquitoes. It is also the home of a number of rare or newly discovered species of animals.<sup>2-5</sup>

Rueda et al<sup>6</sup> reported a total of 101 species from Laos, including newly recorded *Aedes (Stegomyia)* species collected from Khammuane province, ie *Aedes (Stegomyia) albopictus* (Skuse) and *Ae. (Stg.) pseudoscutellaris* (Theobald), and those reported in the literature, ie, *Ae. (Stg.) aegypti* (Linnaeus), *Ae. gardnerii imitator* (Leicester), *Ae. (Stg.) seatoi* Huang, *Ae. (Stg.) annandalei* (Theobald), *Ae. (Stg.) craggi* (Barraud), *Ae. (Stg.) malikuli* Huang, *Ae. (Stg.) perplexus* (Leicester), *Ae. (Stg.) desmotes* (Giles), *Ae. (Stg.) pseudalbopictus* (Borel).<sup>7-9</sup> All voucher specimens of these newly recorded species, as reported by Rueda et al,<sup>6</sup> were deposited in the Smithsonian Institution, National Museum of Natural History, Washington, DC, and in the Entomology Laboratory, Institut Pasteur Laos. Tangena et al<sup>10</sup> added an additional 51 species to the list of Lao mosquito fauna. However, all voucher specimens from that report were, unfortunately, damaged or lost, and we were not able to conduct any further morphological examinations or DNA analysis of those specimens from this study.

The subgenus *Stegomyia* of genus *Aedes* has 128 valid species worldwide,<sup>11</sup> with 24 species found in the Greater Mekong subregion of Asia (Cambodia, China, Laos, Myanmar, Thailand, and Vietnam), including 12 species from Lao PDR. Several species of subgenus *Stegomyia* are major vectors of various organisms that cause human infectious diseases such as dengue, yellow fever, chikungunya, Zika viruses, and filariasis.<sup>12-14</sup>

*Aedes aegypti* is the primary vector of dengue throughout the tropical and subtropical regions of the world.<sup>15</sup> *Aedes albopictus* is also an important vector in dengue epidemics.<sup>16-18</sup> *Aedes malayensis* Colless is widely distributed in many parts of Asia,<sup>19</sup> particularly Cambodia, India, Malaysia, Singapore, Taiwan, Thailand, and Vietnam.<sup>11</sup> A recent study showed a high susceptibility of *Ae. malayensis* and its vectorial capacity for both dengue serotype 2 and chikungunya.<sup>20</sup> This species is recorded for the first time in the Lao PDR. In this study, we confirmed the identification of *Ae. malayensis* from Laos based on the cytochrome c oxidase subunit I (*COI*) mitochondrial gene and the morphological diagnostic characteristics of adults, and compared them with *Ae. albopictus*.

## MATERIALS AND METHODS

### Specimen Collection

Larvae of mosquitoes were collected from aquatic habitats along the Nam Noy River (17.768548°N, 105.381989°E), Lao PDR (Figure 1) in March 2017, using standard larval dippers (350 ml, 13 cm diam ( BioQuip,

**FIRST RECORD OF *Aedes (Stegomyia) malayensis* Colless (Diptera: Culicidae) in the Lao People's Democratic Republic, based on morphological diagnosis and molecular analysis**

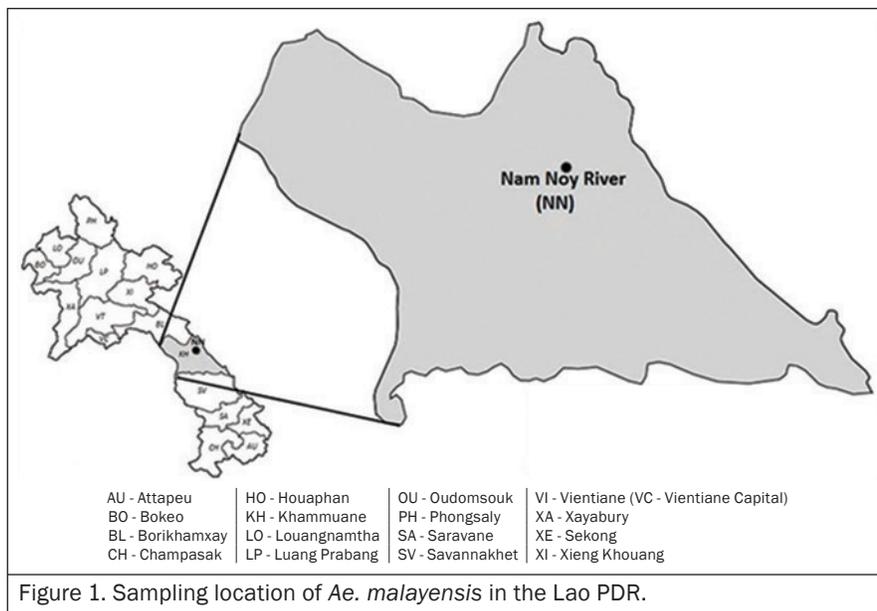


Figure 1. Sampling location of *Ae. malayensis* in the Lao PDR.

Rancho Dominguez, CA, USA). They were carefully transferred into a WhirlPak plastic bag (BioQuip) using pipettes, and transported to the laboratory of Institut Pasteur du Laos.

**Morphological Identification**

Emerged adults were pinned on paper points, each given a unique collection number, properly labeled, and identified using a stereomicroscope (Olympus SZX7, Tokyo, Japan) following the morphological keys of Rattanarithikul et al.<sup>21</sup> Voucher specimens were deposited in the Entomology Collection, Institut Pasteur du Laos, Vientiane, Lao PDR, and the National Mosquito Collections of the Smithsonian Institution, National Museum of Natural History, Washington, DC. Diagnostic characters of *Ae. malayensis* adults were photographed.

**DNA Extraction and Sequencing**

Total genomic DNA was extracted from a single whole mosquito using Macherey-Nagel NucleoSpin Tissue (GmbH & Co KG, Duren, Germany) according to manufacturer's instructions. The fragment of mitochondrial cytochrome c oxidase subunit I (mtDNA *COI*) gene was amplified using the polymerase chain reaction (PCR) Master Mix 2X (Promega Corporation, Madison, WI, USA) utilizing LCO1490 and HCO2198 primers.<sup>22</sup> The PCR protocol consisted of a one minute denaturation at 94°C and 5 cycles at 94°C for 40 seconds, 45°C for 40 seconds and 72°C for one minute, followed by 30 cycles at 94°C for 40 seconds, 49°C for 40 seconds and 72°C for one minute, and a 5-minute extension at 72°C. The PCR amplicons were electrophoresed in 1.5% TAE agarose gels stained with GelRed Nucleic Acid Gel Stain (Biotium Inc, Hayward, CA, USA), and the PCR

products were then cleaned by adding ExosapIT (USB Co, Cleveland, OH, USA). Samples were placed in the thermocycler and ran at 37°C for 30 minutes, followed by 80°C for 15 minutes.

All sequencing reactions were conducted in both directions using the original primers and the Big Dye Terminator Kit v.3.1 (PE Applied Biosystems, Warrington, UK), analyzed on an ABI Prism 3500xL - Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were edited in Sequencher v.5.4.6 (Genes Codes Co, Ann Arbor, MI, USA), and aligned using Geneious 9.1.6.<sup>23</sup> A bootstrapped<sup>24</sup> Neighbor Joining tree<sup>25</sup> was used based on

1,000 replicates. The evolutionary distances were calculated using the Kimura-2 parameters method<sup>26</sup> conducted in MEGA v.7.<sup>27</sup> All 658 base pair (bp) of the barcode fragment were included in pairwise comparisons. Two sequences of *Ae. albopictus* were used as an outgroup.

**RESULTS**

**Morphological Diagnosis**

The adult *Ae. malayensis* is very similar to *Ae. albopictus*, except for the diagnostic characters given below<sup>21</sup>:

1. A supraalar area of thorax with a patch of pale scales extended toward the scutellum in *Ae. malayensis*, and a supraalar area of thorax with spot of pale scales not extended toward the scutellum in *Ae. albopictus* (Figure 2);
2. Abdominal terga IV-VI with dorsal white bands connected to lateral pale patches in *Ae. malayensis*, and abdominal terga IV-VI with dorsal white bands separated from lateral spots in *Ae. albopictus* (Figure 3).

Furthermore, the adults of *Ae. malayensis* are very similar to adults of *Ae. alcasidi* Huang, *Ae. riversi* Bohart and Ingram, and *Ae. scutellaris* (Walker) and share the following diagnostic characters<sup>19</sup>:

1. Midfemur without median white line on anterior surface;
2. Wing with minute basal spot of white scales on costa; and
3. Hindtarsomere 5 entirely white.<sup>19</sup> Unfortunately, we did not have samples of *Ae. alcasidi*, *Ae. riversi*,

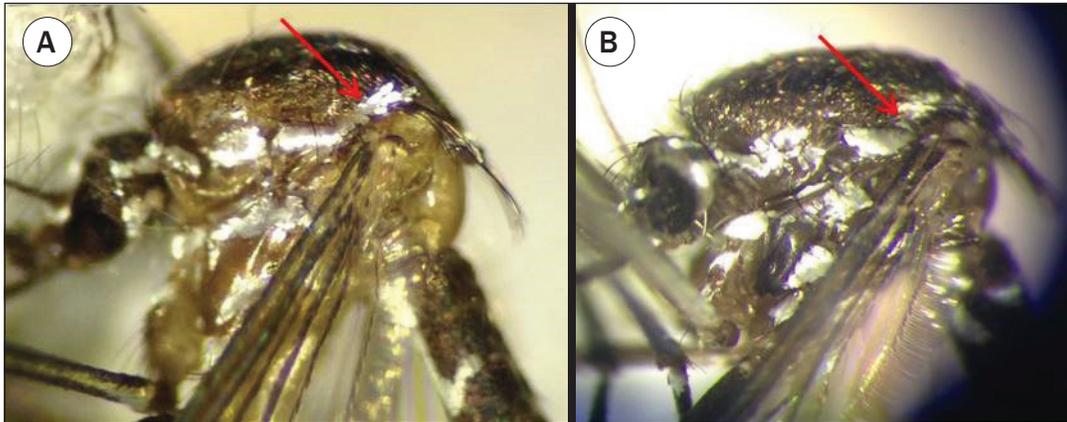


Figure 2. Morphological comparison of the thorax.  
 (A) *Ae. malayensis* - a supraalar area of thorax with a patch of pale scales extended upward toward the scutellum.  
 (B) *Ae. albopictus* - a supraalar area of thorax with spot of pale scales not extended toward the scutellum.

or *Ae. scutellaris* to compare, but we present the photographs of those diagnostic characters of *Ae. malayensis* from the Lao PDR (Figure 4).

**Bionomics**

Larvae (n=31) of *Ae. malayensis* were collected in the NNT NPA from rock holes and rock pools along the edges of the Nam Noy River, in an open area between the river and the forest. The habitats were not completely shaded, but some of them were naturally shaded by the rocks with fresh, clean, and cool water, and without vegetation (Figure 5). Larvae of *Ae. albopictus* were also collected from the same habitats in association with *Ae. malayensis*. Adults (n=17) of *Ae. malayensis* were collected using sweep nets near larval habitats. Adult females were attracted to humans and fed on their blood.

**Molecular Characterization**

The fragment of *COI* was sequenced for 4 specimens of *Ae. malayensis*, and 2 specimens of *Ae. albopictus* were used as an outgroup (GenBank numbers: MG921172-MG921178). We compared them with the DNA barcode sequences of our samples with those published previously (KY420809, KY420810, KY420811; KR349280, KR349282).<sup>20</sup> The amplicon length of the barcode sequence of *Ae. malayensis* was consistent at 658 bp (without primers). The base compositions were similar for all specimens, 14.31% G, 15.98% C, 29.22% A, and 40.49% T. The bootstrap consensus tree confirmed the differences observed in the morphology of the *Ae. malayensis* female adult compared with *Ae. albopictus* (Figure 6).

**COMMENT**

Larvae of *Ae. malayensis* have been found in tree holes in Singapore and Taiwan, and in coconut shells in Vietnam. In Thailand, they were found in rock holes, rock pools, water jars, and bamboo cups, while in Malaysia they were collected from rock pools, bamboo stumps, a coconut shell, and artificial containers.<sup>19</sup> In the Lao PDR, *Ae. malayensis* was found in rock pools (Figure 5) and rock holes together with *Ae. albopictus*. This sympatry

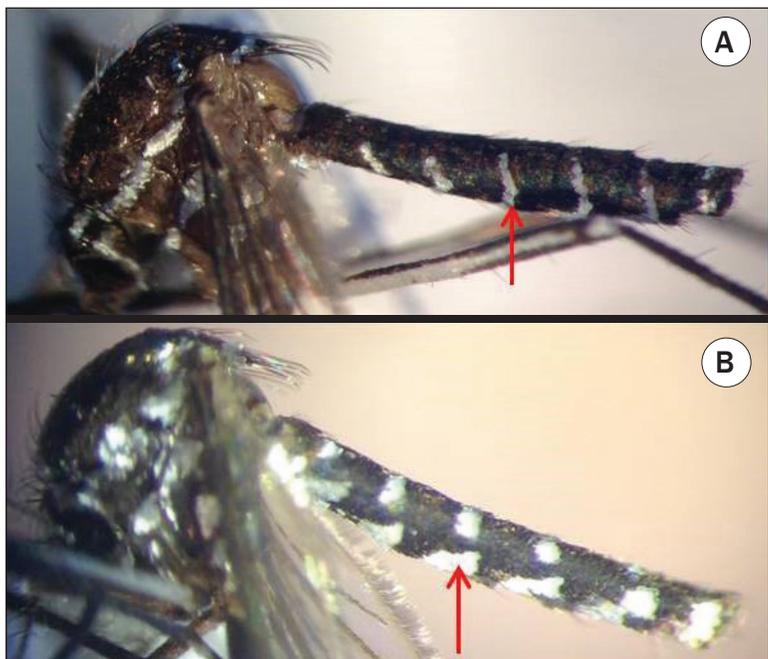


Figure 3. Morphological comparison of the abdominal terga IV-VI.  
 (A) *Ae. malayensis* - dorsal white bands connected to lateral pale patches.  
 (B) *Ae. albopictus* - dorsal white bands separated from lateral spots.

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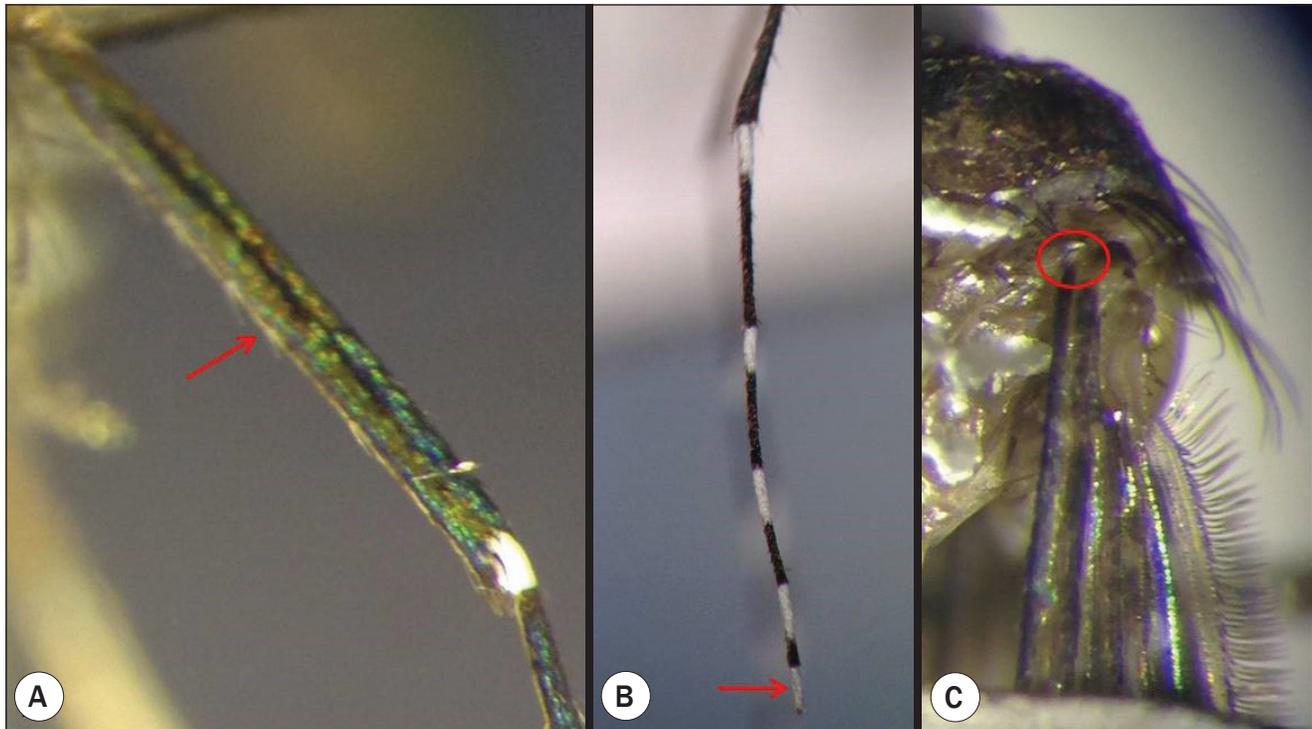


Figure 4. Morphological characters of *Ae. malayensis* that are similar to *Ae. alcasidi*, *Ae. riversi*, and *Ae. scutellaris*:

(A) Midfemur without median white line on anterior surface.

(B) Hindtarsomere 5 entirely white.

(C) Wing with minute basal spot of white scales on costa.

was also observed in Singapore, Malaysia, Thailand, and Taiwan.<sup>19,28</sup> The larval habitat along the Nam Noy River is approximately 40 km from the nearest urban center of Oudomsouk (17.710971°N, 105.15086°E), and could only be accessed by a 3-hour boat trip.

Larvae of *Ae. malayensis* are morphologically similar to *Ae. albopictus* and are very difficult to separate from each other.<sup>28</sup> The body ornamentations of the adults and larvae are highly variable.<sup>28</sup> Despite the fact that *Ae. albopictus* is very similar to *Ae. malayensis*, the combination of some distinct morphological characters differentiate these 2 species<sup>21</sup> (Figures 2 and 3). Likewise, the adults of *Ae. malayensis* are very similar to adults of *Ae. alcasidi*, *Ae. riversi*, and *Ae. scutellaris*,<sup>19</sup> particularly the wings, midfemur, and hindtarsomere 5 (Figure 4). Overall, *Ae. malayensis* can be especially recognized by its abdominal ornamentation (Figure 3).

DNA barcoding of the mitochondrial *COI* has been an efficient and useful marker for mosquito identification and confirmation of new species.<sup>29-31</sup> Herein, the observed morphological differences are corroborated with the *COI* sequences. The *COI* sequences of *Ae. malayensis* from Lao PDR were clustered with the *COI* sequences of the Singapore samples, and distinguished from the

*Ae. albopictus*, thereby confirming the existence of *Ae. malayensis* in the Lao PDR (Figure 6).

Dengue, chikungunya, Zika, and yellow fever viruses are the most important pathogens associated with the species of subgenus *Stegomyia*.<sup>14,32</sup> Because of the important role of *Aedes (Stegomyia)* species in arbovirus transmission, Huang<sup>28</sup> described for the first time the larvae and pupae, and redescribed both adult sexes of *Ae. malayensis* from samples collected from the type locality in Pulau Hantu, Singapore.

Even though the medical importance of *Ae. malayensis* was not well known at the time, it was found to have strong anthropophilic behavior in Bo-Pia, Prachuap Khiri Khan, Thailand,<sup>19</sup> and India,<sup>33</sup> as well as our recent observations in Nakai, along the Nam Noy River. In addition, Rosen et al<sup>34</sup> reported that *Ae. malayensis* was susceptible to all 4 serotypes of dengue virus after oral infection, but the presence of virus in the mosquito saliva was not determined. Moreover, Mendenhall et al<sup>20</sup> compared the vector competence of *Ae. albopictus* and *Ae. malayensis* with *Ae. aegypti* from Singapore after oral infection with dengue serotype 2 and chikungunya viruses and observed the high susceptibility of *Ae. malayensis* and *Ae. albopictus* to both arboviruses.

Importantly, the saliva of infected *Ae. malayensis* contained infectious particles for both viruses. This provided the evidence that *Ae. malayensis* and *Ae. albopictus* from Singapore possess all the necessary traits to transmit these arboviruses.

*Aedes malayensis* remains an understudied species of *Stegomyia*, although it is found in Singapore, Malaysia, Thailand, Cambodia, Vietnam, Taiwan, China (Hainan),<sup>19,29,35</sup> India,<sup>33</sup> and now in the Lao PDR. Because of the wide distribution of *Ae. malayensis* in many parts of Asia,<sup>19</sup> its high vector competence,<sup>20,34</sup> and its anthropophilic behavior,<sup>19,33</sup> more studies are warranted on the significance of this *Stegomyia* species as an arbovirus vector in the Lao PDR.

ACKNOWLEDGMENTS

We are grateful to the staff of the Watershed Management and Protection Authority (WMPA) area who authorized our research in the WMPA area and provided technical support during the fieldwork; the staff of the Public Health Office, Nakai District, Khammuane Province for cooperation and field assistance; and the Institut Pasteur du Laos staff for their support throughout this study. Special thanks go to Dr Sandra Nagaki for reviewing this manuscript and for her valuable comments. We also appreciate the input of Louis Lambrechts of the Insect-Virus Interaction group of the Institut Pasteur de Paris.

This study was partially supported by the US Naval Medical Research Unit Two, work unit number D1428, in support of the Military Infectious Diseases Research Program and Institut Pasteur du Laos.

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Figure 5. Larval habitats (rock holes) of *Ae. malayensis* in the Nakai District.

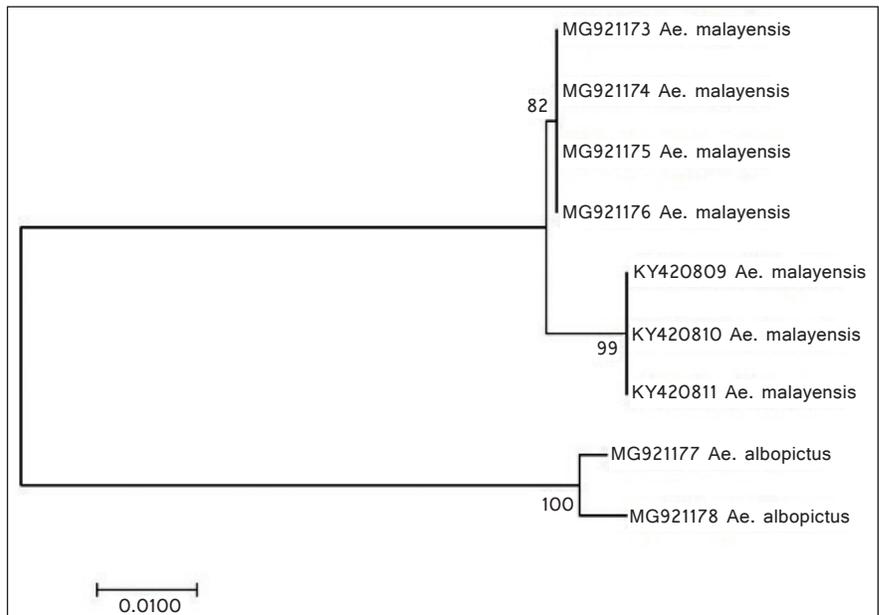


Figure 6. Bootstrapped NJ tree using *COI* sequences of *Ae. malayensis* (MG921172-MG921176 = Lao PDR; KY420809-KY420811 = Singapore<sup>20</sup>) based on 1,000 replicates of Tamura-Nei algorithm. Bootstrap values less than 50% are not shown. Scale bar represents sequence (%) divergence between samples. *Ae. albopictus* (MG921177-MG921178) was used as an outgroup.

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**FIRST RECORD OF Aedes (Stegomyia) Malayensis Colless (Diptera: Culicidae) IN THE LAO PEOPLE'S DEMOCRATIC REPUBLIC, BASED ON MORPHOLOGICAL DIAGNOSIS AND MOLECULAR ANALYSIS**

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# Mosquito Surveillance Conducted by US Military Personnel in the Aftermath of the Nuclear Explosion at Nagasaki, Japan, 1945

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## ABSTRACT

Mosquito surveillance data can be used to develop bionomic profiles of vector species to inform abatement plans. Thus, surveillance was conducted in the months following Allied occupation of Japan at the conclusion of World War II. Mosquito surveillance in Nagasaki, Japan, began one month after the nuclear bomb destroyed much of the city. The resulting specimens housed within the US National mosquito collection are documented here for the first time. Specimen labels were digitized and specimens were photographed to record specimen condition as part of the process for making them readily available to researchers.

On August 9, 1945, the United States military detonated a nuclear bomb over the city of Nagasaki, Japan, marking only the second (and last) time a nuclear weapon was ever used in combat. Five days later, Japan officially surrendered to Allied forces, ending World War II (WWII). On this day, now known as V-J Day (Victory over Japan) President Harry Truman selected General Douglas A. MacArthur to supervise the Allied occupation of Japan. During this occupation in the months after the end of WWII, US military entomologists were tasked with developing and implementing a mosquito control plan for Japan.<sup>1</sup> Challenges related to mosquito control abounded in postwar Japan, many of which had been building during the years leading up to the end of the war. In 1942, there was a Dengue fever outbreak reported from Nagasaki, however, with the destruction of cities and the displacement of populations, information about Dengue fever incidence before American occupation is limited.<sup>2</sup> According to La Casse,<sup>1</sup> Japanese informants claimed that Dengue fever was completely unheard of in Japan before 1942,<sup>1</sup> but approximately 13,000 Dengue fever cases were reported in that year. It is important to note that many houses in Japan were required to keep concrete tanks holding water for fire suppression during wartime and these containers were perfect habitat for container-breeding mosquitoes such as *Aedes albopictus* Skuse.<sup>2</sup> One of the first directives by US military authorities was to fill with dirt, overturn, or destroy these containers.<sup>2</sup> Other important container-breeding sites included cemeteries, particularly for *Ae. albopictus* and *Ae. togoi* (Theobald). Although each gravesite may only have provided habitat for a few larvae, large number of these breeding sites meant large populations of mosquitoes in cities like Nagasaki.<sup>2</sup> Gray articulated

this challenge eloquently, while commenting generally on this challenge impacting people across the world: “It is a strange commentary on human intelligence that the graves of the dead are permitted to bring death, disease and discomfort to the living.”<sup>2</sup> Mosquitoes collected from “foul-water,” including liquid manure tanks, posed another significant challenge. These tanks were present in most agriculture fields and were productive breeding sites for species such as *Culex quinquefasciatus* Say, *Cx. pipiens* Linnaeus, and *Armigeres* species.<sup>2</sup> Finally, rice paddies are of particular concern for the malaria vector, *Anopheles sinensis* Wiedemann sensu lato in and around Nagasaki. Mosquitoes that breed in containers, polluted water, and rice paddies were the primary concerns for Nagasaki under Allied occupation. In a 1947 report prepared by the Commanding Officer of the US Army 207th Malaria Survey Detachment (MSD), CPT Walter La Casse, noted that there was great concern in early 1946 that an epidemic of Japanese encephalitis (JEV) was a major threat to American forces occupying mainland Japan.<sup>1</sup> Thus, a plan was developed to collect as much bionomics data as possible to help develop mosquito abatement plans. The 207th MSD stationed in Kyoto, Japan, conducted mosquito surveys from 1946-1949.<sup>3</sup> Mosquito taxonomy in Japan was not well established before WWII. Until 1945, only 12 mosquito species were described from the islands; however, by 1959, the total number of species known from Japan was 45.<sup>3</sup>

In this article, we present digitized records and data of mosquito specimens housed within the US National mosquito collection that are associated with US military surveillance in the city of Nagasaki during Allied occupation of Japan. The specimens of interest were donated

to the United States National Museum (USNM) collection by Charles A. Triplehorn in November 1973, and are part of the personal collection of Dr Donald J. Borror who is most famous for his work using bioacoustics to track bird distributions. However, in 1945, Dr Borror was a US Navy entomologist deployed to Japan.<sup>4</sup> In correspondence with the South East Asia Mosquito Project (later redesignated as the Walter Reed Biosystematics Unit (WRBU)) staff, Dr Borror mentioned documented paper records by Triplehorn containing detailed collection data. An exhaustive search of the WRBU archives did not discover these records. In 1974, there was at least one attempt to contact Dr Borror to request additional information regarding these collection data, but with no response. This article describes how specimen label data related to these collections were digitized and photographed, and all specimens cataloged and accessioned into the USNM inventory.

#### MATERIALS AND METHODS

The National mosquito collection is comprised of approximately 1.5 million specimens and is managed by the WRBU. Digitization is continuous, however, generally focused on gathering specimen data related to ongoing vector-borne disease outbreaks or taxonomic revisions. The Nagasaki specimens were due for accession but had a low priority for digitization and had remained unnoticed for decades. Upon rediscovering these specimens during a routine search for species distribution updates, their historical importance was immediately recognized and the rest of the collection was systematically inspected for other specimens collected in post-WWII Japan.

Specimen labels were digitized by entering verbatim label data into a standard USNM entomology EMu (electronic museum) data entry form. All specimens were assigned a unique USNM catalog number to be officially accessioned into the collection. Although all specimens were assigned new catalog numbers, each record retains every associated original specimen number within the individual records. Each specimen received a unique number and all associated preparations (eg, slides) were also assigned the same catalog number so that all objects associated with each specimen can be tracked using the same catalog number. The 2D matrix barcode labels with these catalog numbers were affixed to each specimen. Barcode labels for pinned specimens were added under existing labels and can be read by a barcode scanner when the specimen is inverted, while barcodes were affixed directly to slide preparations (Figure 1). Specimen labels were photographed to allow verbatim data to be independently verified and address possible errors in interpretation. Specimens were photographed using a Dino-Lite Edge handheld digital microscope (Dino-Lite

US, Torrance, CA) with extended depth of field capability for vertical imaging to document their current condition. Each specimen was examined and recorded to document its current condition, sex, and if remounting was required. Figures 1, 2, and 3 show 3 specimen records including photographs, digitized label data, and notes.

In order to map these localities, each specimen was georeferenced using the point-radius method<sup>5</sup> which investigates text descriptions of localities on specimen labels as well as any information relevant to the description of the collection site provided in original descriptions. This allows assignment of the most precise gazetteer entry as possible to serve as a centroid of a collection event.<sup>6</sup> To account for the uncertainty of the exact locality, an uncertainty measurement was assigned using the Mammal Networked Information System Georeferencing Calculator (<http://maniset.org>).<sup>5</sup> This method accounts for errors and missing information including coordinate datum, extent of the named place, and precision of the coordinates assigned. All specimen label data that could be used to characterize the collecting events were captured and retained.<sup>7</sup> This approach has been applied in the past to specimens of the USNM Psychodidae collection.<sup>8,9</sup> As details about the exact collection sites are not available, specimens within the WWII Japan collection were generally assigned a centroid for the city of Nagasaki and an uncertainty measurement encompassing the entire city.

#### RESULTS

A total of 452 specimens representing 16 unique taxa with a summary list of habitat descriptions (bionomics) reported for each mosquito species are shown in the Table. Although some specimens showed signs of damage, eg, rubbed setae, broken antennae and/or legs, wings, etc, most were in fair shape with labels that could be read clearly. Nearly all specimens in this investigation were found to have been collected in Nagasaki, Japan, in September and October, 1945. Smaller subsets of specimens from the 207th MSD were collected in Nagasaki, Kyoto, and Isahaya-shi between 1947 and 1950. Of the 16 taxa found in this collection, 8 are known to vector pathogens, including *Plasmodium* sp. (*An. sinensis*), Japanese encephalitis virus (*Ae. togoi*, *Cx. tritaeniorhynchus* Giles, *Cx. vishnui* Theobald) and Dengue fever virus (*Ae. albopictus*). In addition, the Table includes the vector status for each taxon.

#### COMMENT

When considering the time and place of these collections, it is natural to wonder whether these specimens show any signs of radiation exposure that can be measured today. However intriguing, it is not likely that these specimens

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will have any recordable radiation. One reason is that after the initial explosion over Nagasaki, only low levels of radiation were recorded from areas outside of a 1.5 km radius from ground zero.<sup>10</sup> Additionally, nuclear fallout is expected to affect invertebrates differently depending on their life history; insects that used topsoil would be more adversely affected than those, such as mosquitoes, that use water that is frequently flushed or diluted with rainfall. Fuller et al<sup>11</sup> suggested resilience of aquatic invertebrate populations to radionuclides.

Since data characterizing the risk to insects after a nuclear explosion are rare, studies examining the impact of radiation exposure due to nuclear reactor meltdowns may be more appropriate. Williams et al<sup>12</sup> investigated the effects of the Chernobyl nuclear disaster on local populations of Chironomidae species and found morphological deformities in 60% of field-collected larvae. Although a significant finding, radiation from un-contained nuclear waste was pervasive in this location and had been affecting the



Figure 1.

Specimen information

Catalog Number: USNMENT01240053  
 Scientific Name: *Aedes (Stg.) albopictus* (Skuse, 1894)  
 Sex/Lifestage: Female, Adult  
 Preparation: Pin mounted

Notes: Specimen is in good condition with diagnostic characters clearly visible. Some setae appear rubbed on scutum. Specimen has some dust/debris covering exterior. Glue affixing specimen to pin is stable.

Collection data

Locality: Nagasaki, Japan                      Collection Date: 9 October 1945  
 Collector: Borror, D.J.                          Verbatim Scientific Name: *Ae. albopictus*  
 Additional Information: #8



Figure 2.

Specimen Information

Catalog Number: USNMENT01240364                      Scientific Name: *Aedes (Stg.) albopictus* (Skuse, 1894)  
 Sex/Lifestage: Whole larva                                  Preparation: Slide mounted

Notes: Specimen is in good condition with diagnostic characters clearly visible. Mounting medium appears to be Canada Balsam and has darkened slightly.

Collection Data

Locality: Iron kettle, bombed area, Nagasaki, Japan                      Collection Date: 11 October 1945  
 Collector: Borror, D.J.    Verbatim Scientific Name: *Ae. albopictus*  
 Additional Information: #18C

ecosystem for years after the disaster. A study investigating the possible impact of the Fukushima Dai-ichi Nuclear Power Plant meltdown of 2011 on local populations of the pale grass blue butterfly (*Zizeeria maha*) found some evidence of morphological abnormalities attributed to radiation exposure.<sup>13</sup> However, this site also had persistent radiation exposure risk. Biological asymmetry, known as fluctuating asymmetry (FA) can be used as a measure of developmental stability. A study<sup>14</sup> on stag beetles (*Lucanus cervus*) in Chernobyl found that males in highly contaminated sites had significantly elevated levels of FA in secondary sexual characters compared to males from control sites. A detailed study of FA in mosquito samples from Nagasaki may or may not reveal the physical effects of radiation but data on mating success are no longer possible.

The specimens examined during this study represent a lasting and permanent record for the invaluable work done to characterize and combat the mosquito-borne disease threats during the Allied forces occupation of Japan. Military entomologists and other medical officers during that time made systematic collections to develop bionomic profiles for vector species to inform mosquito abatement plans. Although it has been nearly 75 years since these collections were made, the specimens form a lasting legacy that can be used in taxonomic revisions and to accurately predict species distributions and biology.

ACKNOWLEDGEMENTS

This study was made possible by a FY2017 grant (P0091\_17\_WR\_1.3.1.) from the Armed Forces Health Surveillance Branch and its Global Emerging Infections Surveillance Section.

This research was performed under a Memorandum of Understanding between the Walter Reed Army Institute of Research and the Smithsonian Institution's National Museum of Natural History, with institutional support provided by both organizations.

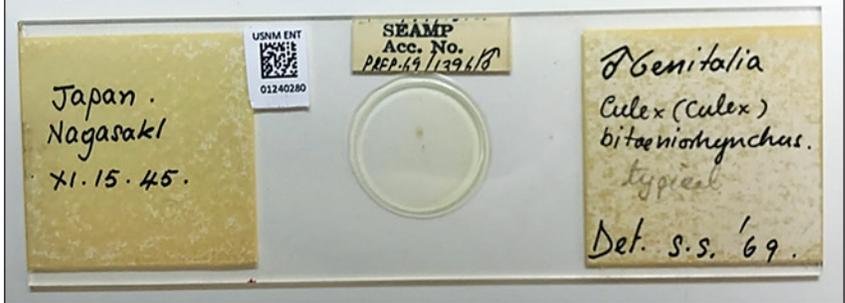


Figure 3.

Specimen information

Catalog Number: USNM01240280  
 Scientific Name: *Culex (Ocu.) bitaeniorhynchus* (Giles, 1901)  
 Sex/Lifestage: Male, Adult  
 Preparation: Pin mounted adult with slide mounted genitalia  
 Notes: Specimen is in fair condition with diagnostic characters clearly visible. Glue affixing specimen to pin is stable. Slide mounting medium appears to be Euparal and remains clear.

Collection data

Locality: Nagasaki, Japan  
 Collector: Borror, D.J.  
 Collection Date: 15 November 1945  
 Verbatim Scientific Name: *Culex (Culex) bitaeniorhynchus*  
 Identified by: S.S. 1973  
 Additional Information: SEAMP ACC No. PREP 69/1396; #1E

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A summary of the mosquito specimens considered within this study. Species names are presented along with the total number of specimens associated with each taxon, each species vector status and the collection site description used to inform species bionomics profiles.

Species Name	Specimen Count	Vector Status	Collection Site Description
<i>Aedes (Hul.) japonicus</i> (Theobald, 1901)	13	WNV	Fire tub, cemetery urn, rock hole
<i>Aedes (Stg.) albopictus</i> (Skuse, 1894)	127	DENV, CHIKV, ZIKV	Bamboo stump, bamboo vase, concrete tub, creek, pan, iron kettle bombed area, cemetery urn, wooden tub
<i>Aedes (Tan.) togoi</i> (Theobald, 1907)	149	JEV	Bamboo stump, bucket, cement tank, ditch, fire tub, rock hole, tank in bombed area, wooden barrel
<i>Anopheles (Ano.) koreicus</i> Yamada and Watanabe, 1918	15		Cement tank, seepage pool
<i>Anopheles (Ano.) sinensis</i> s.l.	31	<i>Brugia malayi</i> and secondary vector of <i>Plasmodium</i> sp.	Ditch, grassy pool, nightsoil pool, pond, rice paddy
<i>Armigeres (Arm.) subalbatus</i> (Coquillett, 1898)	22	<i>Wuchereria bancrofti</i>	Crack, nightsoil pool
<i>Culex (Cui.) pallidothorax</i> Theobald, 1905	21		Cave pool, creek in bombed area
<i>Culex (Cux.) quinquefasciatus</i> Say, 1823	6	<i>Wuchereria bancrofti</i>	Concrete tank, stone pool
<i>Culex (Cux.) tritaeniorhynchus</i> Giles, 1901	23	JEV	Cement tank, fire tub, nightsoil pool, pond, rice paddy
<i>Culex (Cux.) vagans</i> Wiedemann, 1828	7		No data
<i>Culex (Cux.) vishnui</i> Theobald, 1901	1	JEV	Artificial pond
<i>Culex (Eum.) hayashi</i> Yamada, 1917	14		Air raid shelter, cement tank, lily pond, ground pool
<i>Culex (Lop.) infantulus</i> Edwards, 1922	9		Cave pool
<i>Culex (Ocu.) bitaeniorhynchus</i> Giles, 1901	5		No data
<i>Tripteroides (Trp.) bambusa</i> (Yamada, 1917)	8		Bamboo stump
<i>Uranotaenia (Ura.) bimaculiala</i> (Leicester, 1908)	1		No data

WNV indicates West Nile virus; DENV, Dengue virus; CHIKV, chikungunya virus; ZIKV, Zika virus; JEV, Japanese encephalitis virus.

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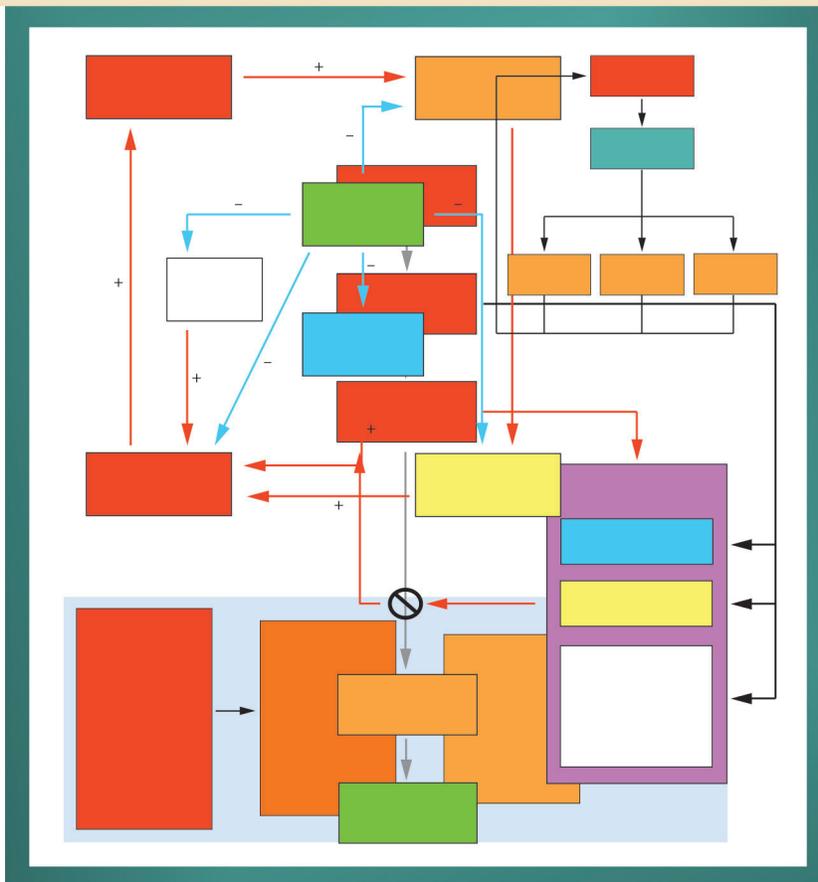
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# Georgia's Collaborative Approach to Expanding Mosquito Surveillance in Response to Zika Virus: Year Two

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## ABSTRACT

With the continued increase in international travel and immigration to Georgia, the Department of Public Health (DPH) continued its mission to prevent and respond to Zika virus (ZIKV) transmission.

**Methods:** We analyzed surveillance data from the DPH to compare the geographical distribution of counties conducting surveillance, total number, and overall percentage of mosquito species collected in 2016 and 2017. Mosquito surveillance in 2017 was mapped by county and species using ArcMap 10.2.0.

**Results:** From 2016 and 2017, mosquito surveillance increased from 60 to 159 counties (165% increase). A total of 145,346 mosquitoes were trapped and identified in 2016 compared to 152,593 in 2017 (5.43% increase). There was a difference in the type of mosquito species found by year. Some species collected in previous years were not collected in 2017, while other species found in 2017 were not previously collected during mosquito surveillance. Also, certain mosquito species were found outside of their expected geographical range.

**Conclusion:** The continued collaborative response to ZIKV by the DPH allowed a continued increase in its surveillance program. Existing and new partnerships continued to develop with military and local health departments to expand and share data. This additional surveillance data allowed DPH to make sound public health decisions regarding mosquito-borne disease risks and close gaps in data related to vector distribution.

Georgia's exposure to emerging mosquito-borne diseases is increasing due to international travel, immigration to Georgia, and out of state residents relocating to Georgia. The Department of Public Health (DPH) maintained its mission to prevent and respond to Zika virus (ZIKV) transmission and used its current funding to prepare for future public health emergencies.

The prevention or reduction of transmission of mosquito-borne diseases is completely dependent on the control of mosquito vectors and limiting person-mosquito contact. Mosquito surveillance is a key component of any local integrated vector management program. The goal of mosquito-based surveillance is to quantify human risk by determining local vector presence and abundance. Historically, the DPH was tasked with dealing with nuisance mosquito complaints with limited funding for mosquito control, not to mention mosquito surveillance. Not until the introduction of West Nile virus (WNV) in 2001 was sufficient funding provided to begin dealing with mosquito vector species.

Unfortunately, WNV funding diminished over the next several years and most mosquito control programs were

either eliminated or downsized. By 2005, only 55 counties in Georgia conducted sporadic mosquito surveillance, and by 2015, the number of counties was reduced to 13. Further, the availability of known mosquito control was limited to 6 counties. However, with the declaration by the World Health Organization in 2016 that the ZIKV threat constituted a public health emergency, funding was provided to increase mosquito surveillance and emergency mosquito control.<sup>1</sup>

## INTEGRATED MOSQUITO MANAGEMENT

Mosquito control is integral to protect the public health. In Georgia, there are  $\approx 60$  different mosquito species. Each mosquito species has a different flight range, host preference, larval habitat, and potential for carrying and transmitting infectious diseases.

Although nuisance mosquitoes can already be considered a health problem in Georgia, mosquitoes that carry infectious diseases like WNV, La Crosse encephalitis (LAC), and eastern equine encephalitis (EEE), as well as those that can transmit new and emerging viruses such as chikungunya and ZIKV are especially considered a public health concern.

One of the practices to control the mosquitoes to reduce the nuisance factor and protect public health is the use of a wide variety of control methods known as Integrated Mosquito Management (IMM). The first part of IMM is trapping and surveillance, which quantifies the numbers, species, and location of mosquitoes.

THE IMPORTANCE OF MOSQUITO SURVEILLANCE

A scientifically driven surveillance program is the backbone of every mosquito control operation. Surveillance for native and exotic mosquito species should be part of mosquito control, regardless of the immediate threat of disease outbreaks. It should be developed proactively to justify mosquito control funding requirements and risk for arboviral disease transmission.

The primary purpose of mosquito surveillance is to determine the species composition, abundance, and spatial distribution within the geographic area of interest through collection of eggs, larvae, and adult mosquitoes. Surveillance is valuable for determining changes in the geographic distribution and abundance of mosquito species, evaluating control efforts by comparing presurveillance and postsurveillance data, obtaining relative measurements of the vector populations over time, accumulating a historical database, pesticide resistance studies, and facilitating appropriate and timely decisions regarding interventions.

METHODS

Mosquito surveillance trapping data provided by the DPH and that collected in collaboration with DPH were analyzed. The geographical distribution of counties conducting surveillance, total number, and overall percentage of mosquito species collected in 2016 were compared to 2017 data. Mosquito surveillance in 2017 was mapped by county and species using ArcMap 10.2.0 (Esri, Inc, Redlands, CA). Human and livestock case data were also compared to surveillance data to detect disease patterns.

RESULTS

In 2017, mosquito surveillance was performed in all 159 Georgia counties (Figure 1). This is compared to surveillance conducted in 60 counties in 2016, and only 13 counties in 2015 (Table 1). This is the first time surveillance data was collected in every county in Georgia, and while

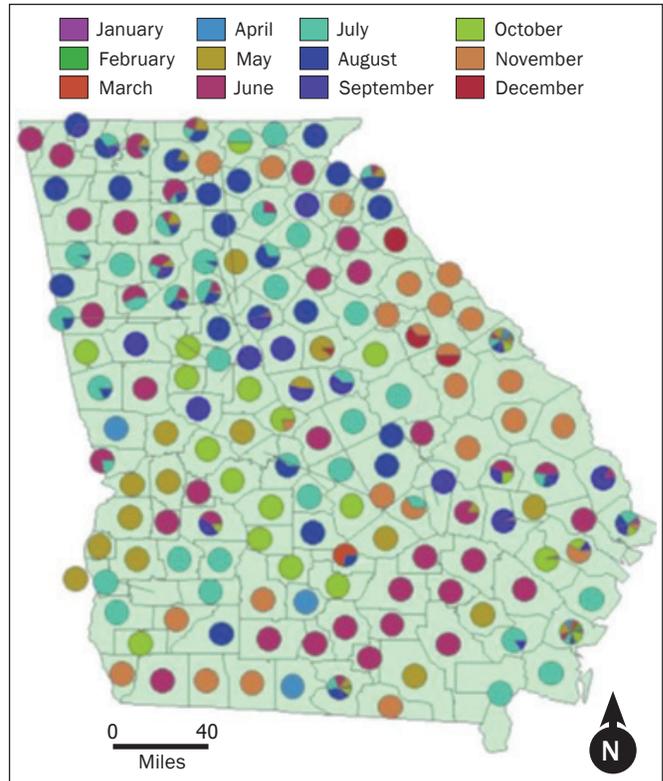


Figure 1. The month in which mosquito surveillance was performed in each county in 2017. Surveillance was performed during several months in some counties.

surveillance was limited in many counties, these data can serve as an initial baseline.

Table 1. Number and percentage of counties with mosquito surveillance performed per year, 2001-2017.

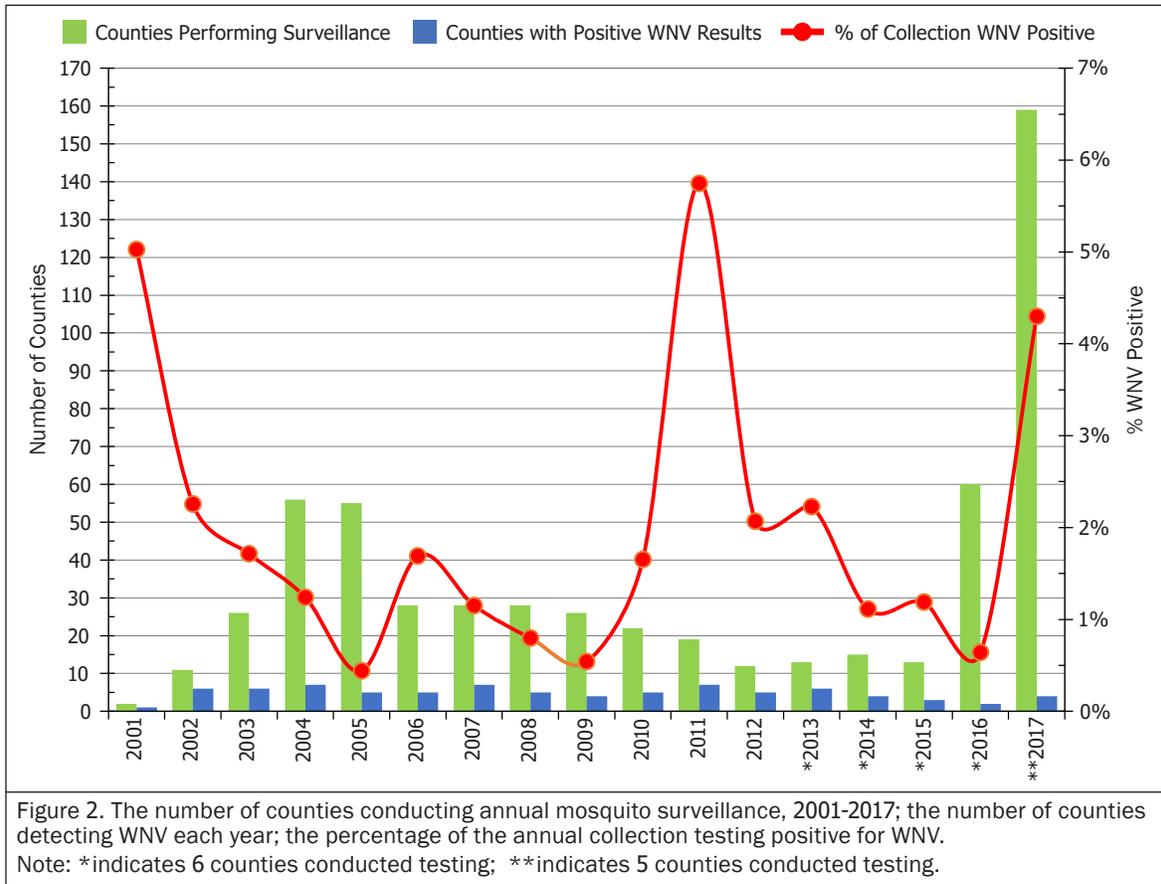
Year	No. of Counties Conducting Surveillance	Percentage of Counties (N=159)
2001	2	1.3%
2002	11	6.9%
2003	26	16.4%
2004	56	35.2%
2005	55	34.6%
2006	28	17.6%
2007	28	17.6%
2008	28	17.6%
2009	26	16.4%
2010	22	13.8%
2011	19	11.9%
2012	12	7.5%
2013	13	8.2%
2014	15	9.4%
2015	13	8.2%
2016	60	37.7%
2017	159	100.0%

The focus of mosquito surveillance has traditionally been on WNV, LAC, and EEE vectors, thus data reported to the DPH from other sources is incomplete, consisting only of those specific vectors sent for arboviral testing. Similar to previous years, including 2016, *Culex quinquefasciatus* (Say) was captured at the highest percentage of mosquitoes collected overall in 2017 (62.5%/56%) (Table 2). Trapping was prioritized in urban and suburban areas to target container-breeding *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse), and the shift in surveillance focus was demonstrated in the gradual increase in *Ae. albopictus* (3.7% to 4.0%) from 2016 to 2017 (Table 2). Although the restriction of limited surveillance was not a problem in 2017 with surveillance being performed in all counties in Georgia, *Ae. aegypti* was found only in Muscogee County,

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Table 2. Mosquito species collected per year, 2015-2017.

Species	2015 Totals from 13 Counties	Percentage of Total Collection from 13 Counties	2016 Totals from 60 Counties	Percentage of Total Collection from 60 Counties	2017 Totals from 159 Counties	Percentage of Total Collection from 159 Counties
<i>Ae. aegypti</i>	82	0.108%	26	0.018%	32	0.021%
<i>Ae. albopictus</i>	1,141	1.500%	5375	3.698%	6,175	4.047%
<i>Ae. cinereus</i>	0	0.000%	4	0.003%	143	0.094%
<i>Ae. vexans</i>	162	0.213%	6,583	4.529%	3,295	2.159%
<i>Aedes/Ochlerotatus spp.</i>	6	0.008%	120	0.083%	243	0.159%
<i>An. barberi</i>	0	0.000%	1	0.001%	0	0.000%
<i>An. crucians</i>	25	0.033%	1,879	1.293%	1,230	0.806%
<i>An. punctipennis</i>	26	0.034%	494	0.340%	1,100	0.721%
<i>An. quadrimaculatus</i>	61	0.080%	268	0.184%	74	0.048%
<i>Anopheles spp.</i>	5	0.007%	135	0.093%	214	0.140%
<i>Cq. perturbans</i>	1,265	1.663%	5,975	4.111%	1,820	1.193%
<i>Cs. inornata</i>	130	0.171%	14	0.010%	12	0.008%
<i>Cs. melanura</i>	906	1.191%	996	0.688%	2,139	1.402%
<i>Culex spp.</i>	4,996	6.569%	10,876	7.483%	9,401	6.161%
<i>Culiseta spp.</i>	0	0.000%	0	0.000%	12	0.008%
<i>Cx. coronator</i>	262	0.345%	629	0.433%	542	0.355%
<i>Cx. erraticus</i>	300	0.394%	2,425	1.676%	2,211	1.449%
<i>Cx. nigripalpus</i>	5,657	7.438%	11,101	7.638%	26,599	17.431%
<i>Cx. peccator</i>	0	0.000%	12	0.008%	0	0.000%
<i>Cx. quinquefasciatus</i>	60,423	79.450%	90,709	62.409%	85,357	55.938%
<i>Cx. restuans</i>	100	0.131%	389	0.268%	460	0.301%
<i>Cx. salinarius</i>	350	0.460%	2,962	2.038%	7,954	5.213%
<i>Cx. territans</i>	1	0.001%	34	0.023%	70	0.046%
<i>Ma. dyari</i>	0	0.000%	0	0.000%	3	0.002%
<i>Ma. titillans</i>	0	0.000%	98	0.068%	244	0.160%
<i>Oc. atlanticus</i>	1	0.001%	758	0.522%	298	0.195%
<i>Oc. canadensis</i>	0	0.000%	117	0.081%	1	0.001%
<i>Oc. fulvus pallens</i>	0	0.000%	1	0.001%	39	0.026%
<i>Oc. infirmatus</i>	2	0.003%	45	0.031%	74	0.048%
<i>Oc. japonicus</i>	8	0.011%	53	0.037%	376	0.246%
<i>Oc. mitchellae</i>	0	0.000%	9	0.006%	8	0.005%
<i>Oc. sollicitans</i>	0	0.000%	0	0.000%	30	0.020%
<i>Oc. sticticus</i>	0	0.000%	31	0.021%	36	0.024%
<i>Oc. taeniorhynchus</i>	0	0.000%	5	0.003%	488	0.320%
<i>Oc. thibaulti</i>	0	0.000%	0	0.000%	2	0.001%
<i>Oc. triseriatus</i>	25	0.033%	71	0.049%	74	0.048%
<i>Oc. trivittatus</i>	0	0.000%	7	0.005%	499	0.327%
<i>Or. signifera</i>	3	0.004%	23	0.016%	117	0.077%
<i>Ps. ciliata</i>	0	0.000%	25	0.017%	46	0.030%
<i>Ps. columbiae</i>	88	0.116%	332	0.229%	225	0.147%
<i>Ps. cyanescens</i>	2	0.003%	30	0.021%	100	0.066%
<i>Ps. discolor</i>	0	0.000%	0	0.000%	5	0.003%
<i>Ps. ferox</i>	10	0.013%	106	0.073%	326	0.214%
<i>Ps. howardii</i>	3	0.004%	34	0.023%	9	0.006%
<i>Ps. mathesoni</i>	0	0.000%	0	0.000%	9	0.006%
<i>Psorophora spp.</i>	6	0.008%	0	0.000%	9	0.006%
<i>Tx. rutilus</i>	1	0.001%	52	0.036%	21	0.014%
<i>Ur. iowii</i>	0	0.000%	13	0.009%	2	0.001%
<i>Ur. sapphirina</i>	2	0.003%	118	0.081%	43	0.028%
unknown	3	0.004%	2,411	1.659%	426	0.279%
Total	76,052		145,346		152,593	



again at low numbers (n=32) similar to 2016 (n=26) (Table 2). The statewide results suggest that *Ae. albopictus* has outcompeted this species, an occurrence reported previously in current literature.<sup>2</sup> We continue to explore why this small pocket of *Ae. aegypti* persists in Muscogee County.

The expected continuing increase of ZIKV throughout the years was actually not observed in 2017. The first travel-associated case of ZIKV was reported in Georgia in December 2015. In 2016, there were 113 travel-associated cases reported in Georgia. In 2017, there were a total of 6 travel-associated cases. To date, there have been no locally transmitted (mosquito to human) cases of Zika in Georgia. For travel-associated cases, a response protocol was developed and followed where vector surveillance coordinators (VSC) performed adult mosquito surveillance around the residence of each case, as well as applying larvicide once mosquito breeding sites were identified. Barrier spraying with adulticide was also performed if the residence was in a high risk area.

#### West Nile Virus

The WNV is a mosquito-borne disease of birds. Humans are occasionally infected with WNV through mosquito bites. Approximately, 1 in 5 people infected with WNV

develop symptoms of “West Nile fever,” often characterized by fever, headache, fatigue, and muscle pain or weakness.<sup>3</sup> Less than 1% of people infected with WNV develop neurologic disease such as meningitis, encephalitis, or flaccid paralysis.<sup>3</sup>

As shown in Figure 2, West Nile virus was first documented in Georgia in July 2001. Six human cases of WNV encephalitis were reported in Georgia that year, including one death. Cases have been reported each year since, with varying numbers of human deaths. To improve identification of Georgians infected with WNV, surveillance for WNV illness in humans was expanded for the 2003 transmission season to include all acute infections of WNV. In addition, routine screening of the national blood supply began in 2003, resulting in the identification of individuals infected with WNV prior to the development of symptoms (some WNV infections are asymptomatic). While the majority of human infections with arboviruses have resulted from bites by infected mosquitoes, other rare modes of transmission have been identified, including blood transfusion and organ transplantation. The increase in WNV among mosquitoes, humans, and birds represents the importance of mosquito surveillance to monitor disease patterns, facilitating rapid response to disease outbreaks.

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In 2017, the number of WNV cases increased significantly, making it the state's second most active year after 2012. Georgia reported 48 cases of WNV and 15 WNV presumptive viremic blood donors (PVD), with 7 deaths in 2017. The PVDs were asymptomatic at the time of blood donation, but tested positive for the presence of select arboviruses.

Forty-three (87.5%) of the 48 cases experienced WNV neurologic illness (altered mental status, paralysis, encephalitis, Guillain-Barré syndrome and/or meningitis) and 5 (10.4%) were diagnosed with WNV fever. The average age of the 48 case population was 61.4 years (range 17-87). The average age of those with WNV neurologic illness (n=5) was 64.6 years (range 26-87). Forty (83%) of the 48 cases were male. The majority of cases were reported in July, August, and September, with the peak in August.

California serogroup (CS) viruses, including California encephalitis, Keystone, La Crosse encephalitis (LAC), Jamestown Canyon, snowshoe hare, and trivittatus, are all mosquito-borne arboviral infections. In the United States, LAC is the most common of the California serogroup viruses. There were 2 cases of LAC reported in Georgia in 2017 and zero cases of LAC in 2016.

In 2017, two confirmed cases of EEE and one of PVD were reported in Georgia, compared to one case of EEE in 2016.

### Invasive Mosquito Species

One of the benefits of mosquito surveillance is determining where mosquito species are found, as well as monitoring a change in species range. This is especially important for vector species and for invasive species, which may become involved in arboviral disease cycles.

*Culex coronator* (Dyar and Knab) was first detected in Georgia in 2006. It was found initially in counties below the Fall line (Figure 3), a geological boundary dividing Georgia's Coastal Plain from its Piedmont region.<sup>4</sup> Mosquito surveillance conducted in 2017 showed that this species can now be found in most regions of Georgia (Figure 4). It is important to monitor *Cx. coronator* as it has the potential to be involved in the WNV transmission cycle.

*Ochlerotatus japonicus* (Theobald) was first detected in Georgia in 2002. Since this species lays its eggs in rock pools, it was initially found only above the Fall line (Figure 5). Mosquito surveillance conducted in 2017 showed that this species can now be found in most regions of Georgia (Figure 6). Similar to *Cx. coronator*, it

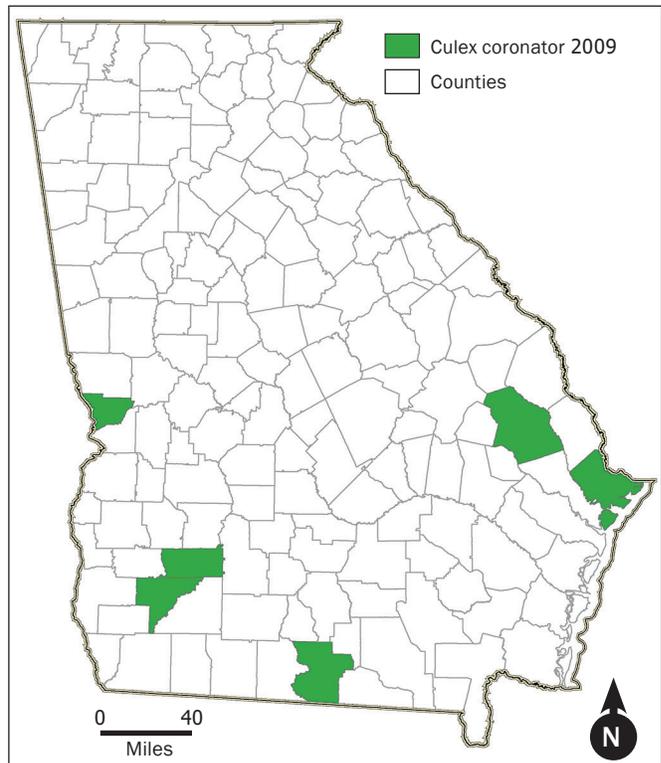


Figure 3. Counties where specimens of *Culex coronator* species were collected in 2009.

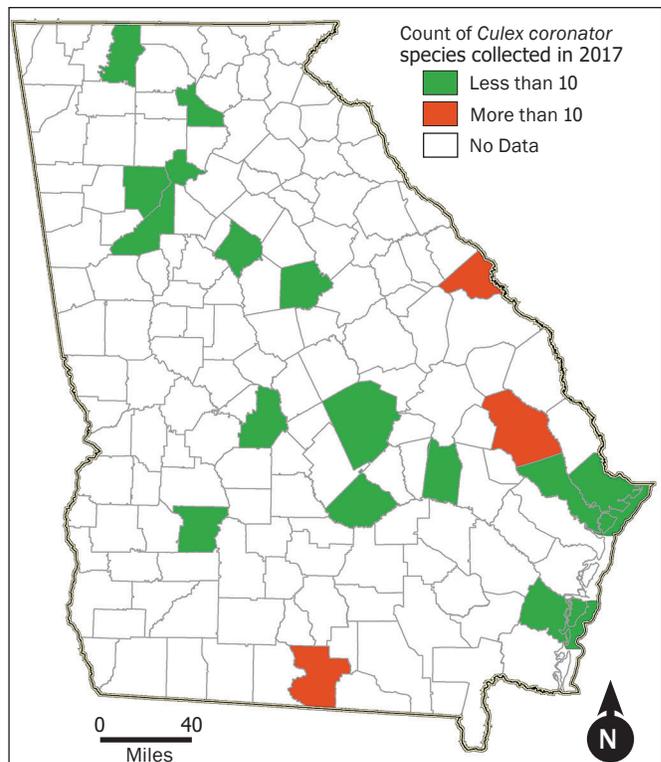


Figure 4. Counties where specimens of *Culex coronator* species were collected in 2017.

is important to monitor *Oc. japonicus* because it also has the potential to be involved in the WNV transmission cycle.

Comprehensive statewide surveillance allowed DPH to observe the geographic distribution of these important species and for the first time document their distribution to areas of the state where they had not been found before.

COMMENT

Resources and Preparedness

Prior to 1970, adequate infrastructure, funding, and public support to fight mosquito-borne diseases such as yellow fever, dengue, and malaria were generally available. However, once these diseases were eradicated in the United States, public health policy decisions greatly decreased the resources for surveillance, prevention, and control of vector-borne diseases. This was understandable because control programs had reduced the public health threat from these diseases, thus lowering the priority for allocation of resources. Those decisions, notwithstanding the technical problems of insecticide, drug resistance, and excessive emphasis on insecticide sprays to kill adult mosquitoes, contributed greatly to the resurgence of diseases such as malaria and dengue, and the introduction and rapid spread of diseases such as WNV. Decreased resources for vector-borne diseases in general resulted in the discontinuation or merger of many programs, and ultimately to the deterioration of the public health infrastructure necessary to deal with those diseases. Moreover, training programs in vector-borne diseases decreased dramatically after 1970. Thus, the DPH was faced with a critical shortage of specialists trained to respond effectively to the resurgence of vector-borne diseases, such as Zika virus.

The likely consequence to Georgia of a continued lack of good vector surveillance and control programs is the knowledge gap as to which mosquitoes and which diseases were present in specific areas of the state. The inability to provide accurate information regarding risk of disease, the detection of new arboviruses that were being introduced to Georgia, and which new diseases were competently vectored would also be of great concern. Since arboviral pathogens could not be detected early, before they infected humans, Georgia experienced cases of arboviral disease that could have been prevented, and, because some of those pathogens are singularly lethal, the state also experienced unnecessary morbidity and mortality. Georgia recognized this challenge and, with additional Zika funding from the Centers for Disease Control and Prevention (CDC), began building a team of vector surveillance and control staff that could respond to the threat of Zika virus.

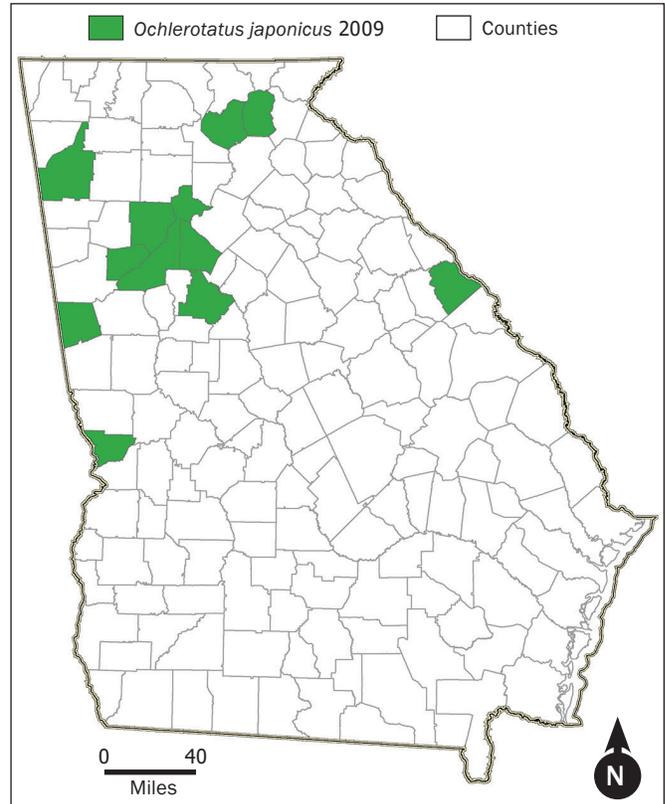


Figure 5. Counties where specimens of *Ochlerotatus japonicus* species were collected in 2009.

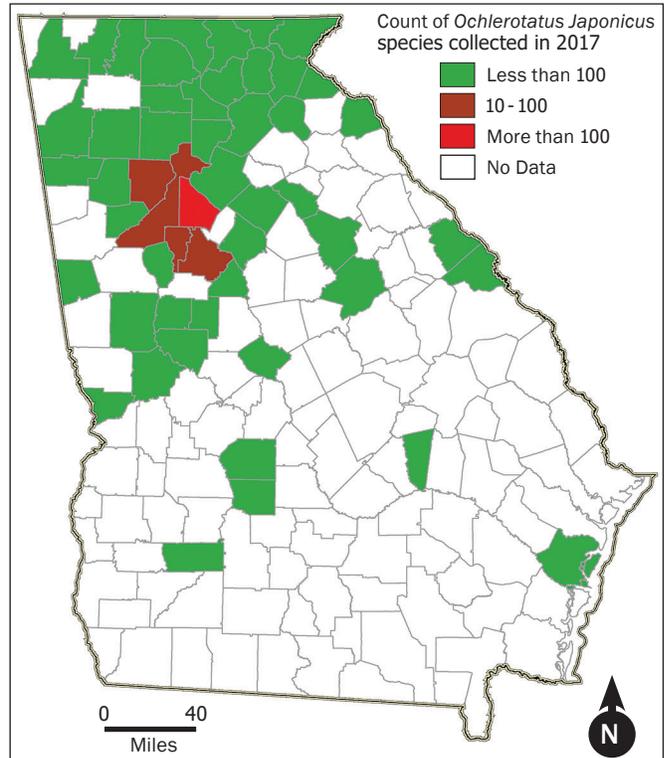


Figure 6. Counties where specimens of *Ochlerotatus japonicus* species were collected in 2017.

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### Vector Surveillance Coordinator Districts

The establishment of 4 regional VSC districts has begun to rebuild Georgia's capacity to detect and respond to both existing and newly introduced vector-borne diseases. The vector-borne disease prevention program from 2016 has been strengthened with the hiring of additional staff for 2017, providing the ability to conduct further mosquito surveillance in more counties around the state.

Nine of 18 health districts were assigned a VSC, whose responsibility is to conduct and improve mosquito surveillance for arboviral diseases such as WNV, EEE, LAC, Zika and other mosquito-borne diseases. Due to limited funding, not all health districts were assigned a VSC to assist with mosquito surveillance; these districts were assigned to the state entomologists. However, some of the districts already had mosquito surveillance programs, and some had an environmental health director or environmental health specialists (EHSs) who had an interest in conducting mosquito surveillance within their district or county, and a sound collaboration was formed.

The VSCs were placed in areas of potential higher risk and regions with little to no mosquito control in most of the included counties to provide more thorough surveillance in the designated districts. Additional and more comprehensive training was provided to the VSCs to strengthen their knowledge based on both larval and adult mosquito identification.

The DPH established a goal for the 2017 mosquito season to conduct surveillance in all 159 counties. Surveillance started in May 2017, and together with EHSs and mosquito control, surveillance was conducted in every county in Georgia, accomplishing our goal. To better prepare for the next mosquito-borne virus to emerge, mosquito surveillance equipment and training in mosquito surveillance, identification, and control was arranged for all interested people in every health district. In response to the threat of possible Zika transmission, the DPH set up 10 additional trailers (total of 11) with mosquito-borne disease threat response equipment, a moveable laboratory with both surveillance and identification equipment, and emergency control supplies and equipment.

### The Future

Any vector control should be guided by robust mosquito surveillance to evaluate the effectiveness of interventions. Surveys performed by the CDC found an increase in the number of US counties with *Ae. aegypti* and *Ae. albopictus* mosquitoes that are competent Zika-transmitting vectors.<sup>5</sup> On June 20, 2017, the CDC released a

statement on the survey findings that show an increase in the number of counties in the southern United States reporting the presence of *Ae. aegypti* and *Ae. albopictus* as compared to a previous report.<sup>5</sup> This demonstrates a continued risk of mosquito-borne diseases and the importance of statewide surveillance.

### CONCLUSION

The small increase in overall mosquito prevalence in 2017 could be due to low numbers in rural areas where breeding sites for anthropophilic mosquito species are not as prevalent. The data support the decisions made for previous mosquito surveillance programs where surveillance was only performed in high risk suburban areas when limited funding was provided.

In Georgia, statewide mosquito surveillance was conducted in all 159 counties in 2017. The goal of vector control was to suppress *Ae. aegypti* and *Ae. albopictus* mosquito populations in a coordinated and effective manner to prevent or interrupt Zika virus transmission. The CDC developed guidelines on their surveillance and control in the United States. The magnitude of activities used in a vector control response depends on the extent of mosquito-borne transmission, as measured by the number of Zika cases and their geographic and temporal distribution. The increase in Zika vector species collected in 2017 compared to previous years represents the progress and success of rebuilding the vector surveillance program in Georgia.

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# An Excel Spreadsheet Tool for Exploring the Seasonality of *Aedes* Vector Hazard for User-Specified Administrative Regions of Brazil

Desmond H. Foley, PhD  
David B. Pecor, BS

## ABSTRACT

*Aedes*-vectored viruses are a major concern for active-duty military personnel working in South and Central America at certain times of the year. Knowledge about the seasonal changes of vector activity is important as it informs time-sensitive vector control, prophylaxis, and travel decisions. To assist in-country and extralimital efforts to anticipate when vector hazards and the risks of transmission are highest, we developed an Excel spreadsheet tool that uses published monthly habitat suitability models to display various aspects of average *Aedes* seasonality for user-defined second order administrative areas of Brazil. This tool expands on those previously developed by the authors for the contiguous United States, with the aim of translating global habitat suitability models into user-friendly formats to provide actionable intelligence for areas of interest.

The risks to military personnel deployed to the US Southern Command (SOUTHCOM) countries from *Aedes* vectored viruses such as chikungunya (CHIKV), dengue (DENV), yellow fever (YFV), and Zika (ZIKV) should not be underestimated. A recent study<sup>1</sup> found active-duty travelers and those visiting friends and relatives among US Department of Defense (DoD) beneficiaries travelling to chikungunya-outbreak regions in the Americas had a composite attack rate for CHIKV, and DENV infection of 3.7%. The authors point out the risk of returning travelers with subclinical infections causing secondary transmissions in nonendemic regions (contiguous United States), and the importance of pretravel counseling to ZIKV-outbreak regions due to the shared vector, *Aedes aegypti* (L.) of CHIKV, DENV, YFV and ZIKV. Although *Ae. albopictus* (Skuse) is thought to be a competent vector of ZIKV,<sup>2</sup> *Ae. aegypti* has been implicated as the primary transmitter of the virus in human populations in the recent outbreak in the Americas.<sup>3,4</sup>

In response to the 2016 World Health Organization declaration of a public health emergency of international concern over ZIKV linked disease, the Walter Reed Biosystematic Unit produced MS Excel tools ([http://vec.tormap.si.edu/Project\\_ESWG\\_ExcelZika.htm](http://vec.tormap.si.edu/Project_ESWG_ExcelZika.htm)) that use published average yearly habitat suitability models and average monthly temperature data to predict the timing of *Aedes* vector hazards for contiguous United States (CONUS) military facilities.<sup>5</sup> Excel-based tools were constructed to assist entomologists and other health

personnel understand whether ZIKV transmission is likely to occur at a location and when they should conduct vector surveillance and control. Foley and Pecor<sup>5</sup> concentrated on DoD facilities but emphasized that the approach could be used with any habitat suitability models and for any area of interest. The ability to incorporate near real-time and forecast temperature data improved accuracy in the short term compared to the use of average monthly temperature data, which were more suited for longer term planning.

For tropical areas compared to temperate ones, temperature is less dominant among climatic drivers of intra-annual changes in mosquito suitability, which limits the applicability of the approach of Foley and Pecor<sup>5</sup> for many areas of SOUTHCOM. However, the availability of recently published global monthly habitat suitability models for *Ae. aegypti* and *Ae. albopictus*<sup>6</sup> presents an opportunity to extend to new areas our approach for providing vector hazard seasonality data.

Bugoch et al<sup>6</sup> used monthly climatic suitability models for autochthonous transmission of ZIKV around the world, conditional on the predicted occurrence of competent *Aedes* mosquito vectors. To account for seasonal variation in the geographical range of ZIKV suitability, they produced maps for each month of the year. They used mosquito species distribution models for *Ae. aegypti* and *Ae. albopictus*<sup>7</sup> (originally fitted to annual data and covariates) to make monthly predictions by use of new monthly covariates for temperature-persistence

suitability,<sup>8</sup> relative humidity, and precipitation. They refined these seasonal maps by scaling their values so that the sum of all monthly maps equaled the annual mean map of Kraemer et al.<sup>9</sup> Final global monthly vector maps show predictions of areas with high likelihood for observation or detection of mosquito populations, which were assumed to be sufficiently abundant to enable transmission of vector-borne diseases to humans.

The extended Figure 6 in the article by Faria et al<sup>10</sup> shows the 12-monthly suitability maps for ZIKV transmission of Bugoch et al<sup>6</sup> for the Americas. Faria et al<sup>10</sup> used linear regression to find that, for each Brazilian region, there was a strong association between estimated climatic suitability and weekly Zika notified cases (adjusted  $R^2 > 0.84$ ,  $P < .001$ ). They also found that, similar to previous findings from DENV outbreaks, notified ZIKV cases lag climatic suitability by about 4 to 6 weeks in all regions, except northeast Brazil, where no time lag was evident.

In this article, we demonstrate the potential utility of global monthly habitat suitability models to explore *Aedes* seasonality for administrative areas of Brazil. Building on our experience with the Excel user interface developed for CONUS military facilities, we designed a tool that shows the intensity of *Aedes* hazard at any time point throughout the year.

#### MATERIALS AND METHODS

Twelve monthly raster layers of 0.04166665 degrees resolution were obtained for *Ae. aegypti* habitat suitability.<sup>6,9,10</sup>

We used the Global Administrative Areas (GADM) v 2.8\* Administrative regions 2nd order administrative level polygons for Brazil, but some editing of the file was necessary before use. Some names were used multiple times so it was necessary to create a field in ArcMap 10.4 (Environmental Systems Research Institute, Redmond, CA) that concatenated Admin 1 (State) and 2 (District) so that each row in the feature table had a unique name combination. Of a total of 5,505 polygons, 8 were outlying islands (Espírito Santo\_Ilha Trindade, Espírito Santo\_Ilhas de Martim Vaz), and others completely overlapped the raster coverage (Bahia\_Madre de deus, Minas Gerais\_Santa Cruz de Minas, Rio Grande do Norte\_Fernando de Noronha, Rio Grande do Sul\_Capela de Santana, Santa Catarina\_Floriniapolis, São Paulo\_Suzanápolis). These were not considered further in the analysis. Santa Catarina\_Florianópolis, which is comprised of a peninsula and nearby island are present

in GADM as 2 polygons; the island (Florianópolis) and the mainland portion (Floriniapolis [note difference in spelling]).

We created a point layer from the polygon layer by using Data Management Tools>Features>Feature to Point tool and added longitude and latitude to the points by using Data Management Tools>Features>Add XY Coordinates. For the XY coordinates of centroids, the island row was selected and the mainland XY was deleted. However, for the population analysis (LandScan) data from the 2 polygons were combined. Other issues involved the need to aggregate some polygons and correct the names of several others. Extraction of all administrative area centroid raster values (ie, average values) was first obtained for polygons using the Zonal statistics as Table tool and, where needed, the Extract values to points tool. This approach was needed because smaller polygons would not produce results using the Zonal statistics as Table tool, which necessitated using the raster data associated with the points for these facilities administrative areas. We used the human population density according to LandScan 2011.<sup>†</sup> This was accomplished using the summary output in the Zonal Statistics as Table tools in ArcMap.

#### Seasonality: Ranking

Seasonality can be presented as a graph of the 12-month pattern of habitat suitability, abundance, etc. Interpretation and comparison of the pattern in one area to other areas can be subject to human error, due to variation in the y-axis, which may mask the underlying pattern. For this reason, we explored the use of rank and related metrics to define important aspects of seasonality for use in semiautomated calculations of vector hazard. For sites that exhibit a seasonal pattern in *Ae. aegypti* habitat suitability, monthly data were standardized by ranking (1 to 12) in Excel (RANK.EQ) to allow comparison within and among areas of interest, both in terms of the relative suitability and the direction (rise=1, fall=-1) of change in suitability with the next time period. Ideally, metrics automatically derived from the rank should inform the user that for the month in question, suitability scores are near the annual peak or lowest point, scores are set to increase or decrease, or scores are moving in

<sup>†</sup>LandScan 2011. People/1 sq km. This product was made using the LandScan (2011) High Resolution Global Population Data Set, the copyright to which is held by UT-Battelle, LLC, which operates the Oak Ridge National Laboratory under contract to the US Department of Energy. The US Government has certain rights in the data set. Neither UT-Battelle, LLC, nor the US Department of Energy, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of the data set. Information about LandScan is available from the Oak Ridge National Laboratory at: <http://www.ornl.gov/sci/landscan/>.

\* <http://www.gadm.org/>

## EXCEL TOOL FOR AEDES VECTOR SEASONALITY

a certain direction. Scores derived for one location can readily be contrasted with those from another.

Average habitat suitability values were calculated for 2 consecutive months to create finer temporal resolution (2 weeks) that were ranked (1 to 24). In addition to rank (1 to 24) and rise/fall (1, -1), we constructed metrics that measured accumulated rise/fall values, to show the duration of the rise and fall extending into the future (future trend or forecast) and the past (past trend or hind cast). We scored a rise as 1 and a no rise as -1, and considered that if change in rank was unidirectional, the rise/fall value would be the same as the preceding rise/fall value.

Instances where a plateau or trough was present can be disguised by the ranking process, due to the exaggeration of minor differences in values. One solution was to round up suitability values to three decimal places to tie the ranks of similar scores. Equal Rank ascending in Excel sometimes resulted in the maximum being less than 24 due to tied ranks. To address this, we calculated the maximum rank in a separate column and changed the highest rank values to 24. This had the effect of exaggerating the jump in rank to the maximum of 24 in a minority of cases, but simplified the identification of peaks and troughs. Figure 1 displays an idealized seasonal pattern of rank values with 6 important points in a typical seasonal cycle and the output for various metrics.

### Seasonality: Multivariate Analysis

To help visualize seasonality spatially, we created Interpolated surfaces (Spatial Analyst Tools>Interpolation>Kriging) for the rank of each month (12 months) using the point shapefile for each second order administrative area, and then created a mask around each point of 100 km (Analysis Tools>Proximity>Buffer), with all buffers dissolved together. Then we clipped interpolated surfaces by the mask (Spatial Analyst Tools>Extraction>Extract by Mask), and created a 3-band (red, green, blue) principle component layer summarizing the 12 interpolated surfaces to give a summary of the yearly seasonal pattern (Spatial Analyst Tools>Multivariate>Principle Components). We also developed iso clusters (Spatial Analyst Tools>Multivariate>Iso Cluster Unsupervised Classification) based on 2 and 6 classes for the 12 interpolated surfaces, then conducted zonal statistics for these iso clusters on the original 12 monthly habitat suitability models. The overlay map show the 5 regions described in Faria et al,<sup>10</sup> the outlines of the states, and the color-coded *Aedes* seasonality map for Brazil. The graphics capabilities are discussed further in the Seasonality Graphics Presentations section on page 25.

## RESULTS

An Excel tool was constructed (Figure 2) with the following components:

### User Controlled Inputs

These comprise drop-down lists and dependent drop-down lists:

- Select State
- Select District
- Select Date

### Automatically Generated Values

**Aedes hazard this date:** The more red that extends to the right, the higher the *Aedes* hazard at that location during the user-defined time period. The sum of the 12-month values equals the yearly hazard value. Bars indicate the probability of mosquito occurrence (0-1.0).

**Annual Aedes hazard this place:** The more red that extends to the right, the higher the *Aedes* hazard at that location across the whole year. Bars indicate the probability of mosquito occurrence (0-1.0).

**Rank this date (1 low, 24 high):** The rank from the lowest *Ae. aegypti* probability (=1) to the highest (=24) for each of 24 two-week periods for the particular location selected.

**Maximum rank this location:** The maximum rank over 24 two-week periods for the particular location selected. Note that maximum rank can be less than 24 due to tied scores.

**Future trend (rising 1, falling -1):** Is the *Ae. aegypti* probability going to rise (1) or fall (-1) in the next 2-week period? Stationary values do not change the prevailing score.

**Past trend (hind cast):** Ascending number of consecutive times that the probability of *Ae. aegypti* has risen (positive) or fallen (negative) as time advances, eg, "12" means that the following period will be the 12th successive time that values have increased, and -2 means that the following period will be the second successive time that values have decreased. Flat (zero trend) periods are counted as a continuation of the prevailing trend.

**Future trend (forecast):** Descending number of consecutive times that the probability of *Ae. aegypti* is predicted to rise (positive) or fall (negative) as time advances, eg, "1" means that 1 successive period of rising values is predicted to occur, -12 means that 12 successive periods of falling values are predicted to occur. Flat (zero trend)

periods are counted as a continuation of the prevailing trend.

Vector Hazard score (0-1.0):

$(\text{Rank}) / (\text{Maximum rank}) \times (\text{Annual } Aedes \text{ hazard for the district chosen})$

In theory, this scale could range from 0 to 1.0 based on the highest rank of vector distribution and activity throughout the year at the location of interest. Users should not rely solely on this metric to assess hazard.

Area (sq km): Area of the second order administrative region.

Number of people: Estimated number of people within the second order administrative region according to Landsat.

Average density of people (sq km): Average density of the second order administrative region derived from Landsat.

Control advice: A text description of where in the seasonal cycle of *Aedes* hazard this time and place represents. Warning: text advice is based on changes in rank, which can result in unexpected results. For example, although probability may change over time in a minor way, as in the off-season, this will be translated to different ranks, which can exaggerate the perception of change and lead to spurious advice. The user is advised to always consult the accompanying graph to gauge the veracity of the automated advice. Advice for each position of the cycle can be tailored by editing contents of cells EJ17:EJ24.

Possible advisory warnings that could be triggered for the 6 time periods shown in Figure 1 include:

1. *Aedes* hazard is at the highest expected level for this location. Maximize vector control and community education.
2. *Aedes* hazard is high for this location but falling. Maintain vector control and community education.
3. *Aedes* hazard is near a low point (could be more than one) for this location. Minimize vector

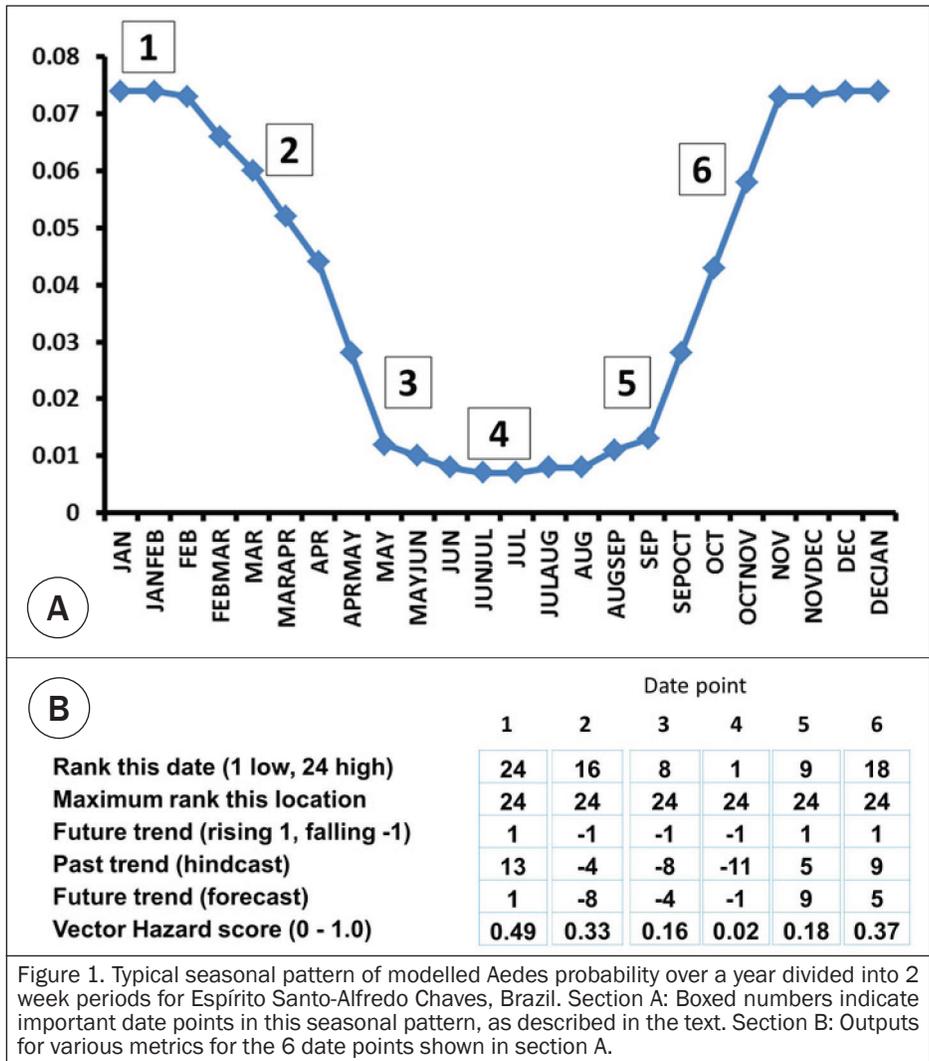


Figure 1. Typical seasonal pattern of modelled *Aedes* probability over a year divided into 2 week periods for Espírito Santo-Alfredo Chaves, Brazil. Section A: Boxed numbers indicate important date points in this seasonal pattern, as described in the text. Section B: Outputs for various metrics for the 6 date points shown in section A.

control and community education. Be aware that peak in cases can lag peak in vectors.

4. *Aedes* hazard is the lowest for this location. Prepare for scaling up vector control.
5. *Aedes* hazard is starting to rise for this location. Initiate vector control and community education.
6. *Aedes* hazard is near a high point for this location. Maximize vector control and community education.

Further refinement of these advisory warnings should include consultation with vector control and public health personnel.

#### Seasonality Graphics Presentations

In addition, Figure 2 presents a graph which displays the seasonal pattern in *Aedes* suitability (blue line) and the time period selected (red line), and a map of Brazil with

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the location of the administrative region selected. Figure 3 shows the results of clustering *Aedes* seasonality into 2 and 6 clusters and according to the first 3 principal components (PCs). With 2 iso clusters, Brazil is divided into a northern cluster and a geographically larger southern cluster. The northern cluster shows a peak in March to April and a trough in July to December, while the southern cluster peaks in December to March and exhibits a pronounced trough in May to September. With 6 iso clusters, the northeastern region shows a more complicated pattern for Brazil. Mean monthly values for each cluster are shown in Figure 3. The continuous PC map shows the most complicated pattern.

The Excel file for Brazil is freely available via the VectorMap website, <http://vectormap.si.edu/>.

### COMMENT

Knowledge of vector seasonality is powerful intelligence about when vector to human contact is expected, thereby providing clarity on the timing of vector hazard and disease transmission risk. In this article, we demonstrate the potential utility of global habitat suitability models to predict *Aedes* (*Ae. aegypti*) seasonality for administrative areas of Brazil. We created a simple Excel spreadsheet interface that calculates metrics and displays various aspects of seasonality for hazard and risk assessment for *Ae. aegypti* vectored diseases such as dengue, chikungunya, yellow fever, and Zika. We believe that this approach adds value to monthly habitat suitability models by allowing a wide variety of users to explore seasonal patterns for any area of interest. Knowledge about the seasonal changes in vector hazard

is important as it informs time-sensitive vector control, prophylaxis, and travel decisions.

Faria et al<sup>10</sup> found that, for each Brazilian region, there was a strong association between estimated climatic suitability and weekly Zika notified cases and that notified ZIKV cases lag climatic suitability by about 4-6 weeks in all regions, except northeast Brazil, where no time lag was evident. The observation that *Aedes* seasonality iso clusters (Figure 3) are most diverse in northeast Brazil may account for the absence of lag, for example, if transmission occurred in iso clusters 3, 5 or 6 that experience earlier suitability for *Aedes*, cases may appear around the time of peak suitability for the other iso cluster areas.

This tool could be extended for other areas of interest where a polygon or point feature delimiting the area is available. The authors are currently exploring extending this tool to other countries such as Colombia, Puerto Rico, and Thailand. If monthly models of habitat suitability or abundance for *Ae. aegypti* or other vector species become available, these can be used with the current Excel interface.

There are a number of limitations with our approach, a few of which are discussed herein: As the primary outputs of the zonal statistics used here were mean values, areas of interest should not be too large, or cover topologically diverse areas, as the range of values can show high diversity not adequately captured in the mean. Ideally, areas should show a clear seasonal pattern, as areas that are not suitable for the vector or have uniformly low

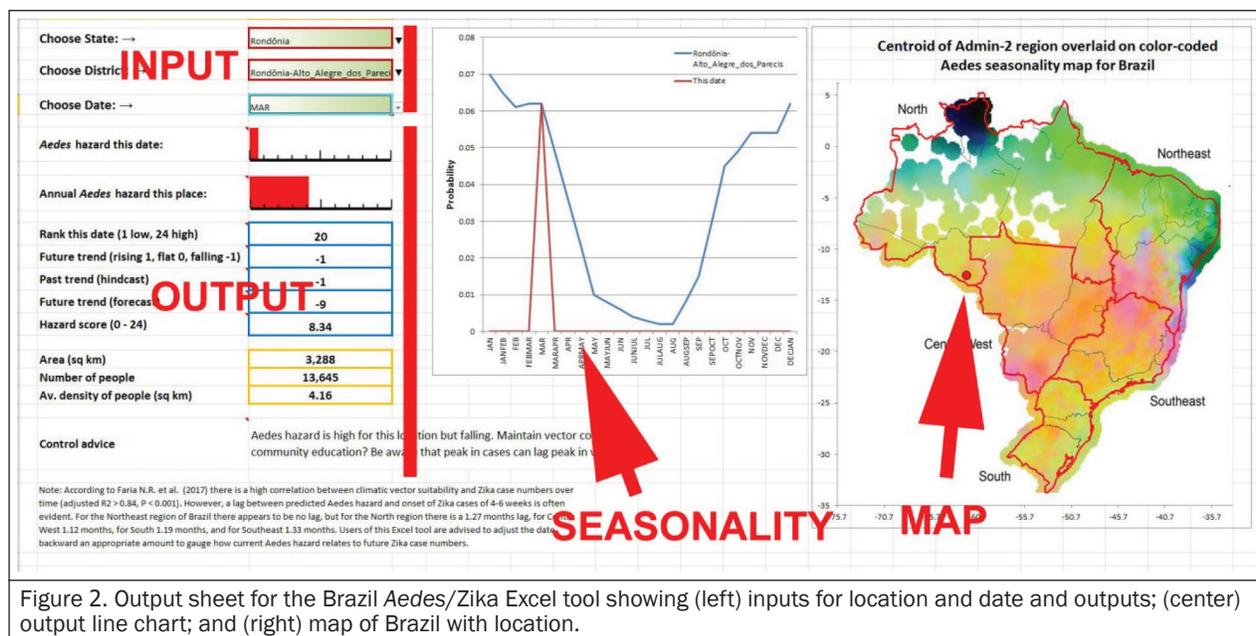


Figure 2. Output sheet for the Brazil Aedes/Zika Excel tool showing (left) inputs for location and date and outputs; (center) output line chart; and (right) map of Brazil with location.

or high suitability will give spurious results for the metrics that rely on ranking used here. The use of ranks can introduce a false sense of seasonal change and should always be considered alongside the actual values; the graph of change in habitat suitability values in the tool is provided for this purpose. Auto generated textualized suggestions regarding response to the position in the annual seasonal cycle of vector hazard is an aspect of this tool under development and the user should show caution in following these suggestions. The seasonal predictions are only as good as the model underpinning this tool; the current model may not accurately show what happens in future years. The relationship of vector habitat suitability to disease risk is not always obvious and appears to vary depending on the region. In addition, consideration of lag and congenital Zika syndrome should take into consideration the full 9 months of pregnancy. Users of this Excel tool are advised to adjust the date backward an appropriate amount to gauge how past *Aedes* hazard relates to current Zika case numbers. Finally, ZIKV can be imported and spread by nonvector transmission routes (eg, sexual transmission<sup>13</sup>), so a level of caution is recommended when trying to relate vector hazard with disease risk.

Our aim was to create an easy to use interface between monthly vector habitat suitability models and areas of interest for those lacking the time and skills to use learn and deploy GIS software. Ideally, this platform would be made available via a web-based interface. However, many users need to access this tool in austere environments where internet connectivity is unreliable or completely unavailable. The MS Excel-based platform can be downloaded before deployment and run on any laptop or desktop computer without the need for an internet connection. The Excel tool we developed is an accessible and adaptable platform for entomological decision-making that makes use of readily available data and models. As a new model becomes available, it can be easily incorporated into this tool. Validation of the output from this tool using mosquito surveillance results, and obtaining user feedback, would be useful goals for future research.

ACKNOWLEDGMENTS

We thank Dr Moritz Kraemer and collaborators for access to the models of *Aedes* suitability. We also thank MAJ Wes McCardle (Chief, Walter Reed Biosystematic Unit) for his support during the development of this tool. This study was made possible from a FY2017 grant (P0091\_17\_WR\_1.3.1.) from the Armed Forces Health Surveillance Branch and its Global Emerging Infections Surveillance Section.

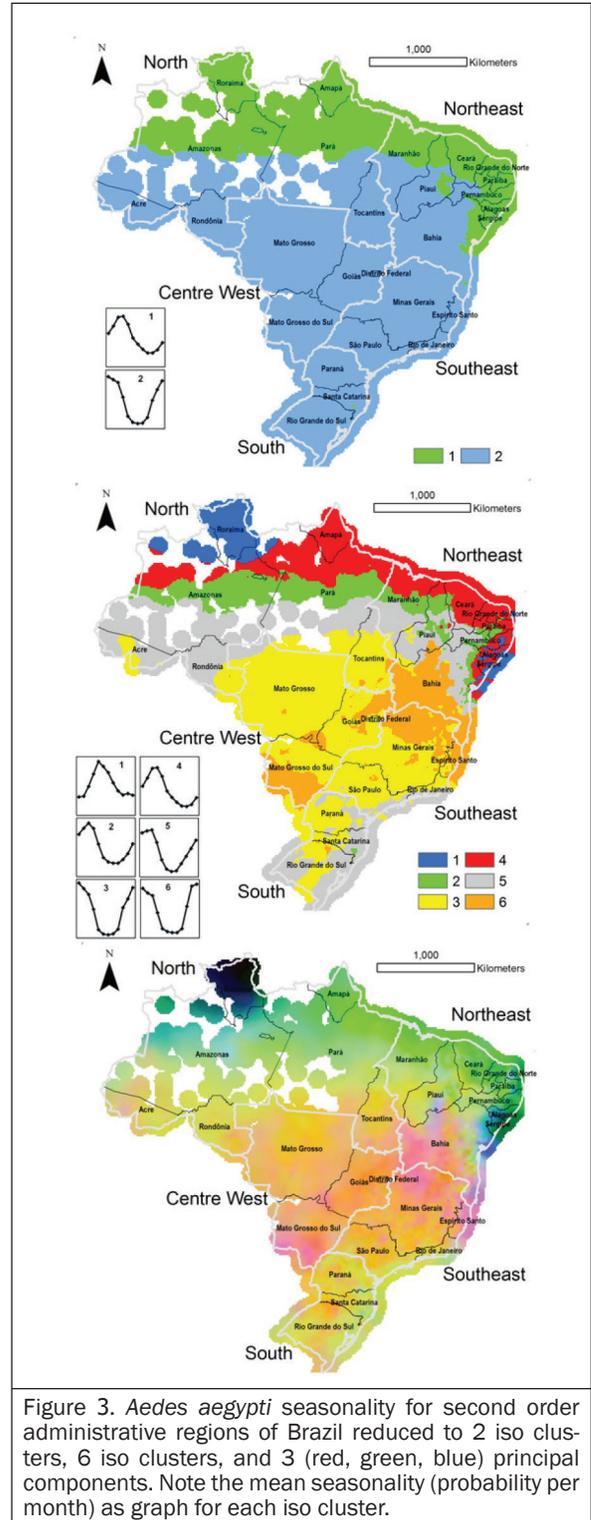


Figure 3. *Aedes aegypti* seasonality for second order administrative regions of Brazil reduced to 2 iso clusters, 6 iso clusters, and 3 (red, green, blue) principal components. Note the mean seasonality (probability per month) as graph for each iso cluster.

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*Aedes aegypti* (courtesy of the CDC)



*Aedes albopictus* (courtesy of the CDC)

# Surveillance for Scrub Typhus, Rickettsial Diseases, and Leptospirosis in US and Multinational Military Training Exercise Cobra Gold Sites in Thailand

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## ABSTRACT

We report findings of field surveillance for disease vectors and the prevalence of *Orientia tsutsugamushi*, the causative agent for scrub typhus, and other *Rickettsial* species that cause murine typhus and spotted fever group rickettsioses, in chigger mites and small rodents; and *Leptospira* in rodent kidney, urine, and environmental water samples. The study sites included various Royal Thai Army military installations and other training sites, and surrounding areas where the multinational military training exercise Cobra Gold was conducted in Thailand in 2017 and 2018. The overall prevalence of *O. tsutsugamushi* and *Rickettsia* infection in chiggers was 1.3% (20/1,594) and 7.5% (119/1,594), respectively. Serum samples of the captured rodents indicated previous exposure to *O. tsutsugamushi* infection with a seropositive rate of 12.2%. *Leptospira* species were isolated from rodent kidneys and water samples collected from catchment areas as well as tap water used for hand washing. Findings from this surveillance are important in determining the potential for scrub typhus, rickettsioses, and leptospirosis risk to military and US government personnel, as well as for informing regional and combatant commanders for prevention, correct diagnosis, prompt treatment, and timely and focused implementation of vector control and personal protective measures.

Thailand serves as a center for a multinational Asia-United States military exercise named Cobra Gold (CBG) that brings together military personnel from the United States and the Asia-Pacific region. It is the largest multinational military exercise hosted by Thailand and the United States. During this exercise, training activities include humanitarian missions such as construction of schools or multipurpose buildings for the community as well as various field-training exercises including survival in new and challenging battlefield environments. In each year since 1982, more than 13,000 troops from the United States and 35 partner countries have participated in the exercises that are held in many regions of Thailand. The training usually occurs between January and March, within the hot and dry season that occurs from October to April, which may alter the distribution of wild animals, rodents, arthropod vectors, and hence pathogen transmission.<sup>1,2</sup> The CBG training sites are usually in rural or semirural areas making them potential habitats for various vector-borne and zoonotic diseases posing risks to the training troops. To address these concerns, the US Army Medical Directorate of the Armed Forces Research Institute of Medical Sciences (USAMD-AFRIMS) in Bangkok, Thailand, initiated a vector-borne and rodent-borne disease surveillance

program in 2017 in support of CBG training exercises. The aim of this surveillance was to evaluate potential risk of vector-borne and rodent-borne disease infections and hence establish a more accurate risk assessment for these diseases. Such information is particularly useful to regional and combatant commanders for timely and focused implementation of personal protective measures and disease prevention and control programs.

Some of the major vector-borne diseases of public health importance in this region include scrub typhus, rickettsioses,<sup>3,4</sup> and leptospirosis; all associated with small mammals and their ectoparasites, especially rodents in rural and semirural settings. Most common clinical signs and symptoms of rickettsial diseases and leptospirosis are reminiscent of other infectious diseases such as malaria, typhoid fever, and dengue fever, which are characterized by high fever and headaches, making differential diagnosis from various other febrile illnesses difficult. Thus, the true burden and risk factors associated with these diseases still remain largely unknown.

Among arthropod-borne diseases, scrub typhus was a major cause of morbidity and mortality in military operations in Asia-Pacific region during World War II.<sup>5</sup> It

## SURVEILLANCE FOR SCRUB TYPHUS, RICKETTSIAL DISEASES, AND LEPTOSPIROSIS IN US AND MULTINATIONAL MILITARY TRAINING EXERCISE COBRA GOLD SITES IN THAILAND

is caused by the infection of *Orientia* bacteria which is transmitted to humans through the bite of infected trombiculid larval mites, commonly referred to as chiggers, which serve as vectors and main reservoirs of this pathogenic bacterium.<sup>1</sup> The chiggers primarily feed on rodents but occasionally on humans, presenting an opportunity to transmit the *Orientia* bacteria. Scrub typhus is predominantly endemic in the Asia-Pacific region, an area commonly referred to as the “Tsutsugamushi Triangle” that extends from northeastern Japan to Pakistan and Afghanistan in the west and northern Australia in the south, although there have been recent reports of scrub typhus outside this region. Approximately one million cases occur in the endemic areas each year and more than a billion people are at risk worldwide.<sup>6</sup> Typically, patients with scrub typhus respond well to doxycycline, ceftriaxone, and azithromycin treatment. However, evidence of potential doxycycline-refractory *Orientia tsutsugamushi* (Tamura) strain have been reported in northern Thailand,<sup>7</sup> raising concerns about efficacy of antibiotic treatment and the growing level of drug-resistant bacteria. Currently, there is no effective vaccine to prevent this disease. The current prevention method is mainly vector control and avoidance of exposure.

Murine typhus is one of the typhus group rickettsiosis. It is a flea-borne disease that is endemic in tropical and subtropical areas of the world.<sup>8</sup> The rat flea, *Xenopsylla cheopis* (Rothschild), has been considered the main vector. Murine typhus is caused by infection with *Rickettsia typhi* which is present in flea feces. The pathogens enter the body through the bite site during feeding of infected flea on host skin, or by accidentally rubbing contaminated feces into the bite site during scratching by the host. Spotted fever group rickettsiosis is a tick-borne or flea-borne infectious disease caused by groups of bacteria in the genus *Rickettsia*, such as *R. honei* (Hone), *R. felis* (Bouyeret), *R. japonica* (Uchida), and *R. helvetica* (Beati). The disease is endemic throughout the world although the prevalence varies from region to region. Information on spotted fever rickettsiosis has only rarely been documented in Thailand,<sup>9-11</sup> although many regions have suitable environment for arthropod vectors and pathogens. The actual burden of murine typhus and spotted fever rickettsiosis in Thailand is still underestimated and not well understood.

Leptospirosis is a worldwide bacterial zoonotic disease that affects humans. Pathogenic *Leptospira* species are the causative agents of leptospirosis, being spread in nature and reflecting maintenance in the kidneys of many wild and domestic reservoir hosts.<sup>12,13</sup> Its transmission occurs when animals are exposed to *Leptospira*

pathogens excreted from colonized renal tubules of infected reservoir hosts via urine into the environment.<sup>14</sup> Generally, humans are accidental hosts and acquire the disease by indirect contact with water or soil contaminated with urine of infected animals or by direct exposure to infected animal tissue or urine.<sup>13,14</sup> Infections vary in severity from mild, self-limiting febrile illnesses to more serious life-threatening conditions. Numerous cases of leptospirosis have been reported within the US military personnel in the Asia-Pacific region as recently as 2014, when more than 81 US service members became infected during an obstacle course at the Jungle Warfare Training Center, Camp Gonsalves in northern Okinawa.<sup>15</sup>

In Thailand, leptospirosis is a reportable disease to the Bureau of Epidemiology, Department of Disease Control, and Ministry of Public Health. Most infections are reported from agricultural workers, primarily rice farmers, due to their tendency to work bare-footed in the fields.<sup>16</sup> The incidence of leptospirosis is underestimated because only clinically diagnosed cases are reported and symptoms often resembles other febrile illnesses leading to misdiagnosis.<sup>13</sup> An overview of the national surveillance system for leptospirosis in Thailand indicates that there is no clear case definition for the disease, laboratory confirmation is infrequently used, and there is an inability to link clinical, epidemiologic, and laboratory data.<sup>17</sup> A lack of knowledge in naturally circulating *Leptospira* compounds the difficulty in linking human host to disease in the rodents. Understanding this relationship is critical to deciphering disease epidemiology and is also useful for risk communication.

Small rodents are widespread and are considered as important reservoir hosts for zoonotic diseases. They also play an important role in the survival and ecology of various ectoparasites including chiggers, ticks, fleas, and lice. Conducting activities in close proximity to rodents' habitats may lead to an increased risk of disease transmission. In this study, we investigated the occurrence of disease vectors and prevalence of scrub typhus, rickettsioses, and leptospirosis in small rodents, chiggers and water samples collected from the CBG exercise areas of Thailand during the 2017 and 2018 training periods.

### MATERIALS AND METHODS

#### Study Sites

The study sites included select sites where CBG training exercise took place. In 2017, surveillance was conducted in 5 different provinces including Chaibaphum, Buri Rum, Khon Kaen, Chanthaburi, and Rayong. In 2018, the selected CBG surveillance sites were in Nakhon

Ratchasima, Chachoengsao, Lopburi, Chanthaburi, and Rayong. Small rodents and ectoparasites were collected from the actual training sites and surrounding areas to cover as many habitats as possible. Also, water samples were collected from water bodies in the vicinity of the training sites as well as from taps used for cleaning and hand washing at students' dining halls and toilet facilities. Summaries of study site, survey period, number of rodents captured and rodent infestation rate per site is presented in Table 1 and Figure 1.

Collection of Small Rodents and Water Samples

All procedures involving animals were conducted in compliance with the animal use protocol (PN15-06: Field sampling of small mammal populations to support zoonotic disease surveillance and ectoparasite collection) approved by the Institutional Animal Care and Use Committee of USAMD-AFRIMS. Locally made wire-live traps measuring 14 cm x 14 cm x 28 cm were used. Rodents were baited with bananas, potatoes, palm seeds, or other types of food that are commonly found in the trapping areas or were recommended by local hunters. A total of 60 to 80 traps were set per night in each site before sunset (around 4 PM) and collected the following morning before 8 AM. Locations of the surveillance sites were recorded using handheld GPS trackers (Garmin Ltd, Olathe, Kansas).

Rodent Processing and Sample Collection

The captured rodents were euthanized with carbon dioxide. Blood samples were collected by postmortem cardiocentesis and aliquoted into serum separator tubes. To prevent potential loss of ectoparasites that might drop off the rodents due to the decrease in body temperature, the carcasses were immediately placed in zip-locked plastic bags and fumigated using ether for 5 minutes. Ectoparasites that fell off in the plastic bags were carefully collected using paint brushes. Carcasses were inspected and the fur of each rodent was thoroughly examined using fine-toothed flea comb to dislodge any ectoparasite into plastic trays. Ectoparasites were stored in collection tubes containing 70% ethanol. Clusters of chiggers on rodent ears, bodies, or genital areas were collected together with the thin layers of skin to prevent damage and preserved in 70% ethanol.

Rodents were identified to species following taxonomic key of small mammals of southeast Asia. For tissue sample collection, the surface of the rodent abdomen was cleaned with 70% ethanol before necropsy. Rodents were dissected and internal organs including lung, liver, kidney, and spleen were harvested. Serum and tissue samples were stored in dry-ice containers and transported to the USAMD-AFRIMS laboratory in Bangkok, where the samples were later stored at -80°C until further analysis.

Table 1. Diversity of small rodents captured from study sites, load of chigger infestations, seropositivity, and *Orientia* and *Rickettsia* sp. positivity rates by rodent species.

Order	Family	Species (No. rodents caught)	Seropositivity (No. seropositive rodents)			Pathogen Detection in Rodent Hosts		Chigger		
			STG	MT	SFG	STG	RT	Infestation	Total Chigger	
Rodentia	Muridae	<i>Bandicota indica</i> (57)	11.1 (5)				5.3 (3) <sup>a</sup>	64.9 (37) <sup>b</sup>	2,441 (1-328) <sup>c</sup>	
		<i>Bandicota savilei</i> (51)	8.9 (4)					76.5 (39)	3,831 (2-338)	
		<i>Berylmys berdmorei</i> (3)	4.4 (25)						100 (3)	18 (4-9)
		<i>Mus caroli</i> (4)	NA	NA	NA					
		<i>Mus cervicolor</i> (6)	NA	NA	NA				16.7 (1)	30 (30)
		<i>Niviventer fulvescens</i> (1)	NA	NA	NA				100 (1)	43 (43)
		<i>Rattus berdmorei</i> (25)	2.2 (1)							
		<i>Rattus exulans</i> (78)	15.6 (7)	25 (1)		1.3 (1)	5.1 (4)			
		<i>Rattus norvegicus</i> (4)	4.4 (25)							
		<i>Rattus tanezumi</i> (189)	53.3 (24)	75 (3)	100 (25)	1.1 (25)	1.1 (25)	65.6 (124)	12,592 (4-595)	
	Sciuridae	<i>Menetes berdmorei</i> (16)	NA	NA	NA			12.5 (25)	341 (5-236)	
Insectivora	Tupaiae	<i>Tupaia belangeri</i> (3)	NA	NA	NA					
		<i>Tupaia glis</i> (25)	NA	NA	NA					
2 Orders	3 Families	13 Species	12.2 (29)	1.3 (3)	0.8 (25)	1.2 (3)	2.3 (6)	64.1 (207)	19,296	

STG indicates scrub typhus group; MT, murine typhus; SFG, spotted fever group; RT, *Rickettsia* species.

Notes concerning numbers in parentheses:

- a. Number of rodents with active infection of the target pathogen.
- b. Number of rodent host with trombiculid chigger.
- c. Range of chiggers collected per hour.

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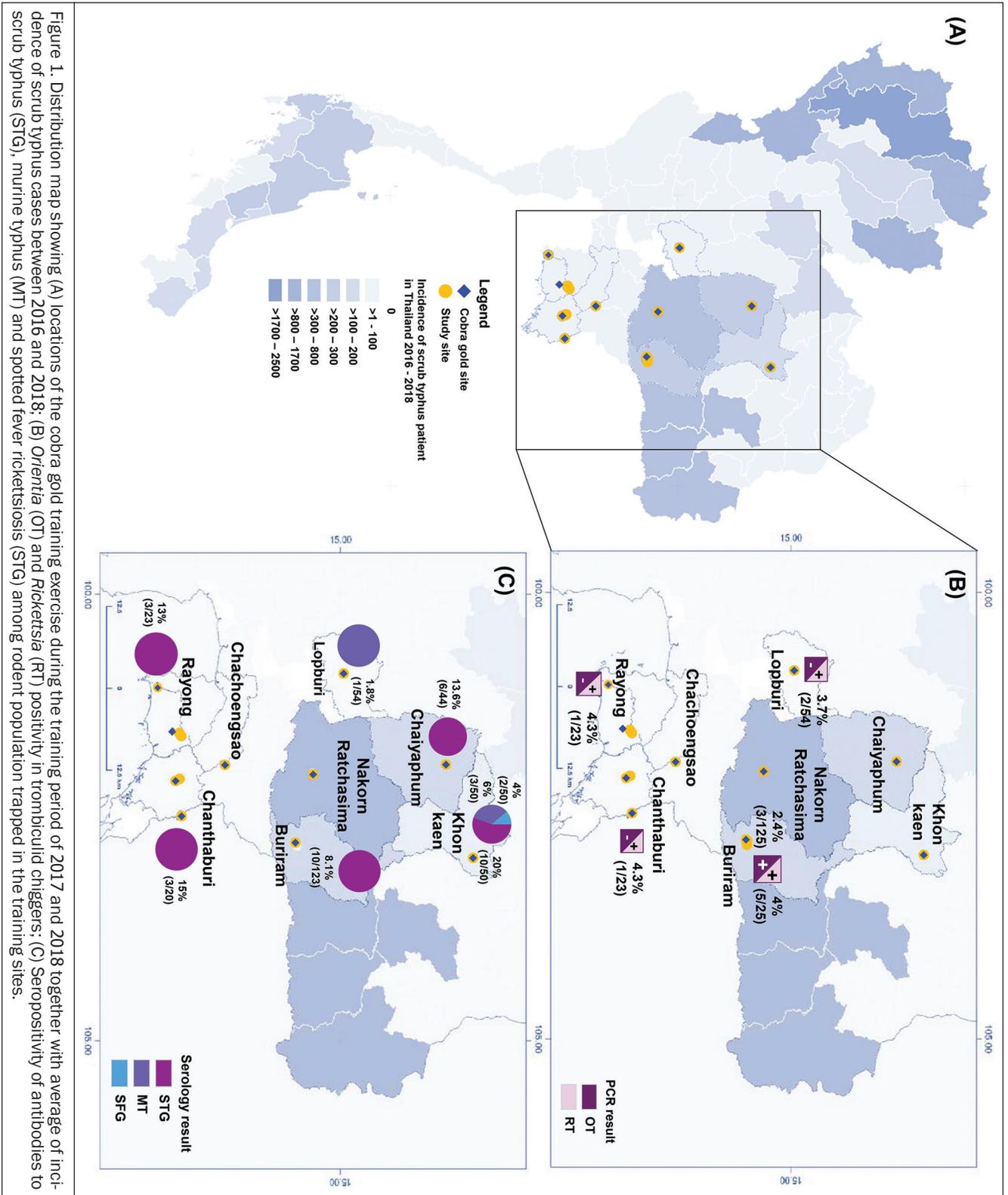


Figure 1. Distribution map showing (A) locations of the cobra gold training exercise during the training period of 2017 and 2018 together with average of incidence of scrub typhus cases between 2016 and 2018; (B) *Orientia* (OT) and *Rickettsia* (RT) positivity in trombiculid chiggers; (C) Seropositivity of antibodies to scrub typhus (STG), murine typhus (MT) and spotted fever rickettsiosis (SFG) among rodent population trapped in the training sites.

Morphological Identification of Chigger Mites

Approximately 10% of chiggers from each rodent were sampled for identification using the standard taxonomic keys previously described by Nadchatram et al<sup>18</sup> and other published taxonomic keys. Slide-mounted chiggers were examined under a dissecting microscope at 400x magnification (Carl Zeiss AG, Oberkochen, Germany) and identified to genus and species level. The remaining chiggers were transferred to 95% ethanol for better preservation of nucleic acid and further pathogen testing.

Detection of Antibodies Reaction against

*O. tsutsugamushi* and *Rickettsia* spp. in Rodents

Serum samples were tested for the presence of specific antibodies to the causative agents of scrub typhus, murine typhus, and spotted fever group rickettsioses using indirect immunofluorescent staining assays (IFA).<sup>19</sup> Types of whole cell antigens used for IFA included a mixture of *O. tsutsugamushi* genotype Karp, Kato, and Gilliam for scrub typhus group antibody assessment, *R. typhi* isolate Wilmington for murine typhus assessment, and *R. honei* isolate TT112 for spotted fever group rickettsiosis. Rodent sera were initially screened at the dilution of 1:50 (seropositivity cutoff). Any positive sample was serially diluted to a final concentration of 1:12,800 and tested for antibody titer.

*Leptospira* Cultures

Isolation of *Leptospira* was performed using 5-fluorouracil-containing Ellinghausen-McCullough-Johnson-Harris (EMJH) medium (Becton, Dickinson and Company, Franklin Lakes, New Jersey) with 5% rabbit serum (Thermo Fisher Scientific Inc, Waltham, Massachusetts). At the field site, sterile environment was maintained by the use of a Bunsen burner containing 95% alcohol. Urine specimens were collected using sterile syringes and needles and dropped in 3 mL of 0.1% semisolid EMJH medium. A section of the cortex from one kidney of each rodent was homogenized using syringe homogenization method. The homogenates were suspended in 5 mL culture EMJH media and debris tissue allowed to precipitate. After 24 hours, 500 µL of the culture was pipetted and subcultured into 3 mL

semisolid EMJH medium and stored at room temperature until transported back to USAMD-AFRIMS. For water samples, EMJH medium was inoculated with 500 µL of the collected water and the bacterial cultures were subpassaged as described earlier. The culture vials were incubated at 30°C for 16 weeks with biweekly dark field microscopic observations to verify the presence of *Leptospira*.

Pathogen Detections by PCR

Detection of *O. tsutsugamushi* and *Rickettsial* Species in Rodents and Chiggers

Nucleic acid was individually extracted from 3 or 4 types of rodent tissues including liver, lung, spleen, and/or kidneys and chigger samples using MagAttract 96 cadior pathogen kit (Qiagen Bioinformatics, Hilden, Germany) following manufacturer protocol. The presence of *O. tsutsugamushi* and *Rickettsia* DNA were evaluated using quantitative real-time PCR (qPCR) assay targeting 47-kDa high temperature transmembrane protein (*HtrA*) gene and 17-kDa genus-specific gene following previously described protocols as shown in Table 2. The qPCR analysis was performed in triplicated reactions and carried out using CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Inc, Hercules, California).

Detection of *Leptospira* DNA in Trapped Rodents and Water Samples

The kidney specimens were subjected to genomic DNA extraction using the QIAamp DNA Mini kit (Qiagen) according to the manufacturer’s instructions. Two different qPCR assays were used for *Leptospira* DNA detection. The *lipL32* qPCR was used to detect the *Leptospira* DNA in the rodent’s kidney specimens,<sup>22</sup> while the qPCR assay targeting 16s rRNA gene (*rrs*) gene was used to detect the *Leptospira* DNA in the water samples (Table 2).<sup>23</sup> For collected water evaluation, 50 mL of water was centrifuged at 3,000×g for 30 minutes to concentrate the bacterial cells. The pellet was resuspended in 140 µL sterile water, and nucleic acid was extracted from the suspension using the QIAamp Viral RNA Mini Kit (Qiagen) following the manufacturer’s protocol. Positive controls included DNA samples prepared from reference *L. interrogans* culture, *Leptospira*-infected rodent kidney

tissues, and *Leptospira* positive water samples collected during previous surveillance studies. The DNA prepared from nonpathogenic *L. biflexa* culture and from noninfected rodent’s tissue was used as negative controls.

Pathogen	Target Gene	Detection Method	Type of Samples	Reference Protocol
<i>Orientia tsutsugamushi</i>	47-kDa <i>HtrA</i>	qPCR	Rodent tissue, chigger	Jiang et al <sup>20</sup>
<i>Rickettsial</i> sp.	17-kDa	qPCR	Rodent tissue, chigger	Wright et al <sup>21</sup>
<i>Leptospira</i> spp.	<i>lipL32</i>	qPCR	Rodent tissue	McAvin et al <sup>22</sup>
	16s rRNA	qPCR	Water sample	Smythe et al <sup>23</sup>
	16s rRNA	nPCR	<i>Leptospira</i> culture medium	Boonsilp et al <sup>24</sup>
	16s rRNA	nPCR	<i>Leptospira</i> qPCR positive sample	Boonsilp et al <sup>24</sup>

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The nested single tube PCR assay targeting partial rRNA gene was performed on positive *lipL32* and *rrs* qPCR assay samples as previous described.<sup>24</sup> Amplicons were determined using 1.5% gel electrophoresis followed by staining with GelStar (Lonza Rockland Inc, Rockland, Maine). Positive PCR samples were purified by GeneJET PCR Purification Kit (Thermo Fisher). After purification, PCR products were sent to Macrogen, Inc (Seoul, South Korea) for sequencing. The DNA sequences of partial *rrs* amplicons were analyzed using Sequencher v 5.0 software (Gene Codes Corporation, Ann Arbor, Michigan) and trimmed to the 433 base pair length fragments. For molecular identification of *Leptospira* species, the DNA fragments were compared to available *Leptospira* sequences in the GenBank database using the Basic Local Alignment Search Tool (BLASTn) algorithm.

### Identification of Recovered *Leptospira* Isolates Using 16S Rrna Gene Amplification

The isolated *Leptospira* was regrown in liquid EMJH at 30°C for 7 days and total genomic DNA extracted from

the cultures using the GeneJET Genomic DNA Purification kit (Thermo Fisher) according to manufacturer's instructions. The nested single tube PCR assay and analysis were performed as described earlier.

### Data Management and Statistical Analysis

Digital map for seropositive rodents and ectoparasites infected with *Orientia* and *Rickettsia* pathogens were created using qGis v 2.18.16 (<https://qgis.org/en/site/>). The epidemiological parameters and indices were calculated according to the guidelines recommended by the World Health Organization.<sup>25,26</sup> The parameters included rodent infestation rate (number of captured rodents from number of traps), mean abundance (number of chiggers collected per number of examined rodents), chigger infestation rate (percentage of rodents infested with chiggers from the total number of captured rodents), specific chigger index (number of chiggers of specific genus recovered from specific rodent host species per number of individual hosts), and overall chigger index (total number of chiggers collected [regardless of species] per total

Table 3. Overall percentage infestations of rodents and trombiculid chiggers, and seropositivity and pathogen positivity by collection sites.

CBG	Collection Site (No. rodents caught)	Collection Periods	Rodent		Chigger			Seropositivity (No. of assayed)			Pathogen Positivity	
			Infestation Rate*	Chigger Infestation†	No. Chiggers	Chigger Index	STG	MT	SFG	STG	RT	
2017	Buri Ram (125)	2017: April, October	23.6 (125/530)	33.6 (42/125)	7,974	189.9	8.1 (10/123)			2.4 (3/125)	4 (5/125)	
	Chaiyaphum (48)	2017: January, March, July	11.4 (48/420)	75 (36/48)	1,763	49.3	13.6 (6/44)					
	Chanthaburi (23)	2017: January, September	1.3 (6/480)	21.7 (5/23)	20	4.0	15 (3/20)				4.3 (1/23)	
	Khon Kaen (62)	2017: January, March, July	13.8 (62/450)	21 (13/62)	1,132	87.1	20 (10/50)	6 (3/50)	4 (2/50)			
	Rayong (3)	January 2017	1 (2/200)									
	Total: 261				36.8 (96/261)	10,889	113.5	12.2 (29/237)	1.3 (3/237)	0.8 (2/237)	1.2 (3/261)	2.3 (6/261)
2018	Chachoeng Sao (9)	December 2017		44.4 (4/9)	111	27.8						
	Chanthaburi (51)	January 2018	22.4 (51/228)	72.5 (37/51)	3,110	84.1	25.5 (13/51)					
	Lopburi (54)	December 2017	15.4 (54/350)	77.8 (42/54)	3,804	91.9		1.8 (1/54)			3.7 (2/54)	
	Nakhon Ratchasima (18)	November 2017		44.4 (8/18)	97	12.1						
	Rayong (23)	January 2018	17.3 (23/133)	87 (20/23)	1,285	64.3	13 (3/23)				4.3 (1/23)	
	Total: 155				71.6 (111/155)	8,407	76.3	10.8 (16/148)	0.7 (1/148)			1.9 (3/155)
Grand Total: 416				49.8 (207/416)	19,296	93.2	0.72 (3/416)	2.2 (9/416)	0.72 (3/416)	0.72 (3/416)	2.2 (9/416)	

CBG indicates Cobra Gold; STG, scrub typhus group; MT, murine typhus; SFG, spotted fever group; RT, *Rickettsia* species.

Notes concerning numbers in parentheses:

\*number of captured rodents/number of traps deployed

†number of rodents infested with chiggers/total number of captured rodents

number of infested hosts). The index was multiplied by 100 to give the percentage index. Correlation analysis was performed to determine association between seropositivity, prevalence of chigger infestation on the rodent, chigger index, and qPCR positivity rate of target pathogen in chiggers.

RESULTS

Abundance of Rodents and Chigger Mites Were Found in CBG Training Exercise Sites

In total, 416 small rodents including rats, mice, and tree-shrews representing 7 genera and 13 species were collected (Table 1). A total of 261 rodents (62.7%) were collected in 2017 (CBG 2017) and 155 rodents (32.3%) trapped in 2018 (CBG 2018) (Table 3). Rodent and ectoparasite populations were surveyed for 37 trap nights at 39 sites covering most of the CBG training exercises sites and surrounding areas (Figure 1). Rodents were successfully trapped from all of the training sites with variations in infestation rates among sites. The CBG 2017 training site located at Buriram and areas adjacent to the training sites were heavily infested with rats with the overall infestation rate of 23.6%. For CBG 2018, the

rate of rodent infestation was highest in Chanthaburi (22.4%) and Rayong (17.3%). The Asian house rat, *Rattus tanezumi* (Kloss), considered the main reservoir host of many zoonotic diseases, was the most abundant rodent species trapped (45.4%, 189/416). Of the collected rodents, 207 were infested with chiggers which corresponded to 49.7% prevalence of chigger infestation. These findings suggested that all of the training sites should be considered as areas at high or moderate risk for rodent-borne and ectoparasite-borne diseases.

Diversity of Chigger Population

Chiggers were found in all study sites, except the CBG 2017 Rayong site. Seven of 13 rodent species were infested with chiggers. The main reservoir hosts of trombiculid chiggers included *Berylmys berdmorei* (Blyth), *Bandicota savilei* (Thomas), *R. tanezumi* (Temminck), and *B. indica* (Bechstein). The overall chigger index indicated that chiggers were most abundant in the CBG 2017 Buriram site (189.9), followed by CBG 2018 Lopburi site (91.9), and CBG 2017 Khon Kaen site (87.1).

A total of 19,296 chiggers belonging to 7 genera were recovered from the rodent hosts (Table 3). Out of 207 chigger infested rodents, 158 (76.3%) rodents were infested with trombiculid chiggers from a single genus, while 43 (20.8%) and 6 (2.9%) rodents harbored chiggers from 2 and 3 genera, respectively. On average, rodents hosted 1.27±0.5 genera (mean±SEM) of chiggers. The most abundant chiggers belonged to the *Walch* species (26.9%) followed by chiggers from *Ascoschoengastia* sp (33.3%), *Leptotrombidium* sp (21.7%), and *Scheogastia* sp (13.5%). Chiggers from *Helenicula* sp (1.4%), *Blankaartia* sp (1%) and *Trombiculindus* sp (0.5%) were found in only few rodents (Table 4).

Prevalence of *O. tsutsugamushi* and *Rickettsia* DNA in Trombiculid Chiggers

A total of 1,594 nucleic acid samples extracted from 3,489 chiggers were tested. The sample set consisted of 1,297 samples extracted from individual chiggers and 297 samples extracted from pools of 2,192 chiggers (5 to 10 chiggers per pool). *Orientia* positivity rate in chiggers was 1.3% (20/1,594) (Table 5). The mean of *Orientia* pathogenic bacteria load detected in individual chiggers ranged from 13.8 to 2,251.6 copies/μl. The positive chiggers were recovered from 18

Table 4. Chigger genus composition among small rodent species.

Rodent Species (No. of rodents caught)	Genus of Chigger (No. of rodents infested with the specific genus of chigger)						
	Lepto	Scheo	Ascosp	Walch	Blank	Helen	Trombi
<i>Bandicota indica</i> (57)	194 (7)	70 (1)	12 (3)	2,162 (36)	3 (1)		
<i>Bandicota savilei</i> (51)	154 (4)			3,677 (39)			
<i>Berylmys berdmorei</i> (3)				18 (3)			
<i>Menetes berdmorei</i> (16)	336 (1)	5 (1)					
<i>Mus cervicolor</i> (6)				30 (1)			
<i>Niviventer fulvescens</i> (1)	43 (1)						
<i>Rattus tanezumi</i> (189)	1,006 (32)	4,706 (26)	5,164 (66)	1,614 (41)	2 (1)	98 (3)	2(1)
Total: 323	1,733 (45)	4,781 (28)	5,176 (69)	7,501 (120)	5 (25)	98 (3)	2(1)

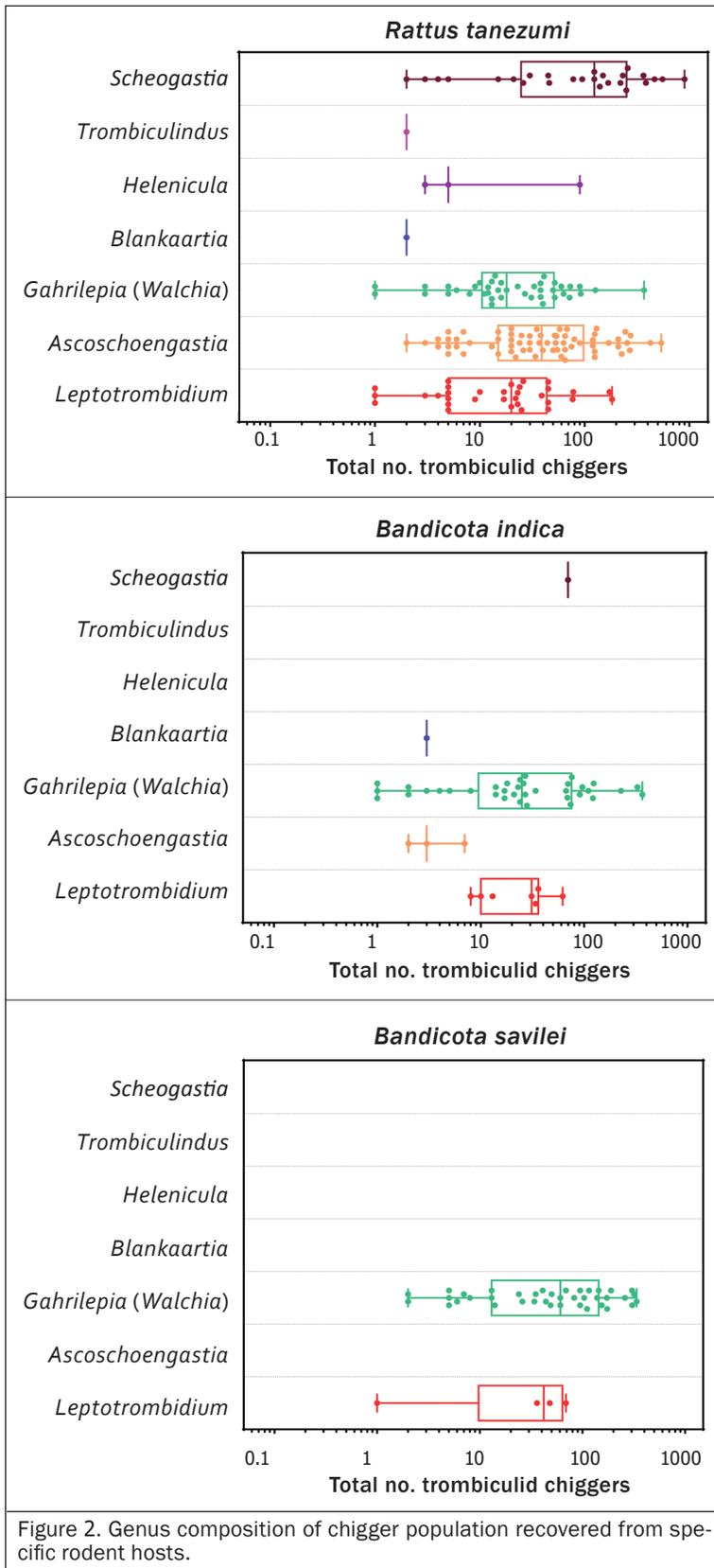
Lepto indicates *Leptotrombidium*; Scheo, *Scheogastia*; Ascosp, *Ascoschoengastia*; Walch, *Walchia*; Blank, *Blankaartia*; Helen, *Helenicula*; trombi, *trombiculindus*.

Table 5. Numbers of trombiculid chiggers positive for *Orientia* and *Rickettsia* by genus.

Genera	Number of Positive Samples		Study Site
	RT	STG	
<i>Ascoschoengastia</i>	-	4	Chaiyaphum, Khon Kaen
<i>Gahrliepia</i>	70	6	Chaiyaphum, Buriram, *Chanthaburi, Lopburi, †Rayong, Chachoeng Sao
<i>Helenicula</i>	1	2	Khon Kaen
<i>Leptotrombidium</i>	39	5	Chaiyaphum, †Chanthaburi, Khon Kaen, †Rayong
<i>Scheogastia</i>	9	3	Buriram
Total	119	20	

RT indicates *Rickettsia* species; STG, scrub typhus group.  
\*Cobra Gold 2017 †Cobra Gold 2018

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individual rodents. 66.6% (12/18) rodents were caught in the training exercise sites, indicating that there may be a high risk of scrub typhus disease transmission. The rodent species with positive chiggers were *R. tanezumi* (72.2%, 13/18); *B. savilei* (22.2%, 4/18); and *B. indica* (5.6%, 1/18), respectively. *Rattus tanezumi* was the only species complex that showed correlation between seropositivity to *O. tsutsugamushi*, load of chigger, and its prevalence infection in chiggers. A significant finding during this surveillance is the first time detection of *Rickettsia* DNA in trombiculid chigger samples in Thailand. Rickettsial pathogens were detected in 119 (7.5%) samples with an average bacterial load between 16.4 and 425.4 copies/ $\mu$ l. One pool of *Ascoschoengastia* sp. chiggers had *Orientia* and *Rickettsia* bacteria coinfection.

**Seroprevalence of *O. tsutsugamushi* and *Rickettsia* Infection in Wild Rodents**

Secondary antibodies for some of rodent species such as *Tupia* species are not commercially available, hence 7.4% of the rodents were excluded from serological assay. Of the 385 rodents examined, 45 rodents (11.7%) captured from 6 of 10 CBG training exercise sites were seropositive for antibodies to *O. tsutsugamushi*-antigen group (Table 1). The highest seroprevalence to *O. tsutsugamushi* was the CBG 2018 Chanthaburi site with (25.5%), followed by the CBG 2017 Khon Kean (20%), Chanthaburi (15%), and Chaiyaphum (13.6%) sites, the CBG 2018 Rayong site, and the CBG 2017 Buriram site (8.1%). Seropositive rodents against *O. tsutsugamushi* antigens were detected in 7 rodent species: *R. bermorei* (50%, 1/2), *R. norvegicus* (50%, 1/2), *B. bermorei* (66.7%, 2/3), *R. tanezumi* (12.7%, 24/189), *R. exulans* (9%, 7/78), *B. indica* (8.8%, 5/57), and *B. savilei* (7.8%, 4/51). Seropositivity differed significantly among rodent species, but not among the study sites. Correlation between chigger and seropositivity was only found in 3 rodent species; *R. tanezumi*, *B. indica*, and *B. savilei* ( $P < .0001$ , 95% CI) (Figure 2). Only 1.6% of the rodents were seropositive to rickettsial pathogens with 1% of *R. typhi*<sup>4</sup> and 0.5% of *R. honei*.<sup>27</sup> Almost all of the seropositive rodents were caught from the training site in Khon Kaen, except one rodent that was seropositive to *R. typhi* which was collected from the Lopburi training site. Mixed infections of 2 different types of bacteria were found in rodents captured from the Khon Kaen

site, where one rodent had specific antibodies to *O. tsutsugamushi* and *R. honei*, while another rodent had antibodies to *R. typhi* and *R. honei*.

The presence of *Orientia* and *Rickettsia* DNA in rodents was examined. The efficiency of qPCR assay for detection of 47-kDa HtrA gene and 17-kDa gene was 97% and 101.3%, respectively. R<sup>2</sup> of the standard curve was 99.4 for both of 47-kDa HtrA and the 17-kDa genes. *Orientia* species DNA was detected only in lung samples of 3 rodents (0.7%, 3/416). Only 2 rodent species, *R. tanezumi* (1.1%, 2/189) and *R. exulans* (1.3%, 1/78) were positive. All of the *Orientia* positive rodents were caught from the training areas in Buriram province. A total of 9 rodents had active rickettsial pathogens infections (2.2%, 9/416). All *Rickettsia* positive rodents were collected from traps from villages and plantations near the training sites of Buriram, Chanthaburi, Lopburi, and Rayong (Table 3). *Rickettsia* DNA was found in many organs with the highest prevalence in lung (66.7%, 6/9), followed by kidney (22.2%, 2/9) and liver (11.1%, 1/9), respectively from 3 rodent species, including *R. exulans*,<sup>4</sup> *B. indica*,<sup>3</sup> and *R. tanezumi*.<sup>27</sup>

Detection of *Leptospira* spp. in Rodent Tissue and Water Samples

During the 2017 surveillance, all of the rodents were shown to be *Leptospira* negative by the qPCR assay targeting the *lipL32* gene. Of the 36 water samples collected from puddles, rivers, canals, and tap water around the training sites and tested for *Leptospira* by qPCR assay targeting *rrs* gene, none of the samples was positive for *Leptospira*. However, 3 samples were positives in the EMJH media culture (Table 6). Two of these samples were recovered from the pond in the school and water source in the village around training site in Chaiyaphum. The other isolate was recovered from the paddy field water samples in Khon Kaen. The partial *rrs* gene was amplified from the 3 positive water cultures. Results obtained from BLASTn search and phylogenetic analysis of *rrs* gene sequences confirmed bacteria as the intermediate *L. licerasiae*.

During the 2018 surveillance period, a total of 155 rodents and 105 water samples were determined for *Leptospira* DNA. Two rodents (1.3%), *Mus cervicolor* (Hodgson) from Nakhon Ratchasima and *R. tanezumi* from Chanthaburi, were positive for *Leptospira* infection. Thirty-three of 105 (31%) water samples from 5

Table 6. Summary of *Leptospira* positive samples.

CBG	Province	Rodents		Environmental Water Samples		
		No. Trapped Rodents	<i>lipL32</i> qPCR Positive	No. Samples	<i>rrs</i> qPCR Positive	Positive Culture
2017	Chaiyaphum	13	0	9	0	2
	Chanthaburi	6	0	10	0	0
	Khon Khan	14	0	7	0	1
	Rayong	3	0	10	0	0
	Total	36	0	36	0	3
2018	Nakhon Ratchasima	18	1	22	3	2
	Chachoengsao	9	0	22	2	1
	Lopburi	54	0	21	5	1
	Chanthaburi	51	1	20	6	2
	Rayong	23	0	20	17	2
	Total	155	2	105	33	8

CBG indicates Cobra Gold.

areas were positive for *Leptospira* by *rrs* real-time PCR detection. Three of the positive water samples were collected from Nakhon Ratchasima, 2 from Chachoengsao, 5 from Lopburi, 6 from Chanthaburi, and 17 from Rayong. Eight isolates of *Leptospira* were successfully recovered from culture. Two isolates from the site in Nakhon Ratchasima were recovered from tap water near the construction site and another one from a canteen in the school. The positive culture sample from Chachoengsao and Lopburi were collected from the ponds near the school. Two isolates from Chanthaburi were recovered from 2 tap water samples from a canteen. The last 2 isolates were the tap water samples collected from the CBG construction site in Rayong.

COMMENT

In this study, rodent trapping and collection of ectoparasites were used instead of the conventional black plate technique to cover larger areas where exposure to vector and pathogen might occur. In addition, due to the military operations, the movement of service members throughout large training areas made it difficult for the black plate method to be used to conduct surveillance in all potential risk areas. By using the rodent collection method, we successfully collected high numbers of trombiculid chiggers as well as mange mites, ticks, and fleas with 49.8% of collected rodents having ectoparasites.

Trombiculid chiggers were recovered from almost all training sites, except the CBG 2017 Rayong site because only few rodents were caught (n=3). A relatively high diversity of chiggers was also found. Our findings indicated that certain rodent species appeared to host more chigger species than others. However, the correlation between these 2 matrices could not be analyzed because the prevalence of chigger load in rodent hosts was not

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distributed evenly. One of the metrics recommended by the WHO is the determination of “arthropod index,”<sup>25</sup> or “chigger index” as analyzed in our study. Multiple studies have demonstrated abundant trombiculid chiggers in various locations in Thailand, although information of chigger indices in different geographic regions is currently lacking. To compare our findings with previous reported data, we reanalyzed the overall chigger indices from the available data previously reported from Thailand. By recalculating the data reported by Coleman et al,<sup>28</sup> we found that the chigger index was 59.5 (153,899 chiggers/2,587 infested hosts). Even though Coleman and colleagues conducted extensive rodent and chigger surveillance studies in many regions in Thailand, most of the rodents (88%) were caught from Chiangrai province. Therefore, the overall chigger index seemed to mainly represent data from specific areas only. Other studies conducted in neighboring province of Chiangmai also showed a relatively similar high chigger index (50.6, 2,277/45).<sup>29</sup> In our study, a higher chigger index was observed (93.2, 19,296/207). Of all the CBG training sites, the CBG 2017 Buriram site showed the highest chigger index (189.9) which was significantly higher than the other sites. This suggested that Buriram site may be considered an area with a high risk of chigger-borne disease transmission to service members.

Many species of trombiculid mites are important vectors of *Orientia* pathogen. Of the trombiculid chiggers collected, 5 genera: *Walchai*, *Leptotrombidium*, *Ascoschoengastia*, *Helenicula*, and *Scheogastia* were positive for *Orientia*. *Rickettsia* sp. infection in a large number of trombiculid chiggers in Thailand was reported for the first time in our study. All of the *Orientia* and *Rickettsia* infected chiggers were recovered from rodents caught approximately 1 to 2 months before the CBG training exercise started.

Based on our serosurveillance study, seropositivity of small rodents to *O. tsutsugamushi* infection was high in many training sites, with the highest seropositive rate in the CBG 2018 Chanthaburi site. Data for scrub typhus seropositivity in the rodent population were analyzed together with data of scrub typhus patient obtained from the Thai Ministry of Public Health. Scrub typhus infection rate in Thailand over a 4-year surveillance period between 2014 and 2017 was high with a total of 31,199 cases reported. The number of scrub typhus cases varied within seasons and regions. The vast majority of the confirmed cases was reported from the northern regions of Thailand, followed by northeastern, southern, and central parts. According to the most recent annual reports in 2016 and 2017, scrub typhus cases were found in all provinces where CBG took place.

The finding and isolation of *Leptospira* pathogens in rodent tissue and water samples may be an indication that activities associated with water, both ground and piped water, may be of concern especially to individuals with wounds or any break in the skin. Human infections occur when they come into contact with the contaminated environment and when the organism penetrates through broken skin or mucosa. A recent report from Dierk et al<sup>15</sup> investigated the association between training activities and the largest record of leptospirosis cases within the US military in 2014. This report indicated that the relationship of water exposure in areas with high leptospirosis levels increased the risk of symptomatic disease for US Marines training in Okinawa, Japan. Rodents are well-known reservoirs of *Leptospira* and are often implicated as the source of human leptospirosis in Thailand. Environmental contamination by *Leptospira* is a more effective route of infection than direct contact, and rodents play an important role as efficient disseminators of *Leptospira* in the environment. Therefore, the prevalence of infected rodents may serve as an indicator of *Leptospira* transmission throughout the environment.

Findings from our surveillance indicate that scrub typhus, rickettsial diseases, and leptospirosis that are widely distributed throughout Thailand pose a more significant threat to military personnel than previously realized. This information is important in determining the potential risk for scrub typhus, other rickettsial diseases, and leptospirosis to military and US government personnel, and for informing regional and combatant commanders for timely diagnosis, treatment and prevention including focused implementation of personal protective measures and vector control in training areas. Continuous surveillance on the ecology, entomology, and epidemiology of these public health important vector-borne diseases is warranted to provide a more accurate risk assessment and determine “hot spots” for these diseases to assist site selection and planning for future CBG training exercises.

### ACKNOWLEDGMENTS

The authors thank Sirima Wongwairot, Nutthanun Aysawasdi, Chawin Limsuwan, Taweesak Monkanna, Opas Thachin, Kasima Wasuworawong, and the staff of USAMD-AFRIMS and the Research Division of Royal Thai Army-AFRIMS for their assistance in the field IFA assay. This research was supported by the Global Emerging Infections Surveillance and Response System, a division of the Armed Forces Health Surveillance Branch of the Department of Entomology, USAMD-AFRIMS under proposal ID 17\_AF\_1.5.2 for fiscal year 2017 and P0081\_18\_AF\_05 for fiscal year 2018.

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# Risk Assessment Mapping for Zoonoses, Bioagent Pathogens, and Vectors at Edgewood Area, Aberdeen Proving Ground, Maryland

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Throughout history, epidemics of infectious diseases have had tremendous effects on the fighting strength of armies, causing delay and cancellation of military operations. From its founding, the US military has employed preventive medicine for force protection in order to conserve its fighting strength. Prior to World War I, the ratio of deaths due to disease versus battle injury was approximately 10:1, which decreased to 1:1 during World War I and 0.01:1 during the Gulf War.<sup>1</sup> During the American Revolution, the Continental Army was greatly affected by typhus and smallpox. Dr Bodo Otto, Brigade Surgeon for General Washington, and ancestor of this paper's authors, was the first to inoculate the Continental Army against smallpox.<sup>2</sup> Today, the mission of the US Army Public Health Center is to "Enhance Army readiness by identifying and assessing current and emerging health threats, developing and communicating public health solutions, and assuring the quality and effectiveness of the Army's Public Health Enterprise."<sup>3</sup>

Most emerging and reemerging disease threats to humans are zoonotic.<sup>4</sup> In 2014, zoonotic diseases comprised 7.4% of all reportable medical events, excluding sexually transmitted diseases, with tick-borne diseases being the most frequently reported.<sup>5</sup> Mosquito-borne diseases, such as West Nile virus (WNV) and Zika virus, also pose a risk to military personnel and their families. For example, in 2015, 6% of *Culex* species pools collected from military installations in the United States were positive for WNV.<sup>6</sup> Influenza and other zoonotic diseases continue to pose a risk to military personnel and their families, particularly where crowding or other unique stressors exist.<sup>7</sup>

Biological agents are a threat to military installations through use of weapons, surreptitious attack, or criminal release. Bioagent databases have been developed for rapid detection and identification of the biological agents during natural outbreaks and criminal/terrorist release, for example, Select Agents List in the United States and the Biological Agents Database in Poland. The accuracy

and applicability of these lists/databases can vary depending on whether the application is military or civilian.<sup>8,9</sup> The Hazard Prediction and Assessment Capability (HPAC) is used by the Department of Defense for training, planning, and tactical operations, and has satisfactorily met the criteria for dispersion modeling.<sup>10</sup> The Bioagent Transport and Environmental Modeling System (BioTEMS) has been used to supplement HPAC to provide greater resolution should a weapon of mass destruction be released, and to fill the gap in HPAC criminal release as well as low volume/low altitude/surreptitious release. For example, vertical resolution can be improved in HPAC for heights of 10 m or less.<sup>11</sup> In a collaborative study between the Defense Threat Reduction Agency (DTRA); the Consequence Management Unit, 415th Chemical Brigade; and the South Carolina Department of Health and Environmental Control, BioTEMS accurately predicted environmental survival and transport of a simulated release, whereas HPAC underestimated environmental survival and transport by 300%.\*

The BioTEMS consists of a set of algorithms and models that have been used as a standalone system for risk assessments of bioagents, infectious diseases, vectors, and to supplement HPAC. The BioTEMS has been used to produce bioagent risk assessments for an overseas naval installation, Fort Detrick, national and international training exercises, national political conventions, and to assist the DTRA during a presidential inauguration in analyzing risk of selected agents and to optimize placement of Biological Integrated Detection Systems (BIDS) units. Risk assessments have also been developed with BioTEMS for several infectious and zoonotic diseases and to distribution of vectors, eg, arboviruses, Ebola, Rocky Mountain spotted fever, avian influenza, plague, *Shigella*, tularemia, mosquitoes, ticks, and mites. It has been used to produce risk assessments in several countries, including Bangladesh, Brazil, Cameroon, China, India, Iran, Libya, Georgia, Turkey, United Arab Emirates, Sierra Leone, and several cities in the United States.

\*Limited access documents not available to the general public.

Recently, BioTEMS was used to identify free-living pathogenic amoebae as the most likely cryptic reservoirs for Ebola virus ( $P > 93\%$ ), identify high risk areas within 15 m, and to determine where to place ProVector devices with antimicrobials.<sup>12</sup> The BioTEMS Ebola model and ProVector anti-Ebola formulation have the potential to reduce the risk of Ebola virus for deployed military forces.

Military personnel and their families can be at risk from vector-borne diseases and release of bioagents on military installations. For example, high risk areas for *Yersinia pestis*, the etiologic agent of bubonic plague, were identified at the US Air Force Academy installation, with some high risk sites adjacent to family housing on the base.<sup>13</sup> Lyme borreliosis poses a significant risk to military personnel and their families where competent vectors are present. In 2015, over 1,000 ticks tested by the Army Public Health Command were positive for *B. burgdorferi*, 21% of *I. scapularis* ticks were positive.<sup>14</sup>

The Edgewood Area, Aberdeen Proving Ground (APG), Harford County, Maryland, is at risk for several zoonotic/vector borne diseases and the release of a bioagent. Through routine sampling of mosquito populations, the US Army Kirk Medical Clinic Preventive Medicine unit at APG detected WNV positive mosquitoes and followed up with mosquito control in 2014.<sup>15</sup> Over 1,100 cases of Lyme disease were confirmed in Harford County from 2000 to 2015.<sup>16</sup> Preliminary modeling of *I. scapularis* populations was accurate to within one square meter at the Edgewood Area.<sup>17</sup> Because of the potential risk at the Edgewood Area from zoonotic pathogens and bioagents, BioTEMS risk assessments were conducted for H5N1 avian influenza, eastern equine encephalitis (EEE), WNV, Zika virus (ZIKAV), unnamed Bioagent X, the lone star tick (*Amblyomma americanum*), and the black-legged tick (*I. scapularis*).

#### METHODS AND RESULTS

There are approximately 1,000 people living on Edgewood Area, with nearly 15,000 people working in both areas of APG. Nearly 20,000 retirees and their families use the recreational facilities at Edgewood.<sup>18</sup> ArcGIS geospatial analysis software, Statistica statistical software, and the BioTEMS were used to analyze geographic information and conduct data analysis. The BioTEMS uses up to several hundred abiotic and biotic factors to produce risk and vulnerability assessments for biological agents and infectious diseases. Examples of biotic and abiotic factors include pathogen strain, vector/host relationship, vectorial capacity, host/vector physiology, colonization ability, population dynamics of hosts and vectors, soil, and weather conditions, such

as wind, temperature, precipitation, and shade. The BioTEMS analysis includes mechanistic and empirical methods, for example, artificial intelligence, fuzzy logic, niche analysis, random forests, general additive regression, and parametric and nonparametric statistics. The BioTEMS is capable of identifying direction of movement of pathogens/vectors, areas for mitigation and management, time for optimal medical countermeasures, and sites for environmental sampling and optimization for placement of BIDS. The BioTEMS TIGER model was used to model ZIKAV. The TIGER acronym represents the 6 stages in the invasion of a mosquito species or haplotype:

- Transport - identifies the point of origin, method, and rate of transport to a locality.
- Introduction - the point or area of initial introduction/immigration of species or haplotypes and preliminary spread into a locality.
- Gap - determines the area where vector/pathogen infiltrates and initially spreads once it has gained a foothold.
- Escalade - incorporates abiotic and biotic factors as possible resistance to invasion.
- Residence and recruitment - incorporates factors and areas where vector/pathogen adds to genetic diversity or becomes endemic and recruits conspecifics/haplotypes.

BioTEMS risk assessment maps (RAMs) were developed for Edgewood for the following infectious diseases and vectors: H5N1 avian influenza, EEE, WNV, ZIKAV, unnamed Bioagent X, lone star tick (*Amblyomma americanum*), and black-legged tick (*Ixodes scapularis*). Both HPAC and BioTEMS were used to model the simulated release of the unnamed bioagent. The BioTEMS risk assessment, based upon abiotic and biotic factors, is given in percentage, and output is visualized in Google Earth.

The BioTEMS risk assessment identified ticks and tick-borne illnesses as the most significant threat to personnel at the Edgewood Area, with *I. scapularis* posing an 18% risk and *A. americanum* a 14% risk, for a combined risk assessment of 32%. The risk assessment of mosquito-borne diseases at the Edgewood Area ranked WNV highest at 1.1% and EEE at 0.2%. The expected direction of movement of ZIKAV introduced through the airport is 349°. The BioTEMS risk assessment for H5N1 Avian influenza was 0.3%. The risk assessment for environmental transport for Bioagent X released into the Edgewood Area from 3 points was 4.2%. Optimal sites were identified for conducting surveillance and control for tick and mosquito-borne pathogens and H5N1 avian influenza. The area of Bioagent X deposition was identified by HPAC which estimated its time to degradation at 24

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Figure 1. BioTEMS risk assessment map: black-legged tick (*Ixodes scapularis*) at the Edgewood Area. Red polygons indicate optimal sites for surveillance.



Figure 3. BioTEMS risk assessment map: West Nile virus at the Edgewood Area. Red polygons indicate optimal sites for surveillance and integrated vector management.



Figure 2. BioTEMS risk assessment map: lone star tick (*Amblyomma americanum*) at the Edgewood Area. Red polygons indicate optimal sites for surveillance.



Figure 4. BioTEMS risk assessment map: eastern equine encephalitis virus at the Edgewood Area. Red polygons indicate optimal sites for surveillance and integrated vector management.



Figure 5. BioTEMS risk assessment map: Zika virus if introduced through the airport located at Edgewood Area. Red polygon indicates high risk area for immediate integrated vector management and surveillance to reduce likelihood of introduction, yellow polygon indicates surveillance areas and control should Zika virus be detected, purple arrow indicates most likely direction of spread of Zika virus in mosquito population.

hours. The BioTEMS predicted the bioagent would last a minimum of 30 days in the environment and spread beyond the initial area of deposition. It also predicted the bioagent could replicate over 10,000 times the initial release amount, resulting in the environmental transport of Bioagent X beyond the initial area of deposition. The BioTEMS identified optimal sites for surveillance and sampling for the vectors and pathogens (Figures 1-7).

COMMENT

Tick-borne Pathogens

The following is a summary of several tick-borne pathogens detected in *I. scapularis* and *A. americanum* from North America as reported by the Centers for Disease Control and Prevention in 2018<sup>19</sup>:

Anaplasmosis is transmitted to humans by tick bites primarily from the blacklegged tick (*I. scapularis*) in the northeastern and upper midwestern United States. *Babesia microti*, the etiologic agent of babesiosis in the United States is transmitted by the blacklegged tick (*I. scapularis*) and is found primarily in the northeast and upper midwest. *Borrelia mayonii*, which also causes Lyme borreliosis, has been found in *I. scapularis* in the upper midwestern United States. *Borrelia miyamotoi*, transmitted by *I. scapularis*, is suspected of causing illness in the United States. Bourbon virus has been identified

in a few patients in the midwest and southern United States and has been detected in *A. americanum*; its distribution in the United States is unknown. Ehrlichiosis is transmitted by *A. americanum* and Heartland virus found in the midwest and the south is most likely transmitted by *A. americanum*. Lyme disease is transmitted by *I. scapularis* in the eastern and upper midwestern United States. Lyme borreliosis, transmitted by *Ixodes* species, is the most commonly reported vector-borne disease in the United States with about 30,000 cases reported annually, with case reports in Maryland ranking consistently among the top 10%.<sup>15</sup> Southern-tick associated rash illness is transmitted by *A. americanum*. Tularmia is transmitted by the *Dermacentor variabilis*, *D. andersoni*, and *A. americanum*. Powassan disease, most cases of which have been reported from the northeast and Great Lakes region, is transmitted by *I. scapularis* and the groundhog tick (*I. cookei*). Rocky Mountain spotted fever (RMSF) is transmitted by (*D. variabilis*),



Figure 6. BioTEMS risk assessment map: H5N1 avian influenza at the Edgewood Area. Red polygons indicate optimal sites for environmental testing and surveillance in waterfowl.

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*D. andersoni*, and the brown dog tick *Rhipicephalus sanguineus*. Spotted-fever group *Rickettsia* have been isolated from *A. americanum*, a species that is capable of transmitting RMSF in the lab, although its role in RMSF transmission is not well understood.<sup>20</sup> Also, an emerging illness, delayed anaphylaxis to red meat, is associated with *A. americanum*.<sup>21</sup> In the November 2017 Vector-borne Disease Report,<sup>22</sup> the Army Public Health Center reported 71 cases of Lyme disease in active duty Army beneficiaries compared to 111 cases during the same time period in 2016. Of 270 ticks tested by the DoD Human Tick Testing Kit Program for *B. burgdorferi*, 25% were positive; 7 of these ticks also tested positive for *Babesia microti* and 9 tested positive for *Anaplasma phagocytophilum*.<sup>22</sup> Evidence from other studies indicate high risk from these 2 species in Maryland. Twenty-six percent of *I. scapularis* in Carroll, Harford, and Howard counties tested positive for *B. burgdorferi*, and *E. chaffeensis*, and *B. lonestari* have been isolated from *A. americanum* at Aberdeen Proving Ground.<sup>23-25</sup>

### West Nile Virus

West Nile virus, family Flaviviridae, was first identified in the United States in 1999.<sup>26</sup> West Nile virus is

an arbovirus, principally transmitted by mosquitoes in the genus *Culex*, with passerine birds serving as the natural reservoir. Transmission to humans, horses, and other mammal species occurs through a bridge mosquito species, such as *Cx. salinarius*, *Cx. pipiens*, and *Cx. restuans*, the primary vectors in the northeastern United States.<sup>27</sup> Over the next decade WNV spread across North America and is now endemic. The first case of WNV in Maryland was reported in 2001, and from 2001 and 2016 there were 334 reported cases.<sup>28</sup> In addition to *Culex* species, *Ochlerotatus japonicas*, an invasive species in Maryland, is capable of transmitting WNV.<sup>29</sup> In addition to *Culex* species, monitoring for and testing of *Oc. japonicas* and *Ae. albopictus* should be conducted at the Edgewood Area. The BioTEMS predicted the risk of EEE to be low at the Edgewood Area. From 2007-2016 there were no reported cases of EEE in humans in Maryland; however, there have been EEE cases reported in horses and in mosquitoes, for example, *Culiseta melanura* and *Culex salinarius*.<sup>30,31</sup> The BioTEMS has been used to predict EEE in other states and is capable of predicting the risk of EEE geographically to within 30 m.<sup>32</sup> Surveillance of EEE at Edgewood Area should be conducted when testing for arboviruses in other mosquito species, such as WNV *C. pipiens*, or when EEE is reported in other parts of Maryland.

### Zika Virus

Zika virus was first isolated in 1947 from a rhesus macaque monkey and in 1948 from *Aedes africanus* from cages placed on a tower in the Zika Forest near Lake Victoria, Uganda.<sup>33</sup> The Zika Forest is a tropical forest, now surrounded by pastures and with small hamlets where research continues on vector-borne diseases, even using the same tower (Figure 8). However, ZIKAV is no longer restricted to transmission of *Ae. africanus* in the tropical forest ecosystem in Africa. Several *Aedes* species have now been implicated in transmission of ZIKAV to humans in urban habitats where the poor are particularly at risk and the disease is spreading globally.<sup>34</sup> Establishing patterns of invasion of vectors and vector-borne diseases into new geographic areas is critical for protecting the public health and naïve human populations. For example, the predictive map developed for *Ae. aegypti* and *Ae. albopictus* globally has a resolution of 5 km.<sup>35</sup> The principle factor responsible for the introduction of disease vectors is air and ship transport.<sup>36,37</sup> Edgewood Area has a small airport, and aircraft travelling from areas within the North America or other regions could introduce infected *Aedes* species infected with ZIKAV. Once introduced into an area, the invasive mosquito species can spread rapidly across regions through ground transport.<sup>38,39</sup> In addition to the import of infected mosquitoes, introduction of ZIKAV into the a new



Figure 7. BioTEMS risk assessment map: Bioagent X if released at Edgewood Area. Red polygons indicate optimal sites for surveillance and environmental transport and a yellow ellipse indicates HPAC deposition model of Bioagent X.

geographic area can occur when local mosquitoes bite infected travelers and become infected or when people become infected through sex or contaminated blood.<sup>40,41</sup> The BioTEMS TIGER model has been used and validated to identify areas at high risk for invasive mosquito species and mosquito-borne diseases, and identify surveillance and integrated vector management in several countries. It is able to predict direction of movement to within 4° or less.<sup>42</sup> From 2016 through May 2017, nearly 4,000 cases with laboratory evidence of recent possible Zika infection were reported in the US territories.<sup>43</sup> As frequent travelers, military personnel and their families are at risk for arbovirus infection and transporting infected mosquitoes or introducing infection to local mosquitoes upon return. Pre-travel counseling should be provided to travelers to ZIKAV-outbreak regions.<sup>44</sup>

#### H5N1 Avian Influenza

Although BioTEMS identified the risk of H5N1 avian influenza as low at the Edgewood Area. There are several sites where the virus could be maintained and serve as a source of an outbreak should it be introduced. Environmental transmission can provide a mechanism for the persistence mechanism of avian influenza even where epidemics cannot be sustained.<sup>45</sup> In a study of avian influenza in Maryland, the risk to backyard poultry was positively associated with exposure to waterfowl and location, particularly in northern Maryland.<sup>46</sup> Edgewood Area lies on a peninsula and is part of the eastern flyway for waterfowl. The possibility of introduction of avian influenza through infected waterfowl should be of concern. Environmental samples and waterfowl should be tested for avian influenza virus at the Edgewood Area. Because of the large area suitable for waterfowl, identifying areas at highest risk for the introduction and survival of H5N1 avian influenza using BioTEMS can optimize surveillance efforts.

#### Bioagent X

The threat of biological warfare and bioterrorism is significant. Small quantities of lethal biological agents can be easily concealed, transported, and released into susceptible populations. Military and civilian populations are vulnerable to biological weapons and it is difficult to provide adequate protection.<sup>47</sup> The BioTEMS was used in conjunction with HPAC to model a computer simulated release of Bioagent X, enhancing the capability of HPAC to accurately predict the environmental survival and transport of the bioagent. For example, HPAC predicted Bioagent X would survive 24 hours and would be



Figure 8. Research tower in the Zika forest and surrounding landscape near Lake Victoria, Uganda. A mosquito vector for the Zika virus, *Aedes africanus*, was first identified in this location in 1948.

restricted in area, whereas BioTEMS predicted the bioagent would survive a minimum of 30 days and would spread outside the affected zone identified using HPAC. The BioTEMS can also be used to identify where environmental samples can be collected for both measuring naturally occurring strains of bioagents and sampling post-release. This capability can assist in consequence management and providing forensic information for law enforcement.

#### CONCLUSION

Identifying the risk of vector-borne diseases and implementing integrated vector management at the Edgewood Area is of significant public health value. The BioTEMS RAMs can be used to assist public health officials identify and mitigate the risk of zoonotic/infectious diseases and bioagents. Once the risk of an infectious disease is determined, medical and preventive measures can be taken to reduce the threat to military personnel and their families. For example, several high risk sites were adjacent to base family housing located in the northeast portion. Particular effort should be conducted in vector

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management in and around this family housing area, including education, surveillance, testing, vegetation management, and, if needed, application of pesticides. To reduce time and cost of surveillance, sites for surveillance that overlap can be chosen. Although not utilized in the current project, the BioTEMS can be used to identify where biological sensors should be placed at Edgewood to optimize detection and mitigate environmental survival. Additional BioTEMS RAMs are being developed for other military installations and cities in the United States and abroad.

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## RISK ASSESSMENT MAPPING FOR ZOOSES, BIOAGENT PATHOGENS, AND VECTORS AT EDGEWOOD AREA, ABERDEEN PROVING GROUND, MARYLAND

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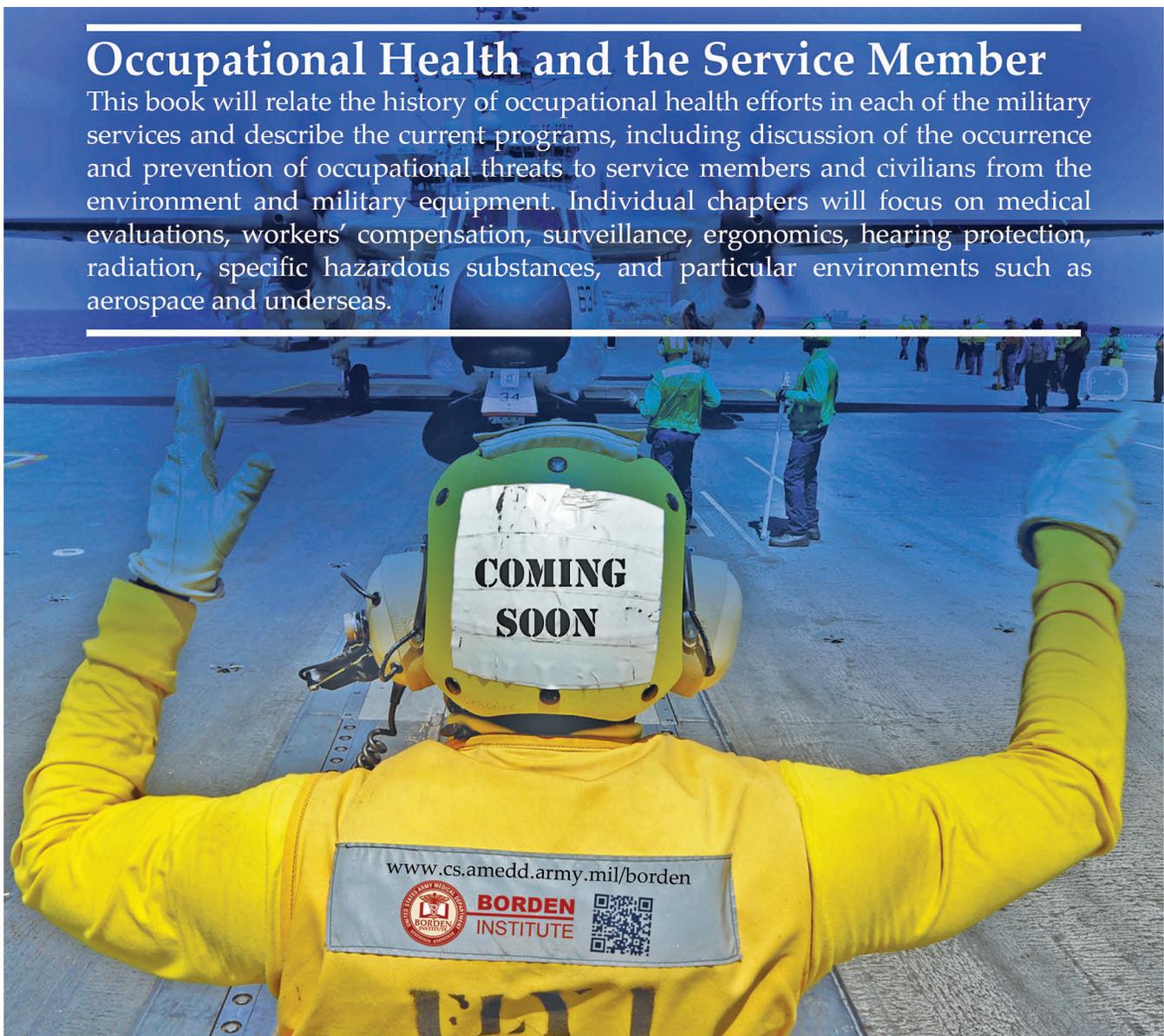
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## Occupational Health and the Service Member

This book will relate the history of occupational health efforts in each of the military services and describe the current programs, including discussion of the occurrence and prevention of occupational threats to service members and civilians from the environment and military equipment. Individual chapters will focus on medical evaluations, workers' compensation, surveillance, ergonomics, hearing protection, radiation, specific hazardous substances, and particular environments such as aerospace and underseas.



# Optimizing Mission-Specific Medical Threat Readiness and Preventive Medicine for Service Members

COL Caroline A. Toffoli, VC, USAR

## ABSTRACT

Deployments and mobilizations of Army Soldiers have been continuing processes and will be sustainable requirements for the foreseeable future. Global deployments often position service members in austere environments that can include exposure to biological threats that can significantly affect their health and medical readiness. Unit commanders and operations personnel bear the responsibility for researching and disseminating up-to-date information on potential biological threats including vector-borne diseases and zoonotic diseases.

It has been a significant challenge to inform and prepare service members for the potential threats in Army Reserve units and units that do not have access to preventive medicine advisors. With the expanding list of known pathogens and the discovery and surveillance of emerging threats, it is extremely important for leaders and providers to have readily-available access to information about current, mission-specific biological threats.

This article discusses the resources that are available to leaders, providers, and service members, and provides an organized resource for obtaining current and complete information on global biological threats that could affect combat readiness.

There are various, disparate resources of information available for consultation regarding potential biological risks associated with various parts of the world; however, there is not a consolidated resource of information that identifies not only the risks, but also the appropriate preventive measures and diagnostic tests that should be performed to identify overt as well as subclinical infection. This article is intended to provide a consolidated resource of information to facilitate the identification, diagnostic testing, and prevention of diseases that pose a risk to military service members. The information is presented to assist medical providers with accessing information needed to best determine pre- and postdeployment medical assessment for preventive medicine and veterinary personnel, and other service members who are at risk for exposure to endemic, zoonotic, and vector-borne diseases.

This article is a resource document providing identification and descriptions of many resources that provide valuable insight into country-specific biological threats, along with specific information on where to submit samples and specimens of concern for identification and diagnosis. Additionally, resources to inform medical readiness and prepare the medical threat brief are provided.

The following fictional scenario illustrates the challenges faced by an Army Reserve unit in achieving mission-specific medical readiness for an impending deployment.

## SCENARIO: MISSION-SPECIFIC MEDICAL THREAT READINESS CONSIDERATIONS

DET 22 is preparing for deployment to Eritrea. The DET Commander, a physician, has requested her Preventive Medicine Officer (PMO) to prepare a medical threat brief outlining the known and potential biological threats that may be encountered during deployment. The Deputy Commander is a Veterinary Corps officer who provides assistance with preparation of the medical readiness plan. The briefing must include information on theater-specific biological threats, resources for diagnostics beyond the scope of what can be supported in theater, and individual medical readiness including:

- ▶ required immunizations,
- ▶ personal protective measures,
- ▶ serology testing,
- ▶ vector identification.

In preparing for the deployment, the PMO faces some challenges in ensuring the information is as current and complete as possible. First, she must determine if there is current information available on the known and potential biological threats in Eritrea. Does the Army Public Health Center have a prepared Entomological and Zoonotic Operational Risk Assessment (EZORA) for Eritrea, or do they have the ability to prepare an EZORA within the next 6 weeks? Also, whom should she contact for assistance with providing the specific information needed for the medical threat brief core and redeployment slides?

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The detachment will require the ability to integrate resources available that provide global biosurveillance, medical readiness, diagnostics, and preventive medicine information.

Is there a way the PMO can find the pieces of information needed and compile them into one resource to provide to the command? The health of the service members depends on the availability of accurate, current information.

The PMO is concerned about how to adequately address the information needed by the Soldiers and leaders during this deployment preparation, especially since many of the Soldiers will venture into austere environments, and may work routinely with potential vectors of disease. Does the information adequately deliver/provide the Soldiers and medical providers with information that will support the mission?

### PROBLEM STATEMENT

How do those responsible for medical readiness of deploying Army units obtain current information on biological threats across the range of military operations, with the variety of exposure to pathogens and in the austere environments in which they serve?

### WHAT IS ADEQUATE MEDICAL READINESS FOR DEPLOYMENT?

Many resources are available for consultation by Soldiers who are deploying to areas of operation that pose medical risks. The evolution of known and emerging pathogens warrants the availability of a contemporaneous resource of information for deploying troops who have the potential to be exposed to pathogens that have vector transmission or zoonotic potential.

Organisms may change their resistance patterns by a variety of natural and induced processes. Additional variation can also occur through migration from other populations of a pathogen, or through a range of cytological and molecular changes. These processes warrant constant vigilance and surveillance of known and emerging pathogens.

Preventive Medicine (PM) and Veterinary Corps (VC) Soldiers bear the risk of exposure to vectors and pathogens that are otherwise of little concern to military service members. The interaction with foreign food sources, animals, and austere environments are a frequent occurrence for PM and VC Soldiers, and exposes them to significant risks for infection with parasites, bacteria, viruses, fungi, molds and protozoans.

### INFORMATION SOURCES

#### World Health Organization

The World Health Organization produces fact sheets on international travel and health, global vector-borne diseases, and information concerning disease outbreak and neglected zoonotic diseases. There are also disease distribution maps. This information is available at <http://www.who.int/en/>.

#### US Army Public Health Center

The US Army Public Health Center (APHC) strives to be a world-class provider of public health services across the US Army and the Department of Defense (DoD). Its mission is to enhance Army readiness by identifying and assessing current and emerging health threats, developing and communicating public health solutions, and assuring the quality and effectiveness of the Army's public health enterprise.

The APHC is an exceptional resource for information that includes major disciplines of concern to deploying service members. These include:

- animal medicine,
- deployment and environmental health,
- diseases and conditions,
- health surveillance and evaluation,
- laboratory sciences and toxicology, and
- region-specific public health information.

The APHC website is a primary site for information on global threats and best practices to promote health and prevent disease. The website is available at <https://phc.amedd.army.mil>.

#### National Center for Medical Intelligence

The National Center for Medical Intelligence (NCMI) is a component of the Defense Intelligence Agency. Its mission is to monitor, track, and assess a full range of global health events that could negatively affect the health of US military and civilian populations. Before troops are deployed to foreign areas for combat, peacekeeping, or humanitarian operations, NCMI's assessment of potential health risks and foreign health care capabilities assists medical providers in planning for the proper medical countermeasures, health care support, and medical personnel support.

The NCMI support to US forces includes:

- ♦ Environmental health: identify and assess environmental risks that can degrade force health or effectiveness.

- ◆ Infectious disease: identify, assess, and report on infectious disease risks that can degrade mission effectiveness of deployed forces and/or cause long-term health implications, and support national security and homeland defense by reporting foreign disease outbreaks that may be naturally occurring or intentional.
- ◆ Life sciences and biotechnology: assess foreign biotechnological developments and medical advances.
- ◆ Medical capabilities: assess foreign emergency and disaster response capabilities, and maintain and update an integrated database on all medical treatment, training, pharmaceutical, and research and production facilities.
- ◆ Medical intelligence products.

The NCMI resources are available at <https://www.ncmi.detrick.army.mil/index.php>.

#### SPECIFIC RESOURCES

##### Entomological and Zoonotic Operational Risk Assessments

Entomological and zoonotic operational risk assessments (EZORAs) are prepared jointly by the APHC Entomological Sciences Program and the Veterinary Services Portfolio to assist public health and military medical planners with predeployment planning and deployments to a country of concern. In addition to vector-borne disease risk, an EZORA also covers zoonotic risks to military personnel and military working dogs.

The EZORA website provides in-depth information on global site-specific diseases and vectors of concern, as well as health risk communication consultation and training. It is available at <https://phc.amedd.army.mil/topics/envirohealth/epm/Pages/EZORA.aspx>.

##### Epidemiology and Surveillance

###### Global Emerging Infections Surveillance and Response System

The Defense Health Agency (DHA) Armed Forces Health Surveillance Branch (AFHSB) plays a critical role in force health protection. As the central epidemiologic resource for the US Armed forces, AFHSB conducts medical surveillance to protect all those who serve our nation in uniform and allies who are critical to our national security interests. The AFHSB collects data about service members and populations of our global partners. The Global Emerging Infections Surveillance and Response System (GEIS) has become a respected authority in surveillance and research about emerging infectious disease and endemic diseases throughout the world. The AFHSB-GEIS provides direction, funding, and oversight to a network of more than 70 partners

based in all regions of the world. Working in conjunction with their host nations, these partners conduct disease surveillance and rapid outbreak response, encourage research and innovation, and build capacity. The GEIS partner network enhances global public health capacity by providing support to:

- ◆ Improve DoD and international partners' capacity to detect and respond to outbreaks of relevant emerging infectious disease.
- ◆ Promote the establishment of electronic surveillance systems that support comprehensive emerging infectious disease surveillance and/or syndromic-based surveillance systems such as the Suite for Automated Global Electronic bioSurveillance.
- ◆ Conduct scientifically valid evaluations of existing and new surveillance and outbreak response systems.
- ◆ Support applied research and development of laboratory diagnostic technologies to rapidly detect potential emerging infectious disease threats (eg, diagnostics, computers and/or laboratory-based systems).
- ◆ Enhance the host nation public health research, clinical investigation and laboratory capacity.

The AFHSB can access epidemiologic information and service member exposure data, and it produces monthly and annual reports on disease occurrence and outcomes. The resources of the AFHSB are available at <https://www.afhsc.mil/Home/About>.

##### Disease Reporting System Internet

The Disease Reporting System Internet (DRSi) is an internet-based resource used to report and keep a historical record of reportable medical events (RME), and a resource for acquiring specific epidemiological data on the incidence of any disease of interest. The DRSi was launched by the Navy and Marine Corps Public Health Center in 2008 and is the system now used by both the Navy and Army for reportable diseases. It uses guidelines and case definitions developed by the AFHSB. Medical assets based in the United States and those deployed are required to use DRSi to report RMEs. The Army Disease Reporting System Internet is the Army's branch of DRSi. Access is restricted to medical professionals who have been granted access in order to perform disease reporting or surveillance. Reportable medical event case definitions and information concerning the DRSi access request process are available at the Public Health Center's website, <https://phc.amedd.army.mil/topics/healthsurv/de/Pages/DRSiResources.aspx>.

##### Global Biosurveillance Portal

The Global Biosurveillance Portal (G-BSP) was developed by the DoD Joint Program Executive Office for

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Chemical and Biological Defense to meet requirements for biosurveillance situational awareness and collaboration capability to support strategic, operational, and tactical users. The G-BSP is an unclassified, web-based enterprise environment that facilitates collaboration, communication, and information sharing in support of the detection, management, and mitigation of man-made and naturally occurring hazards. The web portal:

- ◆ Provides a set of tools and capabilities to enhance the situational awareness of all hazards from the tactical to the strategic level.
- ◆ Facilitates the fusion of multiple unclassified information sources. Hundreds of data sources can be displayed as layers on a map, enabling users to research a specific area of responsibility for both current and historical hazards.
- ◆ Provides a “one stop shop” to access information on specific human, vector, and animal threats; medical countermeasures; facilities capable of providing interventions; and local infrastructure and resourcing.

This resource is accessible by establishing an account at <https://www.biosurveillanceportal.org>.

APHC Entomological Sciences Branch

Located within the US Army Public Health Center Regional Commands, The Entomological Sciences Branch maintains the expertise to address vector-borne, emerging diseases that potentially affect force health and readiness.

Services include:

- ◆ Vector and reservoir surveillance: assists units with setting up surveillance programs for insects and arthropods capable of transmitting disease, and assess pathogen levels in large and small mammal populations on military installations.
- ◆ Identification services: identifies submitted ticks, insects, rodents, reptiles, birds, nuisance plants, or any other unknown specimen.
- ◆ Pathogen diagnostics: coordinates the testing of insects, arthropods, animal blood, and tissue, as well as environmental samples for a wide range of disease causing organisms.
- ◆ Environmental science engineering officers (ESEO) assistance visits: Provides specialized, on-site support to orient newly assigned ESEOs as well as specialized field training for preventive medicine staff to address region-specific pest problems.
- ◆ Installation medical entomology and public health support: reviews/creates documents and program activities to bring pest management programs into compliance with regulatory requirements and sound integrated pest management practices.

- ◆ Health risk communication: provides briefings and other educational material on operational and institutional risk management of vector-borne and zoonotic disease.
- ◆ Deployment training: provides training on entomological issues of preventive medicine for units preparing to deploy.
- ◆ Special consultations and tailored training: subject matter experts who can perform quick-response investigations and training on entomology-related procedures, practices, or perceived issues.

The resources offered by the APHC Entomological Sciences Branch are available at <https://phc.amedd.army.mil/topics/envirohealth/epm/Pages/Deployment-Pest-Management.aspx>. There are also external resource documents that identify specific threats, such as arachnids and venomous snakes, which are prevalent in the areas of operation where service members are frequently deployed.

Diagnostics

US Army Medical Research Institute  
of Infectious Diseases

The US Army Medical Research Institute of Infectious Diseases (USAMRIID) serves as the DoD lead laboratory for medical biological defense research. It serves as a national resource for the isolation and identification of infectious disease agents and is a DoD reference center for information related to arthropod-borne and hemorrhagic fever viruses. While their core mission is to protect the warfighter from biological threats, they also investigate disease outbreaks and threats to public health. Research conducted at USAMRIID leads to medical solutions—therapeutics, vaccines, diagnostics, and information—that benefit both military personnel and civilians. The core competencies of USAMRIID include:

- ◆ Development, testing and evaluation of medical countermeasures.
- ◆ Providing world-class expertise in medical biological defense.
- ◆ Rapid identification of biological agents.
- ◆ Training and educating the force.
- ◆ Maintaining biosafety, biosurety and biosecurity standards.
- ◆ Preparing for technological uncertainty.

The capabilities of USAMRIID include a special pathogens laboratory where clinical or environmental samples can be submitted for identification. The Specimen Collection and Submission Manual provides specific guidelines on sample submission. More information is available at <http://www.usamriid.army.mil/index.htm>.

Centers for Disease Control and Prevention

The Centers for Disease Control and Prevention (CDC) Division of Vector-Borne Diseases maintains The International Catalog of Arboviruses. The Catalog is meant primarily for the description of those viruses biologically transmitted by arthropods in nature and actually or potentially infectious for humans or domestic animals. The Catalog lists, describes, and provides technical information for hundreds of viruses, including the epidemiology and geographic distribution, and provides specific information on specimens required for testing through state health departments. Instructions for submitting specimens to CDC Diagnostic Laboratories for detection of arboviral diseases, bacterial zoonoses, and rickettsial zoonoses are available at <http://www.cdc.gov/ncezid/dvbd/specimensub/index.html>.

EZORA Website

The EZORA website discussed earlier provides information about environmental sample submission, availability of Arthropod Vector Rapid Detection kits, food and bottled water analysis, toxicology assessments, and other evaluations.

Prevention

Centers for Disease Control and Prevention

The CDC maintains a website on global travelers' health that includes information for clinicians regarding medicines and vaccinations that should be administered prior to travel, as well as posttravel evaluation guidance. The CDC Health Information for International Travel (commonly called the Yellow Book) is published every 2 years as a reference for those who advise international travelers about health risks. This reference is available at <http://wwwnc.cdc.gov/travel/page/yellowbook-home-2014>.

Immunizations and Chemoprophylaxis

*Army Regulation 40-562*<sup>1</sup> identifies mandatory vaccinations for military personnel by category, along with guidance on pre-exposure rabies prophylaxis for at-risk populations. Chemoprophylaxis for diseases that have been shown to be of military significance is also discussed. These include anthrax, influenza, meningococcal disease, leptospirosis, plague, scrub typhus, smallpox, traveler's diarrhea, and malaria. Consulting for current information and guidance for appropriate drugs and dosing regimens is essential (for example, the CDC Advisory Committee on Immunizations Practices (ACIP), the NCMI, and the *Control of Communicable Diseases Manual*<sup>2</sup>). Note that the recommendation to consult with the appropriate preventive medicine authority has been omitted from the current (2013) version of *Army Regulation 40-562*.

DHA Immunization Healthcare Branch

The DHA Immunization Healthcare Branch supports all DoD immunization programs and provides clinical consultative services, educational support, and training resources. Its resources are available at <https://health.mil/Military-Health-Topics/Health-Readiness/Immunization-Healthcare/Vaccine-Recommendations/Vaccine-Recommendations-by-AOR>.

Rabies Prophylaxis

Specific guidelines on rabies prophylaxis for veterinarians and animal care providers are found in the *Compendium of Veterinary Standard Precautions for Zoonotic Disease Prevention in Veterinary Personnel*,<sup>3</sup> The document addresses pre- and postexposure prophylaxis as well as titers, and promotes its guidelines in accordance with the CDC ACIP. <http://www.cdc.gov/vaccines/acip/about.html>.

Regulatory Guidance

Army Personnel Policy

The *Department of the Army Personnel Policy Guidance for Overseas Contingency Operations*<sup>4</sup> requires that all personnel deploying to an area of operations are current with theater-specific immunizations which are provided in a table format. Further, the policy mandates that in accordance with ASD(HA) Memorandum dated March 14, 2006,<sup>5</sup> predeployment serum specimens for medical examinations will routinely be collected within one year of deployment. However, if an individual's health status has recently changed or has had an alteration in occupational exposures that increases health risks, a healthcare provider may choose to have a specimen drawn closer to the actual date of deployment. Postdeployment serum specimens for medical examinations should be collected no later than 30 days after arrival at the demobilization site, home station, or inpatient medical treatment facility.

Active duty military activities with medical facilities are usually able to perform some diagnostic testing including immunology; however, specialized tests are often referred to approved laboratories or other military installations.

Geographic Combatant Command  
Medical Entry Standards

Uniformed service members must meet service standards of fitness according to service regulations and policies. Additionally, to deploy to a given combatant command area of responsibility, additional standards may apply based upon operational conditions of the deployed location. For example, Modification 13 to US

## OPTIMIZING MISSION-SPECIFIC MEDICAL THREAT READINESS AND PREVENTIVE MEDICINE FOR SERVICE MEMBERS

Central Command Individual Protection and Individual/Unit Deployment Policy\* defines the standards of fitness for the US Central Command area of responsibility. Each combatant command develops its own standards of fitness for deployment which are available from that command. Leaders of deploying service members must contact the individual combatant command for the fitness policies that pertain to its area of responsibility.

### Medical Threat Briefing

The purpose of medical threat briefings, deployment health guides, and deployment health cards is to help reduce the risk of disease and nonbattle injury during deployment. Per the Army Personnel Policy Guidance,<sup>4</sup> all personnel will receive health threat briefings prior to deployment. Health threat briefings and products should be administered and distributed by preventive medicine personnel.

A medical threat briefing (MTB) contains information on health threats and countermeasures while deployed. A MTB resources website is hosted on Army Knowledge Online (restricted access), available at <https://www.us.army.mil/suite/files/35828267>. Health professionals should use the core slides as a starting point to customize and assemble their own MTB. Use the provided resources list to research specific geographic areas.

### CONCLUSION

Although we will continue to face known and emerging biological threats globally, the Army is able to meet the future challenges of health promotion and medical readiness. With the vast array of resources available for consultation, the challenges of surveillance, diagnostics, prevention, and treatment are manageable. The difficulty of operating in austere environments while maintaining

\*Internal military document not readily accessible by the general public.

health is lessened through the flow of information and exchange of updated methodologies that support advancements in disease awareness, vector identification, and personnel protection.

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# Public Health Response to Imported Mumps Cases – Fort Campbell, Kentucky, 2018

LTC John W. Downs, MC, USA

## ABSTRACT

Mumps is an acute viral disease caused by a paramyxovirus that presents with fever and swelling of one or more of the salivary glands. Although not generally considered a disease of military importance, mumps has been associated with outbreaks among young adults in close living quarters, potentially placing Soldiers at risk for transmission of mumps when living in congregated settings. This article reports a recent public health response to 3 imported mumps cases occurring at Fort Campbell, Kentucky, that resulted in a contact investigation for 109 close contacts across varied settings. No secondary mumps cases were identified. This report highlights the need for continuous preparation for public health emergency response, and the need to develop and maintain strong working relationships with local civilian public health assets, as well as with installation organizations, such as schools, child care centers, and public affairs resources.

Mumps is an acute viral disease caused by a paramyxovirus that presents with fever and swelling of one or more of the salivary glands: parotid, sublingual, or submandibular.<sup>1</sup> Mumps is usually a self-limited, mild disease. However, in some cases, it can develop more severe complications such as meningoencephalitis, or orchitis that results in male sterility.<sup>2</sup> Severe complications are typically more associated with patients who develop disease as adolescents or adults.<sup>2</sup> Mumps had become relatively rare in the post-vaccine era.<sup>3</sup> However, focal outbreaks have become increasingly common, particularly among the unimmunized and students in colleges and universities.<sup>3</sup> Mumps has an incubation period of 12 to 25 days following exposure, and can be transmitted to another person from roughly 2 days prior to symptom onset until about 5 days after symptom onset.<sup>4</sup> The measles, mumps, and rubella (MMR) vaccine is only approximately 78% effective for mumps prevention after one dose, and approximately 90% effective after the standard 2-dose series.<sup>3</sup> However, it is well-described that outbreaks have occurred even among populations where more than 85% of those at risk had received 2 doses of MMR vaccine previously.<sup>3</sup> In October 2017, the Centers for Disease Control and Prevention (CDC) advised that a 3rd “booster” dose of MMR vaccine be provided to a “group or population at increased risk for acquiring mumps because of an outbreak.”<sup>5</sup> However, MMR vaccine has not been shown to be effective for postexposure prophylaxis, and in at least one small study, a 3rd dose given as postexposure prophylaxis was not shown to significantly reduce the mumps attack rate when compared to those exposed individuals who had previously received 2 doses of MMR vaccine.<sup>6</sup>

## BACKGROUND

In spring 2018, the Fort Campbell Department of Preventive Medicine (FTCKY DPM) was notified of 3 suspected mumps cases who presented to the medical activity (MEDDAC) emergency department (ED) on a Friday afternoon (Day 0). The 3 suspected cases involved an active duty Soldier (father, 29 years old), his spouse (mother, 29 years old), and their daughter (5 years old). The Soldier and his spouse reported the onset of fever and left facial swelling within the 24 hours prior to presentation to the ED (Day -1). The child had no overt facial swelling, but complained of jaw pain and tenderness. The Soldier reported that his family had recently traveled to an area of the United States with an active mumps outbreak, staying from about 5 weeks prior to presentation to 2 weeks prior to presentation. The location to which the Soldier and his family traveled is not provided here due to privacy concerns. The Soldier reported that his family stayed with members of his spouse’s family, and that all 4 members of his spouse’s family with whom they resided had become confirmed cases of mumps while the Soldier’s family was present. The Soldier stated that, in fact, he had moved his family to another house in an attempt to prevent transmission of mumps to his family. The Soldier initially felt that he and his family had “gotten lucky” as it had been over 2 weeks since their last contact with a known mumps case, and they had not yet developed symptoms.

A review of available records found that the Soldier had received 2 doses of MMR vaccine at the time of his accession into the Army. His spouse had no evidence of

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MMR vaccine or mumps titer in her military electronic health record. The daughter had received a single dose of MMR vaccine, approximately one year prior. With further questioning, the Soldier revealed that he had returned from a 7-day field exercise on the evening (Day -1) prior to presentation, and that he slept in a communal tent with roughly 20 other Soldiers during this exercise. He also reported that his daughter attended an on-post elementary school for the 4 days prior to presentation. On the basis of symptoms consistent with mumps, and reported epidemiologic linkage to known cases of mumps associated with a well-reported mumps outbreak, a contact investigation was initiated.

### INVESTIGATION

The investigation began with a call to local civilian public health authorities to request assistance with obtaining buccal swab mumps polymerase chain reaction (PCR) testing for confirmation through the Tennessee Department of Health (TNDOH) Laboratory. Civilian public health authorities were very receptive to this request for assistance and immediately began coordination for testing. Buccal swab samples were obtained from the family on Day 1 for processing at the TNDOH Laboratory. The samples were obtained in accordance with published CDC guidance.<sup>7</sup> These samples were collected by TNDOH officials and processed on Day 3. Prior buccal swab samples for mumps viral culture had been obtained during the family's ED visit for processing at a commercial laboratory. However, the presumed turnaround time was excessive, and we could not be certain that the samples had been properly obtained.

On Day 4, laboratory testing conducted by the TNDOH Laboratory confirmed a positive buccal swab mumps PCR in both the Soldier and his spouse. Mumps PCR was negative for the child; however, a serum mumps IgM returned positive for the child later on Day 6. Serum mumps IgM is known to result in both false-positive and false-negative results in previously vaccinated individuals.<sup>4</sup> Thus, this positive mumps IgM may have been secondary to the child having received one dose of MMR vaccine previously. However, given the child's repeated exposures to known mumps cases among her family, and the risk of initially overlooking any secondary cases among other exposed children if we assumed the child to not be infected and therefore cease any further investigation of the child's contacts, FTCKY DPM made the decision to continue the contact investigation as if the child represented a probable case, in accordance with case definitions as discussed later.

For the purposes of this investigation, a confirmed case was defined as a positive mumps laboratory confirmation

via PCR or viral culture in a patient with an acute illness consistent with mumps. A probable case was defined as acute parotitis or other salivary gland swelling lasting at least 2 days in a person with an epidemiologic linkage to another probable or confirmed case, or a person with a positive mumps serum IgM.<sup>3,4</sup> For the purposes of this investigation, presumptive evidence of mumps immunity was defined as either valid documentation of 2 doses of MMR vaccine for contacts over age 4 years, or laboratory evidence of immunity by positive mumps IgG serology, referred to hereafter as "mumps titer."<sup>3</sup> All individuals involved in this investigation were born after 1957; thus, presumptive immunity based on age was not a consideration.

Specific recommendations were tailored to each setting (children and educators, healthcare workers, and other Soldiers). However, our contact and containment plan was generally consistent with the following recommendations for each setting: (1) those contacts for whom evidence of presumptive immunity was available could continue attending work and school without interruption; (2) those contacts who had only received one dose of MMR should receive the second dose as soon as possible, provided the first dose was at least 28 days prior; and (3) those contacts for whom no evidence of immunity could be found were to be excluded from work and/or school from postexposure day 12 through postexposure day 25. Understanding that the MMR vaccine does not provide complete immunity to all even after 2 doses, all contacts were reassessed for symptoms by questionnaire or interview on days 15, 20, and 26 following their respective exposure.

Soldiers who had slept in the communal tent with the case-Soldier were considered close contacts. A list of these contacts was obtained through the case-Soldier's company commander. An immunization review was conducted of these contacts. Of the 27 total contacts identified here (average age 24 years, range 20-37 years), only 4 Soldiers had presumptive evidence of immunity available in the electronic medical record. This was in part due to the realization that in the Army Medical Protection System (MEDPROS), mumps immunity is assumed to be present based on positive testing of measles and rubella titers drawn upon a Soldier's accession into the Army.<sup>8,9</sup> This assumption then transfers to the electronic medical record as the Soldier being "up to date" on MMR vaccine. However, this assumption was not consistent with published guidance for contact investigations.<sup>3,4</sup> Thus, as only measles and rubella titers were available for review, but not mumps titers, we initially assumed that 23 of the 27 (85%) of the exposed Soldiers did not have evidence of immunity to mumps.

Under the assumption that these 23 Soldiers had very likely received MMR vaccine previously, but simply had no documented evidence of mumps immunity per Army policy as described, we opted to send these 23 Soldiers for mumps IgG serology right away in order to clearly document evidence of mumps immunity. We assumed that any significant presence of mumps IgG less than 5 days after exposure would have to be secondary to previously established immunity. Furthermore, if the Soldiers showed evidence of immunity to both measles and rubella, given their young age, it was most likely due to a history of having received at least one dose of MMR vaccine. Of those 23 Soldiers, 18 (78%) had positive mumps titers results. In this manner, we were able to establish presumptive immunity for 22 (81%) of the 27 total Soldier contacts. The remaining 5 Soldiers for which presumptive immunity to mumps could not be established were housed in a separate barracks area, and excluded from contact with other Soldiers for postexposure days 12 to 25. Follow-up symptom interviews were conducted by Army Public Health Nurses (APHNs) on postexposure days 15, 20, and 26. No symptoms consistent with secondary cases were identified.

Six healthcare workers (HCW) were potentially exposed to mumps during the care of the index cases. Fortunately, the need for isolation and droplet precautions was identified immediately upon the family's arrival at the MEDDAC ED, and this likely limited the number of exposed HCWs. Of these 6 HCW contacts, only 3 (50%) initially had presumptive evidence of immunity available in the electronic health record. This was likely due to the fact that the HCWs were hired prior to 2015 when a more rigorous pre-employment vaccination review was implemented at this facility to achieve more consistency with guidance in *Army Regulation (AR) 40-562*,<sup>10</sup> and the 2011 CDC recommendations for the immunization of HCWs.<sup>11</sup> These 3 HCWs were also sent for immediate mumps IgG serology, and all 3 resulted in positive mumps titers. The HCWs were counseled on the signs and symptoms of mumps, and to stay home should they develop any signs or symptoms suggestive of mumps, but were allowed to continue working after presumptive immunity had been established.

The child's school was the source of the majority of contacts identified to investigate. The child attended prekindergarten in a setting where children and staff moved in and out of several different classrooms. This resulted in a total of 62 child contacts (age range 3 to 5 years), and 14 educator contacts. Notification of this potential exposure to a probable mumps case was provided to parents of the other prekindergarten children via a memorandum drafted by FTCKY DPM and a mumps

fact sheet sent home with the students on Day 4 of the investigation. Of the child contacts, 55 (89%) had received at least one dose of MMR vaccine, and 47 (76%) had evidence of 2 doses of MMR vaccine available for review. Seven child contacts were initially identified as having no evidence of presumptive immunity to mumps, and were recommended for exclusion from school for postexposure days 12 through 25. When their parents were presented with this recommendation, evidence of at least one prior MMR vaccination was provided to the APHNs for an additional 5 children. This brought the total with at least one prior MMR vaccination to 60 of 62 (97%) children. Two children were excluded from school at the recommendation of FTCKY DPM for postexposure days 12 through 25. Follow-up mumps symptom interviews were conducted by APHNs on postexposure days 15, 20, and 26. No secondary mumps cases were identified.

Of the 14 educator contacts, only 3 (21%) had presumptive evidence of immunity immediately available for review. Upon notification from their employer, the Department of Defense Educational Activity (DoDEA), directed that they would be excluded from work and placed on sick leave for postexposure days 12 through 25 unless they were able to provide evidence of presumptive immunity to mumps, an additional 7 educators provided sufficient documentation of presumptive immunity to remain working in the school. Four educators (28% of total educator contacts) were excluded from work as they were unable to provide any evidence of presumptive immunity to mumps. As with the other contacts, follow-up mumps symptom interviews were conducted by APHNs on postexposure days 15, 20, and 26. No secondary mumps cases were identified.

We were fortunate that no secondary cases developed during this contact investigation. Heightened awareness and surveillance for additional cases continued until 50 days after the initial case presentations (two 25-day incubation periods) with no additional cases being identified.

#### COMMENT

A number of lessons can be learned from this contact investigation for other Army public health professionals to consider in future communicable disease contact investigations on their installation. First, a strong relationship with local civilian public health officials (at least at the local and regional level, and as necessary, at the state level) is absolutely key to success. Fort Campbell has the unique situation of lying astride the state border between Tennessee and Kentucky. This requires working with public health professionals in both states,

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and maintaining relationships with these professionals through regular contact and interaction. For example, just one month prior to this event, FTCKY DPM had hosted a joint public health emergency preparedness tabletop exercise, which was attended by local public health professionals from several nearby counties in both Tennessee and Kentucky. Fortuitously, nursing staff from Fort Campbell DoDEA schools and Fort Campbell Child and Youth Services also attended. The relationships formed as a result of this event and prior meetings were critical in ensuring that confirmatory testing was readily available, and that local public health professionals were prepared to react had the contact investigation extended to their jurisdictions.

Engaging public affairs professionals early in the process helped to ensure that timely and correct information about the investigation was disseminated to the public. The Fort Campbell MEDDAC Public Affairs office was very effective in translating information about mumps and the investigation into a timely press release to local media that was transmitted through social media almost simultaneously with the letters sent by the school to parents notifying them of potential exposure to mumps. Of note, a press release posted on the Fort Campbell MEDDAC public Facebook page received over 350 shares and nearly 100 comments within the first 18 hours. A press release to a local newspaper was posted on the newspaper's Facebook page and received a similar volume of shares and comments within the first 18 hours. Comments were generally supportive of efforts to prevent additional disease spread. This effort to ensure that accurate information was provided to the Fort Campbell community in an expedient manner likely helped to prevent the development of rumors and inaccuracies that can occur when information flow is less than timely.

A separate notification message was sent to all Fort Campbell clinical providers and nurses through an email distribution list shortly following the press release. This message provided additional, clinically-relevant information about the index cases, and when and where potential exposures would have occurred. It also included a mumps fact sheet, and specific information about incubation periods and when secondary cases could potentially be seen.<sup>4</sup> This information helped clinicians and nurses to appropriately respond to calls from patients and concerned parents who had read the press release and were concerned that they had been exposed and/or that they were currently experiencing mumps symptoms. This was particularly important as the public response, via social media and the MEDDAC call center, generally occurred within the first 48 hours after the press release, almost a week prior to when secondary

cases would have been expected to appear based on incubation period. The FTCKY DPM leadership also met with MEDDAC department chiefs to review signs and symptoms of mumps, and to ensure that incubation periods and exposure locations were understood.

Some areas for improvement were identified as well. As noted previously, the Army does not routinely check mumps titers on Soldiers at initial entry training. As such, presumptive immunity could not immediately be established for most Soldiers. However, some practitioners were unfamiliar with this discrepancy and initially declared presumptive immunity based on an “up to date” MEDPROS immunization report. *Army Regulation 40-562* states “Immunity against mumps is not necessary as a military requirement, but may be appropriate in exceptional clinical circumstances such as outbreaks.”<sup>10(p15)</sup> To this point, the investigation also reiterated that installation occupational health clinics should not use MEDPROS as a reliable means of establishing presumptive immunity for active duty HCWs. The MEDPROS is a system of record for maintaining Soldier readiness, and does not delineate HCWs as having different vaccine recommendations. Clear documentation of a completed MMR vaccine 2-dose series or positive serology is necessary for HCWs in accordance with *AR 40-562*.<sup>10(p15)</sup> This standard meets and exceeds recommendations for HCW vaccination created by the CDC.

In the future, better relationships should be established with local DoDEA leadership. Army Public Health Nurses work frequently with installation Child and Youth Services to ensure a safe and healthy child care environment; however, relationships with DoDEA schools may not be as strong. At Fort Campbell, DoDEA educators did not visit installation occupational health for pre-employment examination, and did not meet with installation occupational health as part of their initial arrival process. As such, Fort Campbell occupational health had no immunization records for these employees, nor did Fort Campbell DoDEA nurses. As on-post educators should be appropriately vaccinated for their work, a better solution may be for these employees to in-process installation occupational health for an immunization review as part of their onboarding process. *Army Regulation 40-562*<sup>10</sup> is the DoD standard for immunization practices with applicability to all DoD uniformed personnel and select civilian employee, as well as the US Coast Guard. Although *AR 40-562* indicates that “as a condition of employment,” DoD schoolteachers will be administered Advisory Committee on Immunization Practices recommended adult immunizations unless already immune,<sup>10(p12)</sup> we found that DoDEA may not routinely enforce this requirement within the contiguous

United States. Efforts to introduce and familiarize installation public health and occupational health assets to local DoDEA officials before an event such as this occurs may allow for greater cooperation in the future and prevent the delay in establishing evidence of presumptive immunity that was noted during this investigation.

#### CONCLUSION

Mumps may not be considered a disease of military importance; however, Soldiers may travel of their own accord or be deployed to locations where mumps outbreaks are occurring. Understanding that the MMR vaccine does not provide 100% immunity against mumps, Soldiers have the potential to develop acute mumps, and may be at greater risk of its complications as adults. Military public health professionals should prepare themselves to respond to mumps cases to prevent the spread of disease, and should be sure to include colleagues in local civilian public health in their planning. This investigation clearly illustrates that the magnitude of a contact investigation can quickly increase, particularly when Soldiers are living in close quarters, and when consolidated child care settings are involved. Thus, continuous preparation for the possibility of these events through public health emergency response exercises is essential so that even if additional cases appear and investigations expand, military public health professionals will have an organized framework for response, and can depend on previously developed collegial relationships to assist in resolving the problem.

#### ACKNOWLEDGEMENTS

The author acknowledges the efforts of Army Public Health Nurses MAJ Simone Edwards, CPT Courtney Buchwald, and Ms Jennifer Williams who were essential in conducting and completing this investigation.

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# Developing Medical Surveillance Examination Guidance for New Occupational Hazards: The IMX-101 Experience

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## ABSTRACT

New materials are constantly being created to address the operational needs of the US Army. These materials provide challenges to occupational health practitioners by presenting unknown health risks and possible effects to workers they evaluate. The responsibility for developing a medical surveillance exam, as part of a comprehensive workplace surveillance program, may become the responsibility of the provider working in a clinic on a military installation where manufacturing, testing, and/or use of the material is being conducted. Insensitive munitions explosive (IMX) has presented such an opportunity for Army occupational medicine providers within the last few years. This article describes the course of action taken by the occupational health clinics, personnel of Army ammunition plants producing IMX-101, and the US Army Public Health Center-Army Institute of Public Health to address the situation of developing a medical surveillance examination during a time when little to no information existed about the components of this new explosive compound.

## DEVELOPING MEDICAL SURVEILLANCE FOR NEW OCCUPATIONAL HAZARDS

When new substances are introduced to the workplace, the potential health risks to exposed workers must be known. If a gap in knowledge exists, an investigation of these health risks must be completed. If the hazard cannot be fully managed using engineering, administrative, and/or personal protective controls, a medical surveillance program must be developed and implemented. This publication will focus on the medical surveillance aspect of an overall occupational surveillance program when new substances are introduced to the workplace.

To begin developing a medical surveillance program, occupational health providers must first conduct a thorough literature search for studies and information related to the substance in question. Toxicologic data, safety data sheets, clinical outcome studies, research and development data, peer-reviewed journal studies or articles, and even popular medicine articles are good places to start gathering information. Included in this step is soliciting help from experts with knowledge of the substance, obtaining information on how the substance will be used (eg, manufacturing and handling), and gathering knowledge of how humans have the potential for developing morbidity related to the substance in question. This step helps to identify what is known and what is not known about the new substance. It also identifies gaps in knowledge that can be filled by requesting new toxicology studies that enlighten occupational health providers about the potential human health effects.

The second step is to solicit assistance from the toxicology and scientific communities. Requesting studies on the new substance that help to determine potential health effects to humans is essential. The focus of these studies should be in areas where there is little knowledge that address the most likely route(s) of exposure, and that explore the health effects of most concern. A medical surveillance program that uses this information will be able to successfully monitor the health of potentially exposed workers, especially if it employs biomarker testing as part of the exam protocol. Examples of Army resources that may be helpful at this step include the US Army Public Health Center Toxicology Directorate, the Army Research Laboratory, and Edgewood Chemical Biological Center.

The third step is to consult with industrial hygiene (IH) and safety personnel at the installation to determine the type of controls that are needed to protect workers who may be exposed to the new substance. If engineering and/or administrative controls are not enough to protect workers from exposure, a recommendation for personal protective equipment (PPE) should be given by IH and implemented by the employer. An exposure monitoring plan should also be developed among industrial hygiene, safety, and medical personnel to address the needs for quantitative data to assess actual worker exposure, validate the selection and use of appropriate PPE (eg, respirators), and compare toxicological information with medical observations and expectations.

Occupational exposure limits should be identified from federal and consensus organizations including, but not limited to: American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values, Occupational Safety & Health Administration (OSHA) Permissible Exposure Limits, Occupational Alliance for Risk Science (OARS) Workplace Environmental Exposure Limits (WEELs), and National Institute for Occupational Safety and Health Recommended Exposure Limits. Many chemical substance exposure values are published but many have yet to be developed. Army occupational health professionals typically follow the most stringent of the OSHA or ACGIH surveillance values.

If IH or safety personnel have initially determined that engineering controls are not enough to reduce the exposure (thus requiring that workers wear PPE), it is recommended that they, along with the employer, work to improve existing engineering controls or to develop a new type of engineering control to reduce or eliminate exposure. The ultimate goal is the reduction or elimination of PPE, if feasible.

Once all preliminary steps are implemented and it is determined that medical surveillance is still required, the last step is to attempt to develop a biological exposure index (BEI) for the substance in question. The reference for this process can be found on the ACGIH website ([www.acgih.org](http://www.acgih.org)). A BEI helps to focus the medical surveillance program towards the health issues that would most concern an occupational health provider. These biologic tests need to have as high a level of sensitivity and specificity as possible. A proper medical surveillance program should be established for workers who have a potential exposure, including those over the exposure limit regardless of PPE use, maintenance workers who may have short duration but very high exposures, and as required to document effectiveness of engineering and PPE control practices.

All of these steps should provide enough information for the medical authority to develop a medical surveillance exam that includes the following:

- ▶ appropriate questions for the subjective portion of the exam,
- ▶ appropriate physical exam focus and lab/diagnostic testing strategy for the objective portion of the exam, and
- ▶ assessment and plan parameters that include frequency of evaluation (eg, annual, biannual, every 5 years), what to do when abnormal results are found, and criteria for limited duty, medical removal, etc.

### CASE STUDY: THE EVOLUTION OF THE IMX-101 MEDICAL SURVEILLANCE EXAMINATION

Insensitive munitions are “munitions which reliably fulfill their performance, readiness and operational requirements on demand, and which minimize the probability of inadvertent initiation and severity of subsequent collateral damage to weapon platforms, logistic systems, and personnel when subjected to unplanned stimuli.”<sup>1</sup> The Department of Defense (DoD) recently launched an initiative to improve the safety of its munitions. IMX-101 (BAE Systems, Inc, Arlington, VA) is one formulation approved by the US Army for use in several weapons systems, including the M795 155 mm artillery projectile.<sup>2</sup>

The components of IMX-101 are 3-nitro-1,2,4-triazol-5-one (NTO), 2,4-dinitroanisole (DNAN), and nitroguanidine (NQ). Army ammunition plants began producing IMX-101 in 2011. The Army also started medical and work environment surveillance at these plants the same year. At that time, there were limited toxicological studies using animal and in vitro models that described the health effects of each individual component. These studies gave insight into potential effects in humans but did not fully explain all possible human health outcomes. Because this was a novel compound, previous medical surveillance data was nonexistent. What was known, however, was that certain components were chemically very similar to other explosive compounds (for instance DNAN and 2,4,6-trinitrotoluene (TNT)). It was reasonably assumed that similar compounds would have similar health effects to humans (which later would be confirmed by toxicological studies and literature review). When deciding how to proceed with performing medical surveillance examinations, the occupational health providers at the plants producing IMX-101 were placed in a unique situation.

### THE FIRST MEDICAL SURVEILLANCE EXAMINATION GUIDELINES FOR IMX-101

Concerns about the health effects and toxicity of IMX-101 components were voiced by Army ammunition plant workers and occupational health personnel at the same time the Army obtained certification to produce the rounds. Although toxicity clearances on all substances had been performed as a part of this process, medical surveillance examinations (MSE) of workers and industrial hygiene survey methods were not well defined after the acquisitions process.

When the first MSE guidelines were developed and implemented at active ammunition plants in 2011, there were few studies or toxicologic data (other than the

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toxicity clearance data) that answered questions about the human health effects of IMX-101 or its components. The approach in this early stage was to monitor multiple organ systems that were suspected to be targets of this new compound. The Joint Munitions Command asked the US Army Public Health Center-Army Institute of Public Health (USAPHC-AIPH) Toxicology Portfolio to conduct additional studies and toxicologic assessments of all IMX-101 compounds to further define the health hazards and help refine the MSE guidelines. Based on findings from these studies, the original set of MSE guidelines appeared to exceed what was clinically necessary. Monk and Mirza outline and discuss the original MSE guidelines in a 2014 USAPHC-AIPH white paper.<sup>3</sup>

### REVIEW OF EARLY MEDICAL SURVEILLANCE DATA

Production of IMX-101 began within months of qualification at Army ammunition plants. As a part of the contract between the manufacturer of IMX-101 and the Holston Army Ammunition Plant, the USAPHC-AIPH received the first set of medical surveillance records on workers for analysis in June 2014. The surveillance time period evaluated was from April 2013 through December 2013. Baseline surveillance exams were completed on workers between April and July 2013, and the first follow-up exam was completed in September 2013. Two additional monthly exams were completed, the first in October, and another in the November-to-December timeframe.

A multispecialty team from the USAPHC-AIPH, including occupational medicine physicians, physician assistants, nurses, and industrial hygienists, analyzed the MSE data. The group was looking for any evidence that the original MSE protocol was able to detect an occupationally-acquired adverse health outcome. Parameters including the workers' exposure length and magnitude based on industrial hygiene workplace surveillance data were also used. This task proved difficult because of its multifaceted nature.

At the conclusion of the evaluation, the USAPHC-AIPH determined that the primary focus should be on developing and implementing engineering controls that control exposures to IMX-101 and its components. Industrial hygiene workplace surveillance data during this time period showed workers were being exposed to NTO over the USAPHC-AIPH occupational exposure limit (OEL) at the time (OEL: 8-hr TWA 0.09 mg/m<sup>3</sup>); workers were also exposed, although less frequently, to DNAN (OEL: 8-hr TWA 1.6 mg/m<sup>3</sup>). There were no exam, lab, or diagnostic test data collected during this time period, suggesting there were health effects to workers in the areas of primary concern related to the known or suspected

toxicological effects of all IMX-101 components (hematologic/hepatic/metabolic for DNAN, male (rodent) reproductive for NTO). Abnormal lab findings discovered during this review were not significantly different from findings found in similar conditions and compared to the general US population. The original PPE recommended (Tyvek or cotton ammunition plant coveralls with anti-static treatment, nitrile gloves, full-face respirators with organic material fillers (for certain operations), and antistatic, grounding footwear) appeared to be protecting workers. The panel recommended continued efforts to find reliable biomarkers for use during MSEs and more studies to determine long-term, occupationally proportional exposure effects to human health. The USAPHC-AIPH panel's final determination was that the MSE protocol that was originally implemented likely exceeded what was clinically important to monitor workers appropriately and that the guidelines should be updated.

During this review, efforts had already begun to retrofit existing plants and rebuild/overhaul plants with improved engineering controls to manage potential exposures. Production rates (quantity) of IMX compound and IMX-containing munitions vary based on demand. Engineering controls for existing, new, and retrofit operations include local exhaust (eg, point source) and canopy hood ventilation systems.

Work continues today on developing monitoring methods for the IMX-101 mixture and its individual compounds. As the occupational health community develops their understanding regarding the routes of entry into a worker's body, the techniques that industrial hygienists use will be refined to reflect the environmental situation. Currently, air sampling is done at the plants to evaluate the potential for inhalation exposure. Wipe sampling has been conducted to evaluate skin exposure to IMX, DNAN, and NTO. Other sampling methods not currently employed may be available in the future.

The PPE for workers exposed to IMX-101 that is currently used at most plants includes vapor and liquid impervious/barrier-type coveralls, full-face respirator masks with air purifying filter cartridges, and nitrile gloves. Some plants still use cotton coveralls and gloves which do not offer as much direct protection from dermal exposure. To date, USAPHC-AIPH is not tracking any cases of adverse health effects believed to be directly caused by IMX-101 or its components in workers who are using any form of PPE.

Although we are lacking studies that can produce BEIs for DNAN, NTO, NQ, or IMX-101, we do have toxicology information, OELs for DNAN and NTO developed

by USAPHC-AIPH, WEELs for DNAN, NTO, and NQ developed by OARS, and past surveillance practices with similar compounds (eg, TNT) that can be used to monitor the health of workers until further research is conducted.

#### UPDATED IMX-101 TOXICOLOGY DATA

The most up-to-date resource for the toxicologic assessment of IMX-101 and its components was published by the USAPHC-AIPH in December 2014.<sup>4</sup> The report summarizes the data from multiple studies completed by the USAPHC Toxicology Portfolio. It also mentions findings from other, nongovernment investigators.

According to the report, DNAN is the main component in the IMX-101 mixture. It has similar health effects to that of TNT; the primary target for both is the hematopoietic system. The secondary targets of DNAN include the hepatic system as well as disruption to the metabolic process caused by the metabolite dinitrophenol (DNP).<sup>4</sup>

NTO primarily targets the reproductive system (at high oral doses, specifically males in rat studies); however, adverse health consequences to humans are not expected given current exposure levels. NTO was demonstrated to affect spermatogenic tissues. Tests investigating the endocrine disruptive potential of NTO were negative, suggesting NTO acts directly on sperm production.<sup>4</sup>

NQ does not appear to have significant health effects. However, one study using mice did associate NQ with a possible diuretic effect. There are also concerns regarding aquatic breakdown products of NQ, and further study is recommended.<sup>4</sup>

Oral and dermal toxicity data are currently the only data available for the IMX-101 mixture. In the subacute oral exposure studies (14-day, repeated oral dose), the mixture displayed the characteristics of the individual components. The hematologic effects (eg, splenomegaly) observed were “likely due to the DNAN component of the IMX-101 mixture; the adverse events occur[ing] at the concentrations similar to those of DNAN alone.”<sup>4(p29)</sup> NTO was noted as the cause for adverse testicular effects (decreased testis and epididymis size, decreased sperm count in rat studies). However, due to the presence of DNAN and NQ in the IMX-101 mixture, the testicular effects of NTO were decreased 10-fold when compared to NTO alone. The *in vitro*, frozen human cadaver, dermal penetration study showed an increased dermal absorption with NTO (0.4 times greater) and NQ (7 times greater) as the IMX-101 mixture as opposed to NTO and NQ alone. This suggests potential enhanced toxicity, possibly due to synergistic, toxicokinetic mixture

effects. *In vivo* effects of dermal exposure to IMX-101 have not been studied to date.<sup>4</sup>

#### PROPOSED REVISIONS TO THE FIRST MEDICAL SURVEILLANCE GUIDELINES

As noted earlier, the medical surveillance approach originally used at facilities that produce IMX-101 exceeded what would generally be considered as an appropriate balance between cumulative sensitivity and cumulative specificity. That is, the current regimen carries an excessive risk of detecting false positive health effects that would be presumed to be an outcome from IMX-101 exposure. Like false negatives, false positives may be harmful to individuals so an artful balance and minimization of both is desirable.

Based on the new toxicology knowledge described above and in response to calls from stakeholders to modify the surveillance, the USAPHC-AIPH published a revised guideline for IMX-101 medical surveillance.<sup>3</sup> While recognizing the still-present knowledge gaps regarding the potential human health effects of IMX-101 on exposed workers, the new guidelines attempt to reconcile laboratory and diagnostic tests that have no applicable biological monitoring capability with ones that have evidence-based studies to support their use.

The revised surveillance guideline focuses on the potential for hematologic and liver effects of DNAN and the metabolic effects of the DNAN metabolite DNP, the potential reproductive effects of NTO, and monitoring the renal system for potential NQ effects.

The differences between the elements of the original IMX-101 medical surveillance guidelines and the proposed revisions to these guidelines are shown in the Table.

#### EXPLAINING THE CHANGES TO IMX-101 MEDICAL SURVEILLANCE

As mentioned earlier, DNAN and TNT have similar effects on human health. Monks and Mirza<sup>3</sup> suggest use of an approach similar to TNT surveillance by monitoring a complete blood count (CBC) primarily focusing on red blood cells (RBCs), glucose-6-phosphate dehydrogenase deficiency status, and liver function tests (LFTs) as advised in Technical Bulletin (Medical) 297.<sup>5</sup> Due to the possible metabolic effects of DNP, a complete metabolic panel was advised. Finally, NQ displayed the potential to affect the renal system in toxicology studies; therefore, a dipstick urinalysis was recommended.

Frequency of testing was another consideration when designing the surveillance guidelines. Hematologic

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metrics require the most frequent evaluation. The original guidelines recommended monthly blood tests. When evaluating CBC results, however, it would be unlikely to see rapid changes in the RBCs (especially the hemoglobin (Hgb) and red cell indices) that are clinically significant and specific enough for DNAN or IMX-101 exposure. A 90-day follow-up after the baseline examination is more in line with the normal RBC lifecycle and early enough to detect a significant drop in Hgb, a change in LFTs related to chemical exposure, or subclinical signs of health changes before a worker becomes symptomatic.

If a worker presents symptomatically sooner than 90 days in the initial 90 days of employment and/or exposure to IMX-101 components, the evaluation should proceed as if it were the 90-day evaluation. Subsequently, semiannual exams should be conducted to include the 90-day exam elements with the addition of regular haptoglobin (Hp) and a reticulocyte count (RC). Optional serum lactate dehydrogenase (LDH) or peripheral blood smear evaluation further strengthens the surveillance regimen of tests to monitor any potential hematological changes with as

much specificity, and in the most likely timeframe of occurrence, for hemolytic anemia as possible. The purpose of watching for a decrease in the Hgb levels (as defined) and Hp levels below the laboratory reference range during the semiannual exam is to detect an exposed worker with the *potential* for hemolytic anemia. The addition of a serum LDH level to the Hp level has a 90% specificity for hemolysis (a normal value for both of these tests has a 92% sensitivity for ruling out hemolysis).<sup>6,7</sup> If hemolysis is confirmed, coupled with findings for anemia on the CBC, an increase in the RC, and abnormalities on the peripheral blood smear (eg, schistocytes, bite cells, spur cells), the anemia is likely of the hemolytic type. The worker's history and physical exam findings, along with the occupational health provider's clinical acumen, are what will then determine if an IMX exposure is the real cause of the described exam abnormalities. In the case of an accidental, gross exposure to IMX-101 components, the postaccident exam should follow the elements of a semiannual exam, with follow-up examinations as determined by the occupational medicine provider (likely every 90 days for 2 or more consecutive cycles).

Comparison of the IMX-101 Medical Surveillance Guidelines. <sup>3</sup>	
Original IMX-101 Medical Surveillance Laboratory and Diagnostic Testing Guidelines (used during April-December 2013 data review)	Proposed Revisions to IMX-101 Medical Surveillance Laboratory and Diagnostic Testing Guidelines
<b>ALL Examinations</b>	<b>Baseline Examination</b>
CBC with manual differential	History and physical (with modified questionnaires)
Comprehensive metabolic panel	CBC with manual differential
Lipid panel	Comprehensive metabolic panel (including AST, ALT, and ALP)
Thyroid panel – thyroid stimulating hormone (TSH), free T4, T3	Dipstick urinalysis
Thyroid peroxidase antibody (TPO antibody)	Gamma-glutamyltransferase (GGT)
Thyroglobulin antibody (TG antibody)	Glucose-6-phosphate dehydrogenase (G6PD)
Follicle stimulating hormone (FSH)	<b>90-day Examination</b>
Luteinizing hormone (LH)	Same as Baseline exam but <b>WITHOUT</b> G6PD
Testosterone (total and free)	<b>Semiannual Examination</b>
Prolactin	Same as 90-day exam with the addition of:
Sex hormone binding globulin (SHBG)	Haptoglobin (Hp)
Men – sperm count (offered but none collected)	Reticulocyte count (RC)
Women – If of childbearing age (if desired): estradiol, 3-day anti-Mullerian hormone (AMH)	Optional Tests
<b>Baseline Examination ONLY</b>	Lactate dehydrogenase (LDH)
History and physical	Peripheral blood smear
Aspartate aminotransferase (AST)	Other tests at provider's discretion as needed
Alanine aminotransferase (ALT)	<b>Termination Examination</b>
Alkaline phosphatase (ALP)	Same as Semiannual exam
Electrocardiogram (EKG)	<b>Additional Surveillance Examination Elements</b>
Chest X-ray	Pulmonary function test (spirometry)
Low testosterone questionnaire (men)	
Obstetrics / gynecological history (women)	
Thyroid questionnaire	
<b>Additional Surveillance Examination Elements</b>	
Pulmonary function test (spirometry; baseline results only)	

Addressing the suspected reproductive system effects of NTO, the USAPHC-AIPH conducted a literature review of infertility and abnormal endocrine findings related to reproduction in the general US population. According to a recent CDC study discussing infertility rates among the general US population, 12% of women and 9% of men aged from 15 to 44 years have sought medical help to have a baby (conceive or prevent miscarriage).<sup>8</sup> Testing for changes in both male and female sexual hormones to date has not shown any adverse effects to exposed workers. Optional sperm count testing was offered to male workers as part of the original surveillance protocol but no samples were collected. It would be difficult for occupational health providers to distinguish IMX-related infertility when compared to the “background infertility” of the general US population. Because there is no long-term safety data regarding any of the IMX-101 compounds and to monitor the unlikely but still possible effects of NTO on reproductive health, Monks and Mirza<sup>3</sup> developed a model questionnaire that could be used to evaluate workers’ reproductive health.

The potential for a rapidly changing plant operations schedule affecting the timing of surveillance exams and changes in exposure profiles in the newly proposed guidance is an issue that poses a challenge. For example, a worker (or segment of workers) in a plant may produce IMX-101 for only 3 months. Once this production cycle is over, they may work in an area that has a different hazard exposure profile. Constantly altering the medical surveillance (or even the need for surveillance) can be difficult from the occupational health perspective. This issue has been identified and will require further investigation.

While it is true that the above testing regime has the potential to detect non-IMX-101 related medical issues, it is believed that this method is more focused on the important and most likely IMX-related health effects. There is also enough confirmatory testing built into the scheme to detect these issues with fair certainty. The goal is to detect IMX-related adverse health issues in the absence of BEIs. If the evaluation focuses on the known (or most likely) health effects without interference of non-evidence-based testing, then the surveillance is achieving its goal until a better method is developed.

One final item that is important to mention was the effort that was placed into producing a clearly written health risk management message explaining why the USAPHC-AIPH was recommending changes to the medical surveillance. It is essential to explain to occupational health clinic providers and workers why their testing regimen would be changing in a way that they can understand and accept the reasoning behind the decision.

#### SUMMARY

This IMX case study represents the difficult situation of retroactively trying to develop a medical surveillance program to address potential exposure to new compounds. Attempting to accomplish all of the necessary steps as discussed in this article to build an evidence-based approach for medical surveillance is made more challenging after production has started and workers are potentially exposed. This approach has potential health risk implications for the workers involved. It appears that no adverse effects related to IMX-101 or its individual compounds have occurred to date. It is possible that we are currently monitoring workers with the most sensitive and specific biologic tests available for IMX-101, but lack the studies to prove that this is the case.

We are in a better position today in defining the health risks of IMX-101 and refining the methods to protect workers than we were a few years ago. There are still knowledge gaps that must be explored before it can be said that there is a “best-case scenario.” That scenario includes industrial hygiene monitoring that is technically reliable and accurately defines the exposure levels, engineering controls that reduce exposures as much as possible, the use of PPE (as appropriate) based on the remaining exposure risk, and an evidence-based medical surveillance program that evaluates and detects the biologic effect on humans with specificity and sensitivity. The Army and all stakeholders remain dedicated to improving their portion of this program and ultimately will focus on what is best to protect the worker.

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#### ACKNOWLEDGEMENTS

I thank Dr Raul Mirza, Dr Pearline McKenzie-Garner, Ms Renita Shoffner, Dr Mark Johnson and his team in the Toxicology Directorate at the APHC, Ms Lindsey Kneten, LTC Joseph Llanos, COL William Rice, and Dr Pete Matos for their contributions to this article.

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This book “demonstrates the commitment of the Army and the Nation to its soldiers. It was a decade in the making, following several special studies, the involvement of external subject matter experts, the development of strong interagency cooperation between the Department of Defense of Veterans Affairs, and the direct input of academic centers. Inside, the reader will find medical doctors and scientists reaching conclusions that are at odds with each other. This book puts these contradicting learned opinions under one cover, for it is important that readers absorb these writings and come to their own conclusions. Science itself exists on the border between what is known and what is not known, so we are constantly rewriting our medical library of knowledge, while recognizing there is no permanent certainty in science’s conclusions. In spite of these difficulties, we are all seeking to understand and to intervene on the side of our soldier patients.”

This book and many others available at:   
[www.cs.amedd.army.mil/borden](http://www.cs.amedd.army.mil/borden)

# Airborne Hazards Related to Deployment



# Missed Opportunities in Human Papillomavirus Vaccination Uptake Among US Air Force Recruits, 2009-2015

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## BACKGROUND

Globally, approximately 2 million new incident cancer cases are caused by infectious diseases every year, and 600,000 of these cases are caused by the Human Papillomavirus (HPV).<sup>1</sup> Interestingly, HPV is also the most common sexually transmitted infection (STI) in the United States with an estimated 14 million new genital HPV infections yearly, half of which are diagnosed among individuals 15 to 24 years of age.<sup>2</sup> From 2003 to 2006, HPV prevalence was as high as 42.5% among females aged 14 to 59 years,<sup>3</sup> the majority of whom (53.8%) were aged 20 to 24 years.<sup>4</sup> Further, studies have demonstrated that HPV seroprevalence is actually low (1% to 8%) in late adolescence but increases with age, reaching up to 15% to 35% by age 40 years.<sup>5</sup> However, HPV acquisition may be accelerated in highly sexually active populations, such as military personnel.<sup>6,7</sup> For example, HPV seroprevalence was reported to be as high as 45% to 51% among active duty (AD) women.<sup>7</sup> Another study found that incident infections of HPV were common with more than one-third of male AD service members seroconverting to one or more of the HPV serotypes.<sup>5</sup> In fact, HPV is also the most common STI among the US military,<sup>8</sup> although HPV vaccination coverage remains consistently low.<sup>9</sup> During the years 2000 to 2012, there were an estimated 304,021 incident cases of HPV (17.5 per 100,000 person-years) among AD service members.<sup>10</sup> To put these data in perspective, the number of healthcare visits due to HPV alone was greater than gonorrhea and chlamydia combined.<sup>5</sup> More recently, a study among female AD service members revealed only 22.5% vaccination uptake of one dose or more.<sup>9</sup>

Although most HPV infections are self-limited and asymptomatic,<sup>11</sup> persistent infection with high-risk HPV types can cause cancers of the cervix, vagina, vulva,

penis, anus, rectum, and oropharynx.<sup>12-14</sup> Specifically, HPV 16 and HPV 18 are high-risk types that are more likely to persist and progress to cancer.<sup>15,16</sup> Whereas, low-risk HPV types 6 and 11 typically cause anogenital warts and respiratory papillomatosis.<sup>17</sup> Thus, cancer prevention and treatment requires public health interventions and early detection.

One primary prevention tool is immunization. Vaccines have been one of the greatest success stories in public health. The Food and Drug Administration has approved 2 cancer preventive vaccines, one for Hepatitis B virus<sup>18</sup> that is a leading cause of liver cancer, the other for HPV. In 2006, a 3-dose HPV quadrivalent vaccine (4vHPV) (Gardasil; Merck & Co, Inc, Whitehouse Station, NJ) was licensed in the United States, protecting against several HPV strains (types 6, 11, 16, 18) that are responsible for about 70% of cervical cancers and 80% of genital warts.<sup>9</sup> The Advisory Committee on Immunization Practices (ACIP) and the US Centers for Disease Control and Prevention (CDC) recommended its use in females aged 11-12 years, and catch-up vaccination for females aged 13 to 26 years who have not been previously vaccinated.<sup>19</sup> A bivalent HPV vaccine (2vHPV) (Cervarix; GlaxoSmithKline plc, Brentford, London, UK) was licensed in 2009.<sup>20</sup> The ACIP also expanded their recommendation in 2009 to include vaccination with the 4vHPV for males between ages 9 and 26 years.<sup>21</sup> In 2014, a 9-valent HPV vaccine (9vHPV) (Gardasil 9; Merck & Co, Inc) became available.<sup>22</sup> The ACIP recommended that any of these 3 vaccines be used for routine vaccinations of females, while only 2 vaccines (4vHPV and 9vHPV) were indicated for males.<sup>23</sup> In 2016, ACIP updated their recommendations regarding dosing schedules and administration of the 9vHPV vaccine.<sup>24,25</sup> The notable change from prior ACIP reports<sup>22,23</sup> was the reduction to a 2-dose schedule for males and females who

The material contained in this study was reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The investigators have adhered to the policies for protection of human subjects as prescribed in Army Regulation 70-25: Use of Volunteers as Subjects of Research, 1990.

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start the series at ages 9 to 14 years. For young adults between the ages of 15 to 26 years, the dosing schedule remained unchanged at 3 doses.<sup>24,25</sup>

The 5 additional types included in 9vHPV vaccine contained oncogenic types 31, 33, 45, 52, and 58 which added primary protection against cervical cancer and cervical pre-cancers, including cervical intraepithelial neoplasia grade 2 or 3 and adenocarcinoma in situ.<sup>23,26</sup> In late 2016, when the 9vHPV vaccine was widely distributed in the United States,<sup>24</sup> the CDC estimated an average of 30,700 HPV-related cancers diagnosed yearly, of which approximately 92% were attributed to these specific HPV types.<sup>15,27</sup> Among females, this translates to prevention of approximately 81% of cervical, 73% of vaginal, and 63% of vulvar cancers.<sup>15</sup> Among males, 57% of penile cancer could be prevented. Among males and females combined, an estimated 88% of anal and 66% of oropharyngeal cancers would be preventable.<sup>15</sup> Overall, an estimated total of 28,500 cancers would be prevented per year with full vaccine coverage with the 9vHPV vaccine and 100% vaccine efficacy.<sup>27,28</sup> Additionally, close to 90% of all anogenital warts would be prevented along with most cases of recurrent respiratory papillomatosis.<sup>17</sup> Even though these vaccines are available, one study revealed an increased incidence of HPV-associated cancers occurring in 11.7 per 100,000 population from 2008 to 2012, compared with the rate of 10.8 per 100,000 population from 2004 to 2009.<sup>27,29</sup>

Although HPV vaccination uptake has increased in both females and males since a licensed HPV vaccine was first made available for each sex,<sup>22</sup> low coverage among adolescent populations continues to exist.<sup>30</sup> During the period 2014-2015, the 1 dose or more coverage was only 62.8% for females and 49.8% for males among the age group 13 to 17 years. Comparatively, the 1 dose or more coverage (56.1%) for females and males combined was less than the coverage for the 1 dose or more (81.3%) of meningococcal 4-valent conjugate vaccine and the coverage for the 1 dose or more (86.4%) of tetanus-diphtheria-acellular pertussis vaccine. This disparity underscores the extent of the HPV vaccination uptake deficiencies among these younger age groups.<sup>30</sup> Thus, catch-up vaccination recommendations herald an opportunity for increased awareness and public health efforts to increase HPV immunization uptake rates.

Several factors support the ACIP recommendation to vaccinate males and females in the catch-up age range. These include studies showing high HPV vaccine efficacy in sexually active women aged 15 to 26 years with prior HPV exposure,<sup>31</sup> reports on the prevalence

of HPV infection in US women,<sup>3</sup> lack of exposure to high-risk HPV types included in the 9vHPV vaccine,<sup>32</sup> and cost-effectiveness analyses on catch-up vaccination within this older population.<sup>33,34</sup> Less is known, however, about HPV vaccine uptake in the catch-up group of older teens and young adults as the National Health Interview Survey only began collecting data in 2013 on age at first HPV dose among female and males aged 15 to 26 years.<sup>35</sup> In one study, HPV vaccination uptake among adults aged 19 to 26 years who reported ever receiving one dose or more of an HPV vaccine was quite low. Among women aged 19 to 26 years, approximately 12% reported receiving their first ever dose of HPV vaccine as catch-up sometime between ages 19 to 26 years, compared to only 2% of men within similar age groups.<sup>36</sup>

Following the ACIP recommendations,<sup>22</sup> the Department of Defense (DoD) made the 9vHPV vaccine available to service members aged 17 to 26 years.<sup>37,38</sup> In 2013, the President's Cancer Panel developed a strategy to accelerate vaccine coverage.<sup>1</sup> As part of this national effort, the DoD created a set of instructions<sup>39</sup> in order to promote these goals. Additionally, the US Department of Health and Human Service's *Healthy People 2020* goals were updated to reach higher HPV vaccination coverage in specific age groups.<sup>40</sup> More recently, the National Cancer Institute issued a joint statement to focus on the low coverage rates of HPV vaccinations and the *missed opportunities* to prevent vaccine preventable cancer. Placing HPV vaccination as a global health priority, the US National Cancer Program has focused on a vaccination campaign against avoidable cancers. The campaign addresses one of the primary and most effective goals to combat preventable cancer: reduce missed clinical opportunities to recommend and administer HPV vaccines.<sup>41</sup>

Despite these efforts, low vaccination uptake rates persist for both the adolescent and catch-up age groups of older teens and young adults. The main goal of this study was to characterize the missed opportunities and past HPV vaccine uptake rates among AD Air Force (AF) recruits (some of whom have prior doses of HPV vaccination documented in their historical electronic DoD immunization records) across a 6-year period (2009-2015) in order to highlight the importance of continued surveillance and catch-up immunization campaigns within the US military. As electronic vaccination and medical records are centralized for the US AF AD and beneficiary populations, our objectives were to determine HPV vaccine uptake by HPV vaccine dose and to quantify missed opportunities for HPV vaccination during basic training among this population in the catch-up age group, aged 17 to 26 years.

METHODS

A retrospective descriptive analysis of electronic records was performed to determine vaccine uptake and the percent of missed opportunities for HPV vaccination among AD AF recruits. The study population was identified using administrative data from the Basic Military Training System and included all AD AF recruits attending basic training at Joint Base San Antonio-Lackland from October 1, 2009, to September 30, 2015. Vaccination codes and other medical encounter data were obtained from the Aeromedical Services Information Management System (ASIMS) and the Air Force Medical Operations Agency (AFMOA). Demographic and military service data, including birth year, age, sex, marital status, race/ethnicity, and military beneficiary status prior to the beginning of basic training, were obtained from the Defense Enrollment Eligibility Reporting System and confirmed using military personnel data. Recruits who were younger than 17 years, older than 26 years, or did not complete basic training were excluded from the analysis.

HPV vaccination uptake among recruits for the period prior to entry into military service and during basic training was calculated using ASIMS data. Vaccine uptake rates were determined according to sex, age group, birth year, race/ethnicity, marital status, year of entry into basic military training, and prior military beneficiary status, stratified by the number of HPV doses. Any recruit with a record of receiving 3 or more HPV doses was considered to have completed the dose series.

A missed opportunity for HPV vaccination was defined as a well-care outpatient encounter (non-HPV infection related) occurring on or after initial intake within a recruit's basic training dates when he or she was eligible for HPV vaccination and the vaccine was not administered. The number and percentage of missed opportunities to initiate HPV vaccination during basic training by demographic and service-related characteristics were calculated using ASIMS and AFMOA data for recruits who never received one dose of HPV vaccine prior to entry or during basic training. Statistical methods included computing

means and proportions to describe vaccine uptake rates and missed opportunities in the study population. Data were analyzed using SAS version 9.4 (SAS Institute Inc, Carey, NC). Approval to conduct the study was obtained from the Institutional Review Boards of Walter Reed Army Institute of Research and Joint Base San Antonio-Lackland.

RESULTS

The study population included a total of 198,888 AD AF recruits aged 17 to 26 years who had completed basic training between October 1, 2009, and September 30, 2015. The mean age of the recruits was 20.16 years (SD 2.16 years), with the majority comprised of males (79%), non-Hispanic whites (67%), and single marital status (69%) (Table 1). Among all recruits in this predominately male population, 0.44% received one HPV dose, 0.30% received 2 doses, and 0.83% had 3 or more doses (Table 2). Female recruits had much higher vaccine coverage

than males (one or more dose(s) was 5.7% for women and 0.47% for men). Coverage for one or more HPV dose(s) was highest in the youngest catch-up age group 17-20 (1.7%) and then decreased somewhat with increasing age (Table 2). A pattern of increasing vaccine uptake with each subsequent birth cohort also was observed. Among race/ethnic groupings, non-Hispanic whites (1.2%) had the lowest vaccine uptake at each vaccine dose (Table 2). Single recruits also had slightly lower vaccine uptake for one or more HPV dose(s) (1.5%) compared with married recruits (1.8%) (Table 2). In addition, vaccine uptake among recruits increased with each year of entry into basic training during the study period, 2009-2015 (Table 2).

Among the subset of 22,720 recruits with prior TRICARE beneficiary status, patterns of vaccine uptake by demographic and service-related characteristics were similar to those found for all recruits; however, HPV coverage was much higher compared with recruits without prior TRICARE beneficiary status. Overall, vaccine uptake for one or more HPV dose(s) among women who were prior beneficiaries was 25.2%, but only 3.8% among men with prior beneficiary status (Table 3).

Table 1. Demographic Characteristics of US Air Force Recruits, 2009-2015 (N=198,888).

Demographic Category	n (%N)
<b>Sex</b>	
Male	157,129 (79%)
Female	41,759 (21%)
<b>Age Group, years</b>	
17-20	129,696 (65%)
21-23	49,222 (25%)
24-26	19,970 (10%)
<b>Cohort Year*</b>	
1983-1986	10,786 (5%)
1987-1990	57,171 (29%)
1991-1994	102,038 (51%)
1995-1998	28,863 (15%)
<b>Race/Ethnicity</b>	
White, non-Hispanic	133,015 (67%)
Black, non-Hispanic	31,135 (16%)
Hispanic	19,372 (10%)
Asian/Pacific Islander	7,770 (4%)
Other	7,596 (4%)
<b>Marital Status</b>	
Married	59,867 (30%)
Single	137,789 (69%)
Other	1,223 (1%)
<b>Year of Entry</b>	
2009	10,504 (5%)
2010	33,486 (17%)
2011	35,100 (18%)
2012	35,272 (18%)
2013	31,627 (16%)
2014	29,692 (15%)
2015	23,207 (12%)

\*Cohort year not determined for 6 recruits.

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Table 4 presents the results of missed opportunities for the first dose of HPV vaccine among AD AF recruits for whom the data showed that they were never vaccinated against HPV. Of the 195,744 recruits who had not initiated the HPV vaccination, 99.9% had one or more missed opportunities, 95.3% had 2 or more missed opportunities, and 76% had 3 or more missed opportunities to receive the first HPV dose.

**COMMENT**

In this study, we determined HPV vaccine uptake and missed opportunities to initiate the HPV vaccination series among a population of AD AF recruits between the ages of 17 to 26 years, a catch-up age group for routine HPV vaccination recommended by the ACIP among those who have not been previously vaccinated. Male recruits comprised 79% of this study population. The particularly low vaccine uptake of 5.7% for women with one or more dose(s) and 0.47% for men with one or more dose(s) was likely due to the inadequate capture of nonmandated HPV vaccine information in a recruit's ASIMS record. In addition, the observed low coverage among males may be the result of ACIP recommendations for HPV vaccination for males in the catch-up age range during the study period.<sup>21-23</sup> As of 2016, ACIP recommends routine HPV vaccination among males up to age 21 if they were not previously vaccinated or had not completed the 3-dose series. However, ACIP states that the HPV vaccine may be offered to males aged 22-26 years.<sup>23</sup>

Other studies that have examined HPV vaccine uptake in military populations have conducted female only studies. In a relatively large study of 270, 257 age-eligible service women in the Army, Air Force, Navy, Marine Corps, and Coast Guard, 22.5% received at least one HPV dose during the study period, 2006-2011.<sup>9</sup> In a smaller study conducted at an Army medical center, 14.8% of eligible AD service women aged 18 to 26 years received one or more HPV vaccine doses during the period 2007-2010,<sup>42</sup> while another study conducted at a Navy medical center and its affiliated clinics found that beneficiaries had higher vaccination coverage than AD service women.<sup>43</sup> While we also found higher vaccine uptake among recruits with prior beneficiary status where electronic records were available, the vaccine coverage was only 25.2% for women and 3.8% for men, an alarmingly

Table 2. HPV Vaccination Uptake Among US Air Force Recruits, 2009-2015.

Demographic Category	Number in Category n	Number of HPV Vaccinations		
		1 Dose n <sub>1</sub> (%)	2 Doses n <sub>2</sub> (%)	3 Doses n <sub>3</sub> (%)
Study Population Total	198,888	881 (0.4%)	605 (0.3%)	1,658 (0.8%)
<b>Sex</b>				
Male	157,129	315 (0.2%)	176 (0.1%)	253 (0.1%)
Female	41,759	566 (1.4%)	429 (1.0%)	1,405 (3.4%)
<b>Age Group, years</b>				
17-20	129,696	598 (0.5%)	421 (0.3%)	1,143 (0.9%)
21-23	49,222	208 (0.4%)	135 (0.3%)	373 (0.8%)
24-26	19,970	75 (0.4%)	49 (0.2%)	142 (0.7%)
<b>Cohort Year*</b>				
1983	642	5 (0.8%)	1 (0.2%)	2 (0.3%)
1984	1,715	6 (0.3%)	1 (0.1%)	6 (0.3%)
1985	3,314	11 (0.3%)	8 (0.2%)	11 (0.3%)
1986	5,115	14 (0.3%)	11 (0.2%)	34 (0.7%)
1987	7,848	28 (0.4%)	9 (0.1%)	34 (0.4%)
1988	11,126	42 (0.4%)	26 (0.2%)	72 (0.6%)
1989	15,785	62 (0.4%)	37 (0.2%)	92 (0.6%)
1990	22,412	79 (0.4%)	57 (0.3%)	155 (0.7%)
1991	27,876	102 (0.4%)	78 (0.3%)	207 (0.7%)
1992	27,524	99 (0.4%)	71 (0.3%)	184 (0.7%)
1993	25,120	129 (0.5%)	79 (0.3%)	230 (0.9%)
1994	21,518	133 (0.6%)	92 (0.4%)	262 (1.2%)
1995	16,300	83 (0.5%)	63 (0.4%)	210 (1.3%)
1996	9,843	66 (0.7%)	51 (0.5%)	120 (1.2%)
1997	2,710	21 (0.8%)	21 (0.8%)	39 (1.4%)
1998	10	1 (10%)	0 (0%)	0 (0%)
<b>Race/Ethnicity</b>				
White, non-Hispanic	133,015	403 (0.3%)	301 (0.2%)	946 (0.7%)
Black, non-Hispanic	31,135	221 (0.7%)	115 (0.4%)	243 (0.8%)
Hispanic	19,372	101 (0.5%)	87 (0.4%)	208 (1.1%)
Asian/Pacific Islander	7,770	71 (0.9%)	45 (0.6%)	87 (1.1%)
Other	7,596	85 (1.1%)	57 (0.8%)	174 (2.3%)
<b>Marital Status</b>				
Married	59,867	275 (0.5%)	217 (0.4%)	572 (1.0%)
Single	137,789	584 (0.4%)	381 (0.3%)	1,069 (0.8%)
Other	1,223	85 (7.0%)	57 (4.7%)	174 (14.2%)
<b>Year of Entry</b>				
2009	10,504	51 (0.5%)	23 (0.2%)	60 (0.6%)
2010	33,486	90 (0.3%)	58 (0.2%)	155 (0.5%)
2011	35,100	127 (0.4%)	80 (0.2%)	238 (0.7%)
2012	35,272	151 (0.4%)	86 (0.2%)	280 (0.8%)
2013	31,627	164 (0.5%)	119 (0.4%)	284 (0.9%)
2014	29,692	142 (0.5%)	129 (0.4%)	327 (1.1%)
2015	23,207	156 (0.7%)	110 (0.5%)	314 (1.4%)

\*Cohort year not determined for 6 recruits.

low uptake among a population that has full access to healthcare.

There were numerous well-care medical encounters during basic training among this population of AD AF recruits which underscores the importance of offering

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Table 3. HPV Vaccination Uptake Among US Air Force Recruits by Prior TRICARE Beneficiary Status, 2009-2015.

Demographic Category	No Prior TRICARE Beneficiary Status				Prior TRICARE Beneficiary Status			
	Total n <sub>1</sub>	1 Dose %n <sub>1</sub>	2 Doses %n <sub>1</sub>	3 Doses %n <sub>1</sub>	Total n <sub>2</sub>	1 Dose %n <sub>2</sub>	2 Doses %n <sub>2</sub>	3 Doses %n <sub>2</sub>
Study Population Total	176,168	0.14%	0.09%	0.32%	22,270	2.79%	1.94%	4.85%
<b>Sex</b>								
Male	140,519	0.03%	0.02%	0.03%	16,610	1.61%	0.92%	1.30%
Female	35,649	0.56%	0.40%	1.46%	6,110	6.01%	4.71%	14.50%
<b>Age Group, years</b>								
17-20	113,942	0.11%	0.10%	0.32%	15,754	2.98%	1.98%	4.96%
21-23	43,719	0.18%	0.09%	0.29%	5,503	2.38%	1.76%	4.51%
24-26	18,507	0.22%	0.10%	0.38%	1,463	2.32%	2.12%	4.92%
<b>Cohort Year*</b>								
1983-1986	10,531	0.28%	0.15%	0.39%	255	2.75%	1.96%	4.71%
1987-1990	51,319	0.18%	0.09%	0.30%	5,852	2.05%	1.42%	3.42%
1991-1994	89,020	0.12%	0.10%	0.33%	13,018	2.72%	1.77%	4.56%
1995-1998	25,275	0.07%	0.06%	0.28%	3,588	4.29%	3.37%	8.28%
<b>Race/Ethnicity</b>								
White, non-Hispanic	119,929	0.10%	0.06%	0.31%	13,086	2.12%	1.72%	4.42%
Black, non-Hispanic	26,898	0.24%	0.14%	0.23%	4,237	3.68%	1.82%	4.30%
Hispanic	17,123	0.13%	0.16%	0.38%	2,249	3.47%	2.67%	6.36%
Asian/Pacific Islander	6,785	0.28%	0.18%	0.46%	985	5.28%	3.35%	5.69%
Other	5,433	0.26%	0.22%	0.57%	2,163	3.28%	2.08%	6.61%
<b>Marital Status</b>								
Married	52,797	0.14%	0.12%	0.40%	7,070	2.87%	2.16%	5.09%
Single	122,291	0.13%	0.08%	0.27%	15,498	2.73%	1.84%	4.74%
Other	1,072	1.21%	0.47%	0.84%	151	5.96%	1.32%	5.30%
<b>Year of Entry</b>								
2009	10,477	0.49%	0.22%	0.57%	27	0.00%	0.00%	0.00%
2010	30,137	0.14%	0.08%	0.25%	3,349	1.40%	0.99%	2.36%
2011	30,999	0.14%	0.13%	0.30%	4,101	2.07%	1.00%	3.54%
2012	30,807	0.11%	0.08%	0.34%	4,465	2.60%	1.34%	3.90%
2013	27,605	0.13%	0.08%	0.35%	4,022	3.21%	2.41%	4.67%
2014	25,898	0.07%	0.08%	0.29%	3,794	3.29%	2.87%	6.64%
2015	20,245	0.11%	0.05%	0.25%	2,962	4.49%	3.38%	8.91%

\*Cohort year not determined for 6 recruits.

HPV vaccination in this catch-up age group. Of note, 99% of recruits had one or more missed opportunities to initiate the vaccination series. A study of women with private insurance also reported that a large percentage (97.1%) of unvaccinated women in the catch-up age range had at least one missed opportunity to receive the first dose of the HPV vaccine.<sup>44</sup> It is important to note that an opportunity for HPV vaccination can be considered an opportunity to reduce risk of several HPV-associated cancers including carcinomas of the cervix and squamous cell cancers of the vagina, vulva, penis, anus, rectum, and oropharynx.<sup>13-15</sup>

A major strength of the current evidence-based study is the inclusion of all AD AF recruits during the period October 1, 2009 to September 30, 2015. This allowed for

standard and comprehensive data collection, utilizing a centralized electronic database, in order to compute HPV uptake and trends. This study is also one of few<sup>45</sup> that describes vaccine uptake and missed opportunities among males in the catch-up age range, an age group that is considered an important component of the US HPV vaccination campaign. Some limitations, however, should also be considered. Information on prior history of HPV vaccination can be captured in ASIMS among recruits who were prior beneficiaries. However, since HPV vaccination is not mandatory for AD AF recruits, this information is provided on a voluntary basis for the majority of recruits. Thus, HPV vaccine uptake rates may be grossly underestimated among recruits that have no prior TRICARE beneficiary status and thus no available documentation of HPV vaccination from

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Table 4. Number of Missed Opportunities for HPV Vaccination Among US Air Force Recruits Who Had Not Been Vaccinated Upon Reporting for Initial Training, 2009-2015.

Demographic Category	Number of Missed Opportunities						
	Total n <sub>1</sub>	1 or More n <sub>2</sub>	%n <sub>2</sub>	2 or More n <sub>3</sub>	%n <sub>3</sub>	3 or More n <sub>4</sub>	%n <sub>4</sub>
Study Population Total	195,744	195,607	99.9%	186,516	95.3%	148,705	76.0%
<b>Sex</b>							
Male	156,385	156,272	99.9%	148,373	94.9%	120,030	76.8%
Female	39,359	39,335	99.9%	38,143	96.9%	28,675	72.9%
<b>Age Group, years</b>							
17-20	127,534	127,434	99.9%	121,964	95.6%	100,019	78.4%
21-23	48,506	48,482	100.0%	45,886	94.6%	34,785	71.7%
24-26	19,704	19,691	99.9%	18,666	94.7%	13,901	70.5%
<b>Cohort Year*</b>							
1983-1986	10,676	10,671	100.0%	10,469	98.1%	8,999	84.3%
1987-1990	56,478	53,448	94.6%	51,443	91.1%	42,579	75.4%
1991-1994	100,372	100,314	99.9%	95,166	94.8%	75,187	74.9%
1995-1998	28,188	28,145	99.8%	26,411	93.7%	21,920	77.8%
<b>Race/Ethnicity</b>							
White, non-Hispanic	131,365	131,268	99.9%	125,795	95.8%	101,227	77.1%
Black, non-Hispanic	30,556	30,529	99.9%	29,018	95.0%	22,443	73.4%
Hispanic	18,976	18,971	100.0%	17,864	94.1%	14,082	74.2%
Asian/Pacific Islander	7,567	7,566	100.0%	7,200	95.1%	5,797	76.6%
Other	7,280	7,273	99.9%	6,639	91.2%	5,156	70.8%
<b>Marital Status</b>							
Married	58,803	58,785	100.0%	56,552	96.2%	45,698	77.7%
Single	135,755	135,636	99.9%	128,975	95.0%	102,218	75.3%
Other	1,186	1,185	99.9%	988	83.3%	788	66.4%
<b>Year of Entry</b>							
2009	10,370	10,350	99.8%	9,975	96.2%	8,196	79.0%
2010	33,183	33,179	100.0%	32,989	99.4%	27,790	83.7%
2011	34,655	34,645	100.0%	34,369	99.2%	29,803	86.0%
2012	34,755	34,732	99.9%	33,134	95.3%	27,414	78.9%
2013	31,060	31,043	99.9%	28,185	90.7%	18,713	60.2%
2014	29,094	29,072	99.9%	26,769	92.0%	19,711	67.7%
2015	22,627	22,586	99.8%	21,095	93.2%	17,078	75.5%

\*Cohort year not determined for 6 recruits.

electronic medical records. Also, this study population is comprised primarily of men in the catch-up age group for which HPV vaccination may not be readily available in the outpatient setting. In addition, validation of the number of HPV vaccine doses by specific dates and information on the timing to receive a second or third dose of the vaccine series during basic training could not be determined for vaccine-eligible recruits. It was only possible, therefore, to estimate missed opportunities among recruits who never received the vaccine, but information was not available to determine missed opportunities for the second or third dose. Moreover, some recorded missed opportunities may have resulted from administrative medical encounters rather than encounters for preventive care. Finally, the study population

was comprised of AD AF recruits only, so the results may not be generalizable to recruit populations in the other branches of the US armed forces.

**CONCLUSION**

Universal access to HPV vaccination among this at-risk population of AD AF military recruits, representing the catch-up age group, will help reduce missed opportunities for HPV vaccination. In turn, this will ultimately lead to the reduction in several cancers that are causally linked to HPV. Targeted efforts should be made to address barriers to administer HPV vaccines in medical care encounters during basic training as this is a readily accessible population.<sup>46</sup> Public health efforts to reach goals of increased HPV vaccination uptake and decreased missed opportunities among the AD recruit population are feasible and necessary. Coordinated efforts by multiple agencies within the AF as well as in the other services are needed to improve HPV vaccine utilization. Goals should include increased initiation of the first HPV dose and completion of all recommended doses for age-eligible service members. Overall, promotion of DoD HPV immunization policies to provide access to vaccination for all age-eligible recruits will benefit the force health protection of US military in order to reach the goals of DoD, *Healthy People 2020*,<sup>39</sup> and the President's Cancer Panel.<sup>1</sup> Further research in HPV vaccination uptake rates in the catch-up age group among the other branches of the armed forces is warranted.

**ACKNOWLEDGEMENTS**

We thank the technical staff of Joint Base San Antonio-Lackland for their assistance in preparing the ASIMS dataset for this study. Particularly, Dr Rebecca Hall and Mr David Tucker were instrumental in the collection and coordination of the ASIMS data. We also thank Ms Janice Gary of the Walter Reed Army Institute of Research who played a key role in the overall management of this research.

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# Barriers to Physical Activity Among Military Hospital Employees

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Much of the world faces a growing obesity epidemic.<sup>1,2(pp92-97)</sup> The consequences of this serious problem are well established and potentially devastating. Conditions associated with an elevated body mass include diabetes, hypertension, coronary heart disease, and certain malignancies.<sup>2(pp97-100),3</sup> For many, obesity and elevated body mass are a consequence of decreased physical activity; industrial advancements (eg, convenient transportation, technological advancements, and decreased need for manual labor) have contributed to an overall decrease in physical activity worldwide.<sup>4</sup>

Multiple studies have examined the reasons for becoming physically inactive, as well as the perceived barriers to activity and overall wellness.<sup>5-10</sup> Common barriers include lack of time, inexperience with exercise, and lack of motivation.<sup>5,6,8-10</sup> Although barriers to wellness and physical activity can affect anyone, certain professions demand an inherently more difficult work schedule that can itself be an obstacle to good health. Healthcare providers often have nontraditional work schedules and cite their challenging schedule as a barrier to healthy behavior.<sup>8</sup> These issues are magnified in the United States, as the American workweek for all industries is already longer than other wealthy industrialized countries and employees are more likely to work odd and/or weekend hours.<sup>11</sup> Long shifts, odd hours, and atypical schedules that are common among healthcare workers are barriers that logically detract from their ability to be physically active, beyond that which is required for their employment.

Before policy change can occur, leaders must understand if and how barriers to physical activity affect their specific population. Researchers have studied barriers in various settings, including corporate and healthcare worksites, but there is sparse evidence about how barriers affect those working at military hospitals.<sup>8,12</sup> Military hospitals are unique in that employees may be active duty military members, civilian employees, or contractors. Each employee type has different training requirements and benefits.

In order to better inform military hospital leadership, the purposes of this study were to describe the common barriers to physical activity for employees at a military hospital, and investigate the association of barriers to physical activity with subjects' perception of personal health status.

## METHODS

The health promotion team at a 42-bed military hospital with 1,147 military and civilian staff\* and a large catchment area serving approximately 39,900 beneficiaries investigated current barriers to physical, nutritional, and spiritual wellness as part of the development of an employee wellness program. In the summer of 2014, the team designed a survey to gather this information in partnership with the Army Public Health Center (APHC). The Injury Prevention Division at the APHC designed the electronic survey using Verint Enterprise Edition software (Melville, NY) and provided a secure link through which employees could access the survey. The study was approved by the APHC Public Health Review Board as public health practice and a data use agreement was formally put in place between the hospital team and APHC. The study was later presented to the Institutional Review Board at the University of North Carolina, which concurred that the investigation did not constitute human research.

The survey was intended to be inclusive of all hospital employees. There were no prerequisites to completing the survey and participation was anonymous and optional.<sup>13</sup> Availability of the survey was announced via digital daily announcements, verbal advertisement to large groups of employees, word of mouth, and specific emails to the staff from hospital executives. The survey was open for a total of 45 days from October to December 2014.

In order to gain a true perspective of the holistic wellness state of hospital employees, subject matter experts

\*As of November 2014: 420 (37%) active duty military personnel, 727 (63%) Department of the Army civilian employees.

in the hospital from public health, physical therapy, dietetics, social work, and religious departments submitted survey questions related to wellness. Although the focus of this article is on responses regarding barriers to physical activity and perceived health, the survey included 49 total questions regarding health behaviors and obstacles to wellness.

The survey team took measures to be as inclusive as possible, but also as efficient as possible. When asked about barriers to healthy behaviors, respondents were instructed to mark all barriers that applied to them. The survey contained predetermined response options with an “other” category to capture write-in responses. To

improve speed and efficiency, certain questions were not presented to the participant if it was appropriate for that person to skip those questions, based on previous responses. The average time to complete the survey was 17 minutes.

Following survey closure, researchers cleaned the data and, when possible, categorized responses where participants marked “other.” From January through March of 2015, data were analyzed using IBM SPSS, version 19 (IBM Corp, Armonk, NY). Initial categorization of data was by state of perceived wellness and stratified by age range and military affiliation. Military affiliation was delineated by military rank, and healthcare occupational specialty was included as a category. Specific barriers to physical activity were highlighted along with the percentage of respondents who indicated that the specific barrier affected their level of physical activity.

Chi-square analysis was performed on individual barriers to physical activity with the dichotomized dependent variable of perceived health. This variable was determined by the answer to the required question “Do you perceive yourself as being healthy?” with the possible answers of “Yes” or “No.” Statistical tests of factors associated with perceived lack of health (ie, “no” responses) were evaluated at the alpha 0.05 level.

Univariate logistic regression was then conducted, examining the association of the number of reported barriers with the dichotomous dependent variable of perceived health. The number of variables was a count based on the number of affirmative answers the participant marked when asked about various types of barriers.

Finally, multiple logistic regression was performed to assess the relationship between a greater number of barriers and perceived health, controlling for demographic variables that were found to be associated with perceived health in the previous univariate logistic regression analyses.

RESULTS

The survey population (N=380) was primarily active duty personnel (officer and enlisted) and government civilians (Table 1). There was minor representation of government contractors and retired military serving as hospital volunteers, identified as “other.” Predominant population subgroups: women (56%); age group 26-39 years (47%); and government civilians (45%). The

Table 1. Demographics of Survey Respondents (N=380).

Demographic	Military Personnel n <sub>1</sub> =205 (%n <sub>1</sub> )	DOA* Civilian Employees n <sub>2</sub> =169 (%n <sub>2</sub> )	Other† n <sub>3</sub> =6 (%n <sub>3</sub> )	All Respondents N=380 (%N)
<b>Sex</b>				
Female	71 (35%)	141 (83%)	2 (33%)	214 (56%)
Male	134 (65%)	28 (17%)	4 (67%)	166 (44%)
<b>Age, years</b>				
18-25	32 (16%)	1 (1%)	1 (17%)	34 (9%)
26-39	109 (53%)	34 (20%)	2 (33%)	145 (47%)
40-54	61 (30%)	76 (45%)	1 (17%)	138 (36%)
55 or older	3 (2%)	58 (34%)	2 (33%)	63 (17%)
<b>Military Affiliation</b>				
Enlisted	118 (58%)	-		118 (31%)
Officer	87 (42%)	-		87 (23%)
DOA civilian	-	169 (100%)		169 (45%)
Other	-	-	6 (100%)	6 (2%)
<b>Education</b>				
High school or GED	49 (24%)	37 (22%)		86 (23%)
Associate's	39 (19%)	41 (24%)		80 (21%)
Bachelor's	42 (21%)	39 (23%)	2 (33%)	83 (22%)
Master's or Doctorate	65 (32%)	33 (20%)	4 (67%)	102 (27%)
Other professional degree	10 (5%)	19 (11%)		29 (8%)
<b>Occupation</b>				
Nurse	31 (15%)	30 (18%)		61 (16%)
Physician	22 (11%)	1 (1%)		23 (6%)
Medic	47 (23%)	3 (2%)		50 (13%)
Technician	18 (9%)	19 (11%)	1 (17%)	38 (10%)
Pharmacy	3 (2%)	5 (3%)		8 (2%)
Other medical profession‡	62 (30%)	13 (8%)	2 (33%)	77 (20%)
Administration	14 (7%)	39 (23%)		53 (14%)
Other nonmedical or unspecified	8 (4%)	59 (35%)	3 (50%)	70 (18%)

\*DOA indicates Department of the Army.  
 †Contract employees and volunteers (retired military).  
 ‡This category includes clinical providers who could not be grouped into broad categories (eg, behavioral health professionals, physical therapists, and dentists).

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survey was largely representative of clinical staff with 86% of those surveyed employed in direct clinical care.

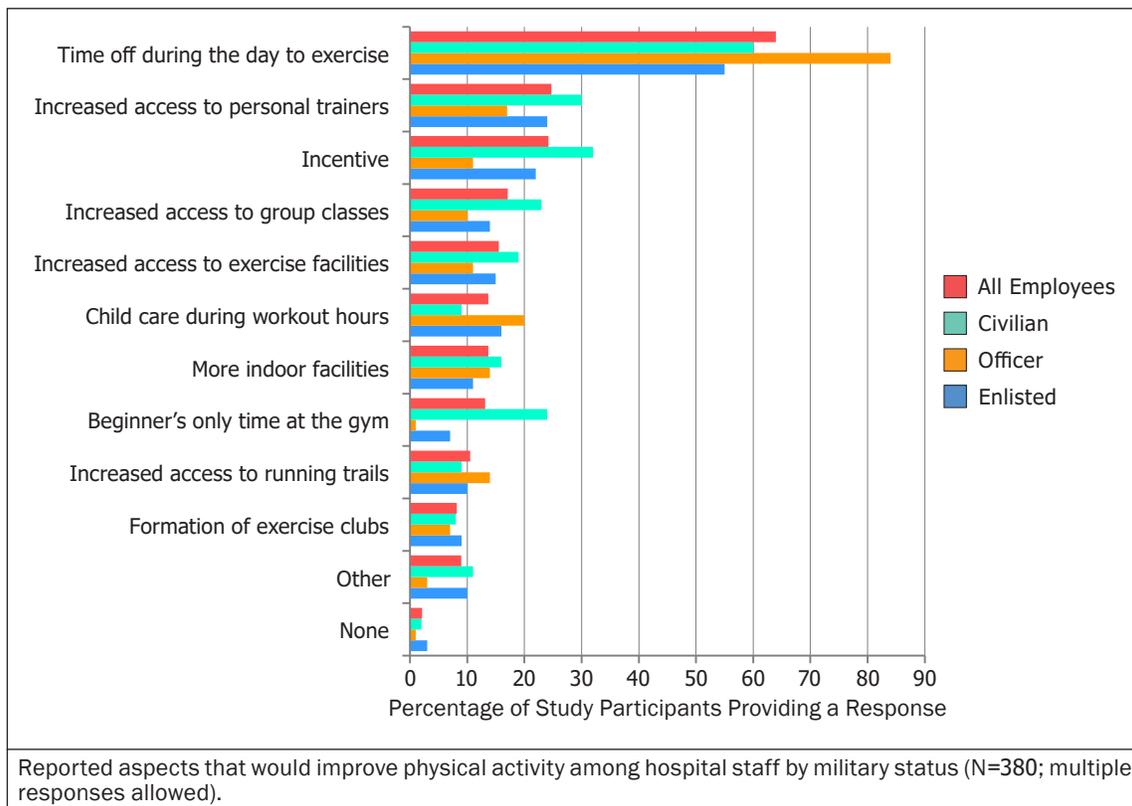
Ninety percent of respondents considered themselves to be healthy, and of those who considered themselves to be unhealthy (n=38), 95% were interested in becoming healthier. Although the survey population largely considered itself to be healthy, many respondents reported unhealthy behaviors such as not enough exercise and poor eating habits. Over half of all respondents (n=222, 58%) indicated that they did not get enough exercise and 158 participants (42%) responded that they were either somewhat or very dissatisfied with their personal physical activity and exercise. As reported by Schuh-Renner et al,<sup>14</sup> 47% of respondents reported at least one injury in the previous 12 months. Active duty military members had greater risk for injury, and activities associated with injuries in this population were similar to those in other military populations (physical training, walking/hiking, and lifting or moving objects).

Despite a long list of possible barriers from which to choose and the freedom to select multiple barriers, as shown in Table 2, the top 3 barriers were nearly twice as prevalent among respondents as all others. Lack of time was common to 65% of participants and lack of motivation affected nearly half (45%) of participants. A previous medical condition was reported as a barrier in just over a

quarter (27%) of all participants. Those citing pain/other medical conditions, lack of experience, financial burden, and discomfort with the gym crowd as barriers to physical activity were more likely to perceive themselves as unhealthy, as 26%, 26%, 25%, and 22% of respondents citing those barriers, respectively, reported perceived lack of health. Enlisted respondents identified previous medical conditions and lack of experience as barriers to physical activity more frequently than other affiliations, while civilians were more likely to cite the financial burden and being uncomfortable with the gym crowd.

Participants also answered questions about aspects of the work environment and available health promotion activities that might improve their physical activity levels. Almost two-thirds of participants (n=243, 64%) indicated that time off during the workday to devote to exercise would improve their physical activity, an aspect nearly 3 times as important as any other as shown in the Figure. This illustrates the importance of time to employees and how closely they associate personal time with their ability to be physically active. Incentives and access to personal trainers, 2 potential factors to improve motivation, were cited as being the next 2 most important aspects that would improve physical activity.

The data suggest that barriers not only affect participation in physical activity itself, but also the organization's



outlook on physical activity. The sample population indicated that adult physical fitness was the health education topic of greatest interest. However, the follow-on questions indicated that nearly 65% of participants anticipated that lack of time would be a barrier to attending health education classes. Respondents who reported a previous medical condition, lack of experience with exercising, or financial burden as barriers to physical activity were more likely to also report a perceived lack of health (26%, 26%, and 25%, respectively), as shown in Table 2.

Given that specific perceived barriers showed correlation to lack of perceived health, the overall number of barriers an individual experienced was examined as another potential factor related to perceived lack of health. Univariate regression analysis showed higher odds ratios with increasing number of barriers indicated (Table 3). This result was statistically significant when 4 or more barriers were reported ( $P \leq .01$ ). Selected demographic variables (age 55 or older, female gender, and civilian employee status) were also significantly or marginally associated with perceived lack of health ( $P \leq .10$ ).

Multiple logistic regression analysis was performed with the variables that were found to be significant in univariate analyses. The presence of 4 or more barriers to physical activity was the only statistically significant factor associated with respondents' perception of health ( $P = .04$ ), as shown in Table 4.

COMMENT

Addressing the obesity epidemic is paramount in preventing devastating disease processes and decreasing barriers to physical activity is a key component of prevention. Previous studies have found that lack of time, motivation, and knowledge are barriers to an individual's wellness,<sup>5,6,8-10</sup> but there is little evidence available about barriers to physical activity among employees of a military medical facility, given the unique aspects of that population. This study confirms that the same barriers (time, motivation, and knowledge) also influence the perceived wellness of military medical facility employees. Additionally, survey participants indicated that they would change aspects of their environment that directly related to these same barriers, if possible, including

Table 2. Barriers to Physical Activity and Perceived Lack of Health (N=380, multiple responses were allowed).

Barrier to Physical Activity	All Respondents N=380 n <sub>1</sub> (%N)	Enlisted n <sub>2</sub> =118 (%n <sub>2</sub> )	Officer n <sub>3</sub> =87 (%n <sub>3</sub> )	Civilian n <sub>4</sub> =175* (%n <sub>4</sub> )	Respondents Reporting Lack of Health (%n <sub>1</sub> )
Lack of time	247 (65%)	65 (55%)	70 (80%)	112 (64%)	21 (9%)
Lack of motivation	171 (45%)	49 (42%)	24 (28%)	98 (56%)	21 (12%)
Pain or previous medical condition	104 (27%)	39 (33%)	18 (21%)	47 (27%)	27 (26%)
Not comfortable with gym crowd	58 (15%)	12 (10%)	7 (8%)	39 (22%)	13 (22%)
Lack of support network	54 (14%)	14 (12%)	9 (10%)	31 (18%)	8 (15%)
Weather	54 (14%)	16 (13%)	17 (20%)	21 (12%)	8 (15%)
No child care	46 (12%)	16 (14%)	14 (16%)	16 (9%)	4 (9%)
Financial burden	20 (5%)	1 (1%)	0 (0%)	19 (11%)	5 (25%)
Lack of experience or knowledge	19 (5%)	9 (8%)	1 (1%)	9 (5%)	5 (26%)
Work	11 (3%)	2 (2%)	6 (7%)	3 (2%)	1 (9%)
No parking at gym	0 (0%)	-	-	-	-
Other	16 (4%)	2 (2%)	4 (5%)	10 (6%)	1 (6%)
None	32 (9%)	19 (16%)	3 (3%)	10 (6%)	1 (3%)

\*Total (n<sub>4</sub>) includes 169 Department of the Army civilian employees and 6 contract employees or hospital volunteers.

finding time for exercise during the day and adding exercise facilities. Furthermore, the current analyses indicated that the barriers not only affect respondents' ability to be physically active, but employees anticipate that similar barriers would affect participation in the organization's offering of physical exercise groups and wellness education sessions.

These barriers not only affect the individual's participation in physical activity, but are also correlated with perception of one's own health. Regression analysis controlling for demographic characteristics indicated that if a person identified 4 or more barriers affecting their participation in physical activity, they were 9 times more likely to perceive themselves as unhealthy. While this result may be intuitive, the consequences are significant. Additional research examining the combinations of perceived barriers to physical activity would increase understanding of their effects on various populations.

The active duty military members in this population (54% of respondents) face unique challenges. The highly transient life of most military personnel often leads to greater distances between the Soldier and family members and traditional social support networks. Furthermore, requirements to maintain physical fitness, mandatory attendance at unit physical training activities, and additional military duties lead to unique challenges for military providers when compared to traditional patient care providers. Despite these differences, our

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results indicate that barriers common to other populations (lack of time and motivation) exist in a military medical facility. Additionally, these very same barriers are statistically related to the employee's perceived health, giving more credence to the idea that barriers must be addressed in a fashion that is meaningful to the individual. Recommendations from the American Heart Association state that employers should seek to reduce or eliminate barriers that discourage use of worksite wellness programs.<sup>15</sup> Further research should be performed to identify the most effective ways to both measure and efficiently address those barriers.

Since workplaces are now taking more prominent roles in advancing the health of their employees,<sup>16</sup> organizational leaders must carefully consider barriers to participating in wellness activities and their impact on the overall health of employees. Large multinational corporations like Google are providing employees access to state-of-the-art fitness centers and multiple opportunities for physical activity.<sup>17</sup> The need to eliminate these barriers is necessary not simply to enhance the employee experience, but also to financially benefit the organization. Financial incentive is gained through decreased healthcare costs, increased productivity, improved morale, increased retention, and decreased absenteeism. As Baicker and colleagues<sup>18</sup> explain, savings are not simply associated with decreased healthcare costs; rather, additional revenue is appreciated when workers are present and well. Decreased absenteeism, for example, allows workers to focus on their own productivity, rather than making up for the work not completed by an absent colleague. Baicker et al found that the return on investment in wellness programs was \$3.27 for every dollar spent through decreased healthcare cost and \$2.73 for every dollar spent through decreased absenteeism.<sup>18</sup>

This study had some limitations. With approximately 32% of the hospital population responding to the survey, it is possible that those who responded did not represent the entire population. The people who were interested enough in wellness to complete the survey may have different barriers than those who chose not to participate. Furthermore, the dichotomized self-assessment of health may have led to a decreased ability to detect differences. Small sample sizes among some subgroups led to large confidence intervals on risk ratios and odds ratios, making it difficult

to draw statistically sound conclusions from the data. With only 10% of individuals reporting themselves to be unhealthy, there may not have been enough power to assess the correlation of all barriers with perceived health. In future studies, the use of a Likert scale may be a more efficient and powerful tool to evaluate self-assessed health metrics.

Table 3. Injury Incidence: Factors Associated with Perceived Lack of Health (N=380).

Variable	Total in Variable Category n <sub>1</sub>	Perceived Lack of Health n <sub>2</sub> (%n <sub>1</sub> )	Odds Ratio (95% CI)	P Value
<b>Number of Barriers to Physical Activity</b>				
0	39	1 (3%)	1.00	
1	108	7 (6%)	2.53 (0.32-19.89)	.36
2	102	9 (9%)	3.44 (0.45-26.27)	.20
3	79	8 (10%)	3.95 (0.51-30.47)	.15
4 or more	52	13 (25%)	9.75 (1.33-71.40)	<.01
<b>Age, years</b>				
18-25	34	1 (3%)	1.00	
26-39	145	10 (7%)	2.35 (0.31-17.70)	.39
40-54	138	16 (12%)	3.94 (0.54-28.69)	.13
55 or older	63	11 (17%)	5.94 (0.80-44.04)	.04
<b>Sex</b>				
Female	214	27 (13%)	1.90 (0.97-3.73)	.05
Male	166	11 (6%)	1.00	
<b>Military Affiliation</b>				
Enlisted	118	8 (7%)	1.48 (0.46-4.74)	.51
Officer	87	4 (5%)	1.00	
DOA* civilian	169	25 (15%)	3.22 (1.16-8.95)	.01
Other†	6	1 (17%)	5.44 (0.77-38.16)	.08
<b>Education</b>				
High school or GED	86	9 (10%)	1.30 (0.53-3.24)	.57
Associate's	80	9 (11%)	1.43 (0.58-14.53)	.43
Bachelor's	83	9 (11%)	1.38 (0.56-3.43)	.48
Master's or Doctorate	102	8 (8%)	1.00	
Other professional degree	29	3 (10%)	1.32 (0.37-4.66)	.67
<b>Occupation</b>				
Nurse	61	6 (10%)	0.98 (0.35-2.77)	.98
Physician	23	0	0	.12
Medic	50	3 (6%)	0.60 (0.16-2.21)	.44
Technician	38	5 (13%)	1.32 (0.45-3.87)	.62
Pharmacy	8	1 (13%)	1.25 (0.18-8.90)	.83
Other medical profession‡	77	6 (8%)	0.78 (0.28-2.21)	.64
Administration	53	10 (19%)	1.89 (0.77-4.63)	.16
Other nonmedical or unspecified	70	7 (10%)	1.00	

\*DOA indicates Department of the Army.

†Contract employees and volunteers (retired military).

‡This category includes clinical providers who could not be grouped into broad categories (eg, behavioral health professionals, physical therapists, and dentists).

Table 4. Multivariable Logistic Regression Results: Factors Associated with Perceived Lack of Health (N=380).

Variable	Total in Variable Category n <sub>1</sub>	Perceived Lack of Health n <sub>2</sub> (%n <sub>1</sub> )	Risk Ratio (95% CI)	P Value
<b>Number of Barriers to Physical Activity</b>				
0	39	1 (3%)	1.00	
1	108	7 (6%)	2.05 (0.24-17.66)	.51
2	102	9 (9%)	3.06 (0.37-25.88)	.30
3	79	8 (10%)	3.51 (0.42-25.88)	.25
4 or more	52	13 (25%)	9.46 (1.14-78.66)	.04
<b>Age, years</b>				
18-25	34	1 (3%)	1.00	
26-39	145	10 (7%)	2.76 (0.32-24.02)	.36
40-54	138	16 (12%)	4.45 (0.49-40.80)	.19
55 or older	63	11 (17%)	6.79 (0.66-70.06)	.11
<b>Sex</b>				
Female	214	27 (13%)	1.34 (0.55-3.27)	.53
Male	166	11 (6%)	1.00	
<b>Military Affiliation</b>				
Enlisted	118	8 (7%)	2.21 (0.59-8.28)	.24
Officer	87	4 (5%)	1.00	
DOA* civilian	169	25 (15%)	2.08 (0.62-6.94)	.23
Other†	6	1 (17%)	3.45 (0.25-46.66)	.35

\* DOA indicates Department of the Army.  
 † Contract employees and volunteers (retired military).

Approaches to improving barriers to physical activity, including environmental changes, are necessary to facilitate an environment of disease, injury, and obesity prevention. Institutional leaders should continue to explore programs that investigate and address common barriers to physical activity. Leaders in military hospitals should consider programs that promote the principles of the Department of Defense initiative Operation Live Well, a program that intends “to make healthy living the easier choice and social norm...”<sup>19</sup> Policies that address barriers of time and motivation, such as authorizing employees 3 paid hours per week to participate in a fitness program, are particularly beneficial and have been successful in other military workplaces.<sup>20</sup> Agreements between leadership and employees facilitate participation in exercise activities, but also require consistent documentation of the workouts, approved routines, and health clearances to continue participation. A cultural change that embraces physical activity and encourages it as a part of each day would be a useful step toward preventing chronic disease processes among military healthcare employees. The analysis and reporting of the successes and failures of these programs will pay dividends financially, emotionally, and physically for many in our communities.

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# Hydration Strategies for the Female Tactical Athlete

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## ABSTRACT

With unprecedented expansion of the roles of women in the military and the longest period of continuous active combat in US history, it is time that research expanded, including the nutritional and hydration requirements of the female tactical athlete. Dehydration has a negative effect on athletic performance, most significantly in high intensity, aerobic endurance activities. There is evidence female athletes may be more prone to the potentially lethal effects of over hydration. The purpose of this article is to provide a review of the literature to ascertain optimal hydration strategies for the female tactical athlete.

Women have always played a crucial role in the support of our nation's military, directly and indirectly. Not until recently, however, have women been allowed to serve in unrestricted roles. Prior to 1993, women were not permitted to serve on combatant aircraft, vessels, or in direct ground combat. One decade later, rescension of outdated policy allowed women into the aforementioned roles including combat units, generating the need for review of occupational standards and assignment policies. On March 10, 2016, the Secretary of Defense announced approval for women to be assigned to direct ground combat roles, including all military services and the US Special Operations Command.<sup>1</sup> With the unprecedented expansion of women's role in combat combined with the longest period of continuous active combat in US history,<sup>2</sup> it is time that research be expanded to include the nutritional and hydration requirements of our female tactical athletes.

Scofield and Kardouni define the tactical athlete as "professionals that require expertise in their occupational skills concomitant with general physical preparedness, which enable them to perform physically demanding occupational tasks while mitigating injury."<sup>3</sup> These tactical professionals are required to possess physical and mental preparedness necessary to effectively operate in their occupation emphasizing the importance of up-to-date hydration guidelines to optimize performance and highlight potential differences in traditional athletes and their tactical counterparts. Uniformity of tactical athletes becomes the explicit requirement of baseline physical fitness, with the potential occupational requirement of confronting and overcoming physical, environmental, and human threats with little to no advanced notice setting them apart from civilian athletes. While traditional players train and compete in seasons, those

considered tactical know their position requires them to operate "in season" constantly.

Anything short of optimal performance for any tactical athlete can be the difference between life and death. However, performance for a new recruit plays a vital role in acceptance and integration into their new unit in addition to survival. An assessment of these guidelines will further facilitate female integration and acceptance by their new team. As combat is frequently in austere conditions and physically demanding, hydration status and strategies are paramount. Not only is hydration crucial to performance optimization, but also prevention of dehydration, as the result can be lethal. This review focuses on what is known in the literature regarding hydration, summarizes the best strategies for optimal performance while avoiding exercise-related illness, and a call for further research in the developing group of female tactical athletes.

## DELETERIOUS EFFECTS OF DEHYDRATION

Water is essential to life down to the cellular level. Without it our species can survive mere days. The lack of a firm definition of dehydration emphasizes the dynamic nature of our regulatory systems. It is understood as "the process of uncompensated water loss via urine, sweat, feces, and respiratory vapor thus reducing total body water below the average basal value."<sup>4-9</sup> However, amongst those who strive to measure hydration status for the purposes of assessing its effects on performance, the accepted value is 2% loss in body weight during activity that otherwise isolates fluid loss as the primary route. Components that lead to dehydration can be broken into categories such as lack of access, physiologic loss, and environmental stressors. Access pertains to fluid unavailability, inadequate time to drink, lack of

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food intake, and unpalatable fluids that can compound the delay in rehydration.<sup>10-12</sup> Physiologic losses are maintained by fluid and electrolyte homeostasis despite wide variations of intake and output. Regarding these measures in the context of rigorous training, the body's renal, hypothalamic, and neurologic control can be taxed to their extremes in a matter of hours.<sup>13</sup> Lastly, and the hardest to control, the environmental factors of ambient temperature, humidity, altitude, air currents, and clothing<sup>14</sup> must be noted when discussing factors affecting water loss. Battle dress uniform and body armor add a minimum of 5°F to the ambient temperature.<sup>15</sup> When envisioning both the training and operations of tactical athletes, the austerity of their locations often impose physical, behavioral, and environmental conditions beyond the bounds of homeostatic norms and demand an optimized hydration strategy.

It is well documented that dehydration has a negative effect on athletic performance.<sup>16,17</sup> While, by definition, dehydration is the loss of 2% body weight, athletes have been documented to have up to 6% to 10% losses or more in rare cases.<sup>18</sup> Under relatively mild dehydration, individuals engaging in rigorous physical activity, even in temperate climates, will experience decrements in performance due to altered thermoregulatory capability, compromised cardiovascular function, and increased fatigue, resulting in reduced endurance, reduced motivation, and increased perceived effort.<sup>5</sup> Inadequate hydration consistently shows a more significant effect on high intensity,<sup>19</sup> aerobic,<sup>20-22</sup> endurance activities such as long-distance running,<sup>23</sup> as well as decrements in accuracy<sup>24,25</sup> with even larger scope application of negative functional effect on team sport performance that demand high levels of skill and precision.<sup>21</sup> Assessment outcomes such as reduced accuracy, speed of complex tasks, and distance judgement were also negatively affected.<sup>24,26-32</sup>

It is less clear to what degree dehydration worsens cognition, originally suggested by Sharma and Gopinathan separately in 1986 and 1988 respectively.<sup>33,34</sup> Lieberman counters with the rational argument that the effect of hypohydration on cognition was rather equivocal until recently due to the difficulty in assessing any independent variable on human behavior, the difficulty of attaining consistent levels of dehydration that do not confound with the stressors used to get there, and our inability to accurately and reliably measure hydration status from moment to moment in addition to our limitations on cognitive measure itself.<sup>35</sup> Research shows that concentration, alertness, and short term memory all decrease while dehydrated.<sup>29,31,35-37</sup> This is in addition to the well documented negative effects on perception of the work

being done, otherwise categorized as mood,<sup>31</sup> and alertness that can be subjectively improved by hydrating.<sup>37</sup>

### DELETERIOUS EFFECTS OF OVERHYDRATION

While not drinking enough fluid can result in underperformance, so too can the consequences of drinking too much. Overhydration, once considered a pre-event strategy, has shown to be an illegitimate solution to dehydration.<sup>29</sup> To date, there is no reported benefit in trials to enhance performance, in either gender, by intentional overhydration. Whatever the marginal competitive advantage may be, it does not outweigh the risks. These risks have been recorded in endurance athletes, hikers, and Soldiers in the form of hyponatremia, seizure, and death. In the wake of a military training-related death,<sup>38</sup> the fluid replacement guidelines were changed and found to be successful.<sup>15</sup> By reducing the incidence of significant serum sodium loss without increasing the risk of dehydration, Kolka et al<sup>15</sup> proposed safer hydrating strategies for strenuous training and expanded the guidelines including female subjects previously unavailable. Similarly, the increasingly high numbers of serious illness and death in female athletes in the last quarter century further emphasize the ongoing problem of overhydrating. In an attempt to prevent heat injury, these endurance athletes are increasing their risk of hyponatremia by way of water overload. Hyponatremia is defined as more than 135 mmol/L of sodium and is associated with a number of sodium and water disorders.<sup>39</sup> Perhaps the most concerning risk is the similarity in presentation of acute overhydration to the antithesis symptomatic dehydration. Mistaking one diagnosis for the other can be devastating, as was demonstrated in the incident of the military trainee; the symptoms of altered mental status, emesis, and nausea were interpreted and treated as if a common case of dehydration or heat-related illness and treated as would have been appropriate for dehydration. Instead, however, his hyponatremia worsened with the treatment for dehydration, and as a result, he died due to irreversible damage of the brain and lungs.<sup>38,40</sup> Another main concern specific to female athlete heat illness is the fact that research shows women are more compliant when it comes to replenishing fluid losses. This might be seen as an advantage; however, research at the other extreme of the spectrum found that female athletes are 25 times more likely to die from hyponatremia during an endurance race.<sup>41</sup> These unfortunate events indicate the need for further research and continued education within the athletic community.

### HOW TO ASSESS HYDRATION STATUS

There are many proposed techniques for measuring hydration status in athletes. To date, there is no one biomarker that is accepted as the gold standard indicator of

hydration. After all, euhydration is the concept of the body in water balance. This is a dynamic state difficult to define physiologically as well as numerically due to its constant flux. The ideal biomarker for athletic standards should be sensitive and accurate enough to detect body water fluctuations of  $\approx 2\%$  body weight in real time, minimally invasive, inexpensive, mobile, expedient, easy to use and require little training. All modern tools hinge around singular measures of the highly dynamic fluid system of interconnected compartments. Armstrong<sup>42</sup> contrasts the importance of accuracy in laboratory measures with the goals of field assessment desiring portability, ease of use, and cost effectiveness. Assessment techniques reviewed for this article included thirst, change in body weight, sweat volume, urine osmolality, urine specific gravity, plasma osmolality, bioelectric impedance analysis, among others.<sup>20,30,42-46</sup>

Water balance is the net difference between water gained and water lost. Daily sources of water include beverages (81%), food (19%),<sup>20</sup> and metabolic production (a negligible volume in comparison, and approximately equal to respiratory water losses of  $\approx 0.12$  g/kcal).<sup>47,48</sup> Water is lost through respiratory, gastrointestinal, renal, and sweat functions.<sup>49</sup> Total body water can be estimated using tracer methods, most commonly deuterium oxide, with an accuracy of 1% to 2%.<sup>8,43</sup> This is the most valid and precise estimation modern science can offer.<sup>50</sup> However, as functional dehydration is 2% or greater body weight (BW) change, this margin of error meets the sensitivity requirement but does not outdo simply tracking body weight fluctuations. Furthermore, tracer methods are less practical in the field due to cost, time-to-result, and technical requirements. Variability due to differences in body composition also make tracer methods a suboptimal technique for operational tactical athletes.<sup>20</sup>

As sweating is the primary avenue of water loss during exercise-heat stress, acute changes in body weight can be used to calculate an athlete's sweat rate.<sup>51</sup> Following the equation used in the 2000 National Athletic Trainers' Association Position Statement, each athlete can, and should, calculate their individual sweat rate: (sweat rate = pre-exercise BW - postexercise BW + fluid intake - urine volume)/exercise time in hours. Sweat rates found in the literature range from 0.5 L/h to more than 4 L/h (0.50 kg/h to 4 kg/h)<sup>5,14,49</sup> with conclusive evidence that, in general, females sweat less than males.<sup>52</sup> As sweat rates vary drastically due to individual metabolic and sweat rates, variance in fitness protocols, type of exercise, and environmental factors, it is imperative that athletes begin to consider hydration with the same regard as nutrition. Many argue that water is in fact the principal nutrient, more important than sources of fuel. It is

advised that athletes, be it an individual or as a team for an average, calculate their sweat rate in various environmental conditions as well as periodically account for conditioning and heat acclimatization.

Without a gold standard, a single assessment technique is inadequate to evaluate body hydration status. Therefore, combining several techniques for multiple points of reference is encouraged. After consideration of barriers such as time required, cost, portability, ease of use, etc, the recommendation is to combine BW changes with urine indices. Body weight measurement should be postvoid but premeal and with as few confounding articles of clothing as possible. A baseline value can be established with at least 3 consecutive morning nude BW measures to approximate euhydration if athletes have access to food and fluid ad libitum.<sup>53</sup> Female athletes may require additional BW measurements as menstrual cycles can alter body weight and fluid distribution.<sup>49,54,55</sup> When acute water losses are estimated by pre- and postexercise BW measures, consideration should be made for sweat trapped in clothing.<sup>56</sup> Body weight measures with calculated percentage of body weight change should be conducted before, during (if exercise exceeds 3 hours), and after exercise sessions to estimate fluid balance.<sup>5</sup>

Pre-exercise urine biomarkers can allow discrimination between euhydration and dehydration.<sup>9,57,58</sup> Urine specific gravity (USG), color, volume, and osmolality (UOsmol) together provide the most insight to an athlete's hydration status. A USG of 1.020 or less is indicative of being euhydrated.<sup>57-59</sup> Osmolality is more variable, but values of 700 mOsmol/kg or less are generally accepted as euhydration.<sup>9,57,58</sup> Color comparison and USG read by a refractometer should be done on midstream samples pre-exercise for consistency. Urine values can be misleading if obtained during rehydration periods, especially during periods of rapid recovery. Consumption of large volumes of fluid will produce copious volumes of hypotonic or dilute urine, falsely reflecting a state of euhydration.<sup>8,58,60</sup> If context considerations are made stacked with consistent BW measures, these techniques provide the most reliable, practical, expedient, easy-to-use, and cost effective methods of assessing hydration status.

#### HYDRATION STRATEGY

The athletic community has no problem accepting that hydration is crucial to exercise performance. This article recommends guidelines consistent with the American College of Sports Medicine (ACSM) Position Stand 2007<sup>49</sup> with a call for continued research on the topic in the direction of both tactical and female athletes. The history of hydration standards is saturated with

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misunderstanding. The commonly quoted “8 by 8,” eight glasses of 8 ounces of water daily, is best explained as a misinterpretation of the 1945 Food and Nutrition Board Recommendation, which was 2.5 L *total* water per day and ignoring the phrase that much dietary water comes from food.<sup>20,61</sup> The most recent issue (2005) by the Food and Nutrition Board of the Institute of Medicine (now renamed the National Academy of Medicine), states 2.7 L per day as the adequate intake for women aged 19 to 30 years, the most inclusive age range for tactical athletes. Because euhydration can be maintained over a wide range of water intakes, it is not possible to determine an estimated average requirement, and this value is based on median total water intake from US survey data.<sup>20,62</sup> The committee recognizes the iterative process and continues to refine these values. An additional consideration when determining baseline is caloric intake. It is often cited and generally accepted that 1 to 1.5 mL of water should be ingested per kilocalorie consumed.<sup>63</sup> This can significantly increase the water demands of tactical athletes. For example, a woman consuming 3,000 kilocalories per day would require 4.5 L of dietary water not including sweat loss deficits. Therefore, hydration must be executed in phases of a continuum: preparation, maintenance, recovery with respect to timing of the athletic event, practice or operation.

### PREPARATION

Hydration before exercise should be at a comfortable rate of  $\approx 5$  to 7 mL/kg at least 4 hours prior to the event in order to allow for sufficient absorption, distribution, and regulation of urine output.<sup>49</sup> Athletes should take note of signs and symptoms of inadequate hydration: dark colored urine or lack of urine production, headache, lightheadedness, or evidence of orthostasis (“head-rush” or light headedness upon standing from a laying or seated position).<sup>5,64</sup> If any are present, the athlete should drink an additional  $\approx 3$  to 5 mL/kg at the same slow rate. If time leading up to event is too short (overlaps significantly with recovery from last exercise), the addition of salted or sodium-containing foods are of benefit as they act to stimulate thirst and retain the consumed fluids.<sup>65-67</sup>

Hyperhydration, with water or glycerol-containing beverages has not been shown to provide physiologic or performance advantages over euhydration. To the contrary, it increases the risk of lowering athlete’s plasma sodium,<sup>68,39</sup> therefore increasing the chance of dilutional hyponatremia. The recommendation remains against its use.<sup>69</sup>

### MAINTENANCE

The goal of hydration in the maintenance phase is to replace fluid at a rate to offset sweat losses, keeping body

weight loss less than 2%, while retaining electrolyte balance. Mathematical analysis of Table 5 in the ACSM Position Stand 2007<sup>49</sup> derived projections that proved satisfactory for both male and female athletes when looking at the variables of runner size, speed, and environmental condition. The upper end of the suggested range of rates is directed towards faster runners with greater BMI, inherently heavier sweaters who are training or operating in warmer environments with the lower suggested rate for the slower, lower BMI, working in cooler conditions. The importance of customized hydration plans cannot be overemphasized, but a suggested starting rate ranges from 0.4 to 0.8L/hour drawn from marathon runners.<sup>49,70</sup> Depending on individual sweat rate, exercise duration and fluid availability, a maintenance phase may not be required. Hydration efforts made during exercise lasting less than 1 hour has shown very little difference in overall hydration strategy.<sup>71</sup> In this case, athletes will transition from preparation directly to recovery.

As for what athletes should drink, in 2005 the Institute of Medicine published the recommended composition of “sports beverages” and electrolytes to be  $\approx 20$  to 30 mEq/L of sodium (chloride as the standard anion),  $\approx 2$  to 5 meq/L potassium, and  $\approx 5\%$  to 10% carbohydrate.<sup>20</sup> Fluid palatability is influenced by temperature, osmolality, and flavoring. Preference varies greatly by individual as well as culture. Preferred water temperature is found to be between 15°C and 21°C. The vessel in which the electrolytes are consumed plays a negligible difference and are up to the preference of the athlete. Sources include drinks, gels, energy bars, and other foods. Ingesting carbohydrate in any of these forms allows the athlete to go harder longer as the provision of carbohydrate decreases the use of stored glycogen.<sup>72-78</sup> A rate of  $\approx 30$  to 60 grams/hour will maintain blood glucose<sup>79,80</sup> and are recommended for exercise lasting more than an hour.<sup>73,81-85</sup> While sports beverages provide the allure of rehydrating as well as a source of carbohydrate, athletes are warned against products that have a carbohydrate concentration greater than 7% as they reduce gastric emptying, the anatomic limiting step to rehydration efforts.<sup>86</sup>

Often times, products used to supplement contain caffeine which are thought to help sustain exercise.<sup>87</sup> It is concluded that caffeine, especially once sensitized, has little effect on hydration status during exercise or even in the 24-hour period.<sup>88-90</sup> Of note, these results were based on unrestricted access to fluids and ability to void as necessary, both of which are not always available to a tactical athlete in the field. Therefore, consideration should be given prior to ingestion of increased doses of caffeine as even a minimal diuretic effect could potentially drive

the athlete into an acute phase of hypovolemia without the ability to replenish the losses. Casa et al<sup>5</sup> also warns against consumption of alcohol and carbonated beverages when prioritizing athletic rehydration.

#### RECOVERY

During the recovery phase, the goal is to fully replace any fluid and electrolyte deficits that have accumulated throughout exercise. If recovery time and opportunity permit, common sodium-containing meals and snacks consumed with a sufficient volume of plain water is enough to bring the athlete back to baseline.<sup>20</sup> While many focus on water, during any recovery period, failure to replace sodium will greatly hamper return to euhydration. In many, if not most situations, tactical athletes will be required to have shortened recovery periods. If time between the end of one exercise and the beginning of the next is less than 12 hours, one should consider this a rapid recovery, and steps should be taken to expedite the athlete's return to equilibrium. While sodium losses are even more difficult to assess than water losses, their intake becomes significantly more important during this rapid recovery and extra salt in meals and recovery fluids may be appropriate.<sup>91,92</sup>

For situations that require faster recovery, ≈1.5 L of fluid should be consumed for every kg of body weight lost during the activity. Due to the increase of fluid loads at a faster than normal intake, the body compensates with increased urine output. The suggested extra fluid half-liter is to offset these losses and bring balance as soon as possible. For best absorption and retention, efforts should be made to consume recovery fluids gradually over longer periods of time as opposed to boluses.<sup>93,94</sup> Contrary to common belief, intravenous fluids do not provide an advantage over oral hydration and electrolyte replacement in the absence of either a dysnatremia<sup>64</sup> or severe dehydration evidenced by greater than 7% body weight loss, nausea, vomiting, or diarrhea.<sup>5</sup>

#### SUMMARY

Very few studies have been done on female athletes or tactical athletes. The overall lack of data on female tactical athletes in the deployment setting simply points to an area needing further discovery. With the current literature, the recommendations in this article are almost entirely gender-specific (male) extrapolation, summarized so athletes, coaches, and those caring for athletes might apply the best data available. While superimposition might be considered a critique of this review, the differences amongst individual athletes is likely more than the differences between genders. The combination of variance in data collection methods and application towards the dynamically imprecise prevents greater

external validity. In summary, this article provides the basics of fluid replacement and recommendations that form the foundation of a hydration strategy to prevent heat illness and avoid overhydration injury. It encourages the combined use of body weight changes surrounding 3 phases of the hydration continuum: preparation, maintenance, and recovery. Included are the latest recommended hydration composition and rate for optimized performance. It cannot be overemphasized that athletes must determine what works best for them as an individual and are encouraged to fine-tune their own hydration strategy. As the female's role in the military expands, so too should the research pertaining to the needs of female tactical athletes.

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# 2017

## Spurgeon Neel Annual Award Winner

The Army Medical Department Museum Foundation sponsors the Spurgeon Neel Annual Award competition for the original essay that best exemplifies the history, legacy, and tradition of the US Army Medical Department. The following essay by Dr Justin Barr was selected as the best submission of the 2017 competition.

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### America's Guerilla Hospitals in the Vietnam War: the CIDG Experience

Justin Barr, MD

In the Vietnam War, the United States military created a parallel hospital system to care for the indigenous armies they raised to fight the Communists. Comprised of ethnic minorities, these militias faced fierce discrimination that denied them adequate access to the Vietnamese health care system; their semiofficial status simultaneously complicated integration into American hospitals. The lack of effective medical care not only led to unnecessary morbidity and mortality but also severely damaged the morale of these units. Relying on extensive archival records in both the National Archives and collections at Ft. Bragg that features hundreds of unique interviews, this paper explores how the Special Forces designed, built, and implemented a hospital system to care for these Soldiers. Evolving into surgery-capable frontline hospitals and using a joint US-Montagnard staff, these establishments served as on-the-job training locations for American and indigenous medics, treating both battle injuries and endemic diseases. Perhaps most importantly, they raised the morale of thousands of Soldiers by guaranteeing superior medical care.

#### BACKGROUND

The Special Forces (SF) were founded in 1952 as a covert unit intended to fight the Soviets in the event of World War III.<sup>1</sup> Based off World War II era groups like the Jedburghs, SF A-Teams would, in theory, parachute behind Russian lines, raise guerilla armies, and fight as a fifth column force.<sup>2</sup> This mission required autonomous medical support given the inaccessibility of conventional military medical resources. Accordingly, the Special Forces established an intensive training regimen designed to prepare Army medics to function as autonomous clinicians.<sup>3</sup> Rigorous, demanding, and with a nearly 50%

failure rate, this medical education included classroom lessons and on the job training in local hospitals.<sup>4</sup>

In the 1960s, the mission focus of the Special Forces evolved from guerilla warfare to counter-insurgency. Communist insurrections flared around the globe, and after the CIA's debacle at the Bay of Pigs, Kennedy placed his faith in the US military and specifically the Special Forces.<sup>5</sup> This shift demanded SF teams stabilize a region by winning the hearts and minds of the local populace.<sup>6</sup> Medicine became especially important to this mission, with counterinsurgency experts envisioning it as powerful tool to appeal to and recruit locals. But this medicine differed substantially from that intended in 1952; rather than just providing trauma care to otherwise healthy guerilla fighters, SF medics were now expected to manage the tropical infections, pediatrics, geriatrics and other sundry diseases associated with any given population. The medic transformed from essentially a trauma specialist to a family practitioner. Training correspondingly expanded and came to include the famous "dog lab" where students could practice surgery on anesthetized animals.<sup>7</sup>

This counter-insurgency focus soon landed the Special Forces in Vietnam.<sup>8</sup> In an effort to staunch the communist infiltration, they began setting up outposts in villages that served a mini-basecamps for patrols and for protecting the local civilian population.<sup>9</sup> SF founded their first camp in Buon Enao in 1961. The medic served as the primary ambassador, ingratiating the SF team into the local village and securing local cooperation and participation.<sup>10</sup> The Special Forces worked to recruit these locals into a militia to help defend the village,

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forming what came to be called Civilian Irregular Defense Groups (CIDG). Efforts focused on the Montagnard populations occupying the highlands of Vietnam.

#### MEDICAL PROBLEMS FOR THE CIDG

The Montagnard makeup of the CIDG units created immense medical challenges for the Special Forces. The majority (87%) of the population of Vietnam is Viet Kinh and lives in the flat, fertile eastern seaboard. The ethnic minorities, dubbed Montagnards, or mountain men, by the French, consist of a constellation of small tribes residing in the Annamite Mountains on Vietnam's western border.<sup>11</sup> The Montagnards' strategic location along the Ho Chi Minh Trail made them valuable allies for whichever side could recruit them, but centuries of discord between the minority people and the Viet Kinh hampered the ability of either the North Vietnamese or South Vietnamese armies to ally with the Montagnards.<sup>12</sup> This same discord undermined medical cooperation between the CIDG and the Vietnamese medical system. As the war expanded in breadth and ferocity through the 1960s, CIDG casualties mounted, but medical care remained markedly deficient.

Preexisting prejudices against the Montagnard people combined with an inadequate health care system for the Vietnamese to create a forbidding medical environment. The Montagnard uprising that killed several South Vietnamese Soldiers in 1963 further inflamed tensions.<sup>13</sup> That same year, the South Vietnamese Army reclassified all CIDG Soldiers as civilians, thus denying them entry into the military hospital system.<sup>14</sup> Given that in 1963 over 50% of Vietnam's physicians wore a uniform and that remaining civilian doctors practiced chiefly in urban areas, the exclusion prevented the men from receiving effective medical care.<sup>15</sup> Furthermore, this change blatantly indicated the government's continuation of its policy of considering Montagnards second-class citizens despite their continued, joint war against the North.

As civilians, the CIDG retained access to provincial hospitals. Hospitals more in name than in reality, the understaffed facilities (there was one physician for every 20,000 people; by comparison, the United States had a rate of 1/743)<sup>16</sup> provided inadequate care for ethnic Vietnamese. Special Forces MSC officer Captain Clayton Peacock found "the [Vietnamese] doctors know little more than some of our medics. The surgeons are butchers by and large...I honestly don't think there is a Vietnamese in all Vietnam who really understands what sterile technique is."<sup>17</sup> While undoubtedly hyperbole, statements like these reflected American impressions of any faith in local health care and came to reinforce the perceived necessity of creating a separate system.

Special Forces personnel disparaged the poor medical care Montagnard received at Vietnamese hospitals. "There were instances...where the CIDG were taken to the hospital there [South Vietnamese military hospital] and assured they would be taken care of, and the patients were found dead in the same place that they had been left three or four days earlier," noted Barry Zindel, an SF physician.<sup>18</sup> Americans regularly recalled anecdotes of Montagnard wounded left in hallways, bereft of food and water, and denied care.<sup>19</sup>

This situation created an immense medical problem for the Special Forces. They could not evacuate wounded CIDG to Vietnamese hospitals because of the inadequate care provided. Montagnards, aware of the situation, actively refused transfer to local hospitals.<sup>20</sup> SF medics tried to provide as much care as possible in the villages themselves, but they did not have adequate training to treat penetrating wounds to the chest, abdomen, or head, nor did they have the facilities to manage complicated postoperative care.<sup>21</sup> United States Army hospitals admitted some of the most desperate CIDG patients, but facilities remained few and far between in the early 1960s and swamped with American casualties, who took priority, by the late-1960s.<sup>22</sup> While frequently flouted, USARV Regulation 40-11 technically prohibited Montagnard Soldiers from accessing American hospitals, and despite much effort, the Special Forces community could not get the Army to rescind that policy.<sup>23</sup> Wounded Montagnards had nowhere to go.

This difficulty extended beyond just medical logistics. From a moral stance, asking men to fight for you without providing them adequate medical support unsettled and frustrated the Special Forces men who lived, and died, alongside the Montagnards. From the ever-important counter-insurgency facet, it hurt the underlying mission. Forces became less aggressive, fearing the consequences of getting wounded. Recruitment into the CIDG units suffered. It proved challenging to win the hearts and minds of a population when unable to provide effective medical care for their arms and legs.

#### IMPROVISING A SOLUTION

Attempting to redress this deficiency, the Special Forces created and operated local hospitals that eventually evolved into a mini-health care system. It started at Bien Hoa, which provided the model that other regions followed. Captain Richard Hobson II, MC and Captain John Slaughter, MC arrived in Bien Hoa in the fall of 1965 and immediately took notice of the disparity in care. "The civilian irregulars were essentially getting very poor medical support, insofar as care that could not be handled at the A-Team," commented Hobson.<sup>24</sup> He

and Slaughter decided a “Civilian Irregular Treatment Center” would best remedy the problem.

They launched this idea without any material support. Certainly, the Government of Vietnam did not offer any assistance, as they wanted to build up the hospital system for the Viet Kinh before attending to the minorities.<sup>25</sup> SF headquarters lacked the budgetary flexibility to contribute resources, pecuniary or otherwise. According to Captain James Gay, MC, who later worked in the Bien Hoa Hospital, “I do not think enough can be said about the ingenuity and aggressiveness of Dr. Hobson in obtaining permission to attempt to build such a complex and convincing his Commanding Officer and fellow staff officers to help him build such a hospital.”<sup>26</sup> Slaughter and Hobson plowed onwards, improvising, begging, and “requisitioning” in true-SF fashion. They received a plot of land in October of 1965 and then used a \$12,500 civil affairs grant to fund construction. The American 93rd Evacuation Hospital at Bien Hoa donated operating room equipment, and a spare anesthesia machine came with the compliments of the 3rd Surgical Hospital. “The beds in the unit came from the 93rd Evac with the help of some flags, cross-bows, and ChiCom machineguns,” recalled Hobson.<sup>27</sup> According to SF medic Clarence Page, who assisted in founding the Bien Hoa hospital, “we had no authorization for a damn piece of equipment. We scrounged, we stole, we borrowed, we used out of our supplies, used S-5 supplies, we did everything.”<sup>28</sup> Through some subterfuge, the cement slated for the Officer’s Club swimming pool in Bien Hoa instead became as the floor of the CIDG hospital there.<sup>29</sup>

Because the SF had never managed or even conceived of opening this type of hospital, Medical Service Corps officers created doctrine and standard operation procedures from scratch.<sup>30</sup> Its unofficial, ad hoc stature also complicated personnel assignments; the table of organization and equipment did not billet any slots for the CIDG Hospital, so they had to second physicians and medics from the field. This action created a tension with the primary mission of providing medical personnel to US and indigenous forces actively patrolling the countryside.<sup>31</sup> In 1968, with increased resources dedicated to the overall war effort in Vietnam, the Special Forces formally acknowledged the CIDG hospitals, enabling them to provide staffing and more advanced medical supplies. Families of the patients provided most of the nursing care, including feeding, bathing, and dressing changes.<sup>32</sup>

What began as a glorified dispensary evolved into a fully functional combat hospital. In December of 1965, Slaughter, Hobson, and engineer Carl Schmidt finished constructing the four structures that initially comprised

the Bien Hoa CIDG Hospital. These included two forty-bed patient wards, a medical supply warehouse (which doubled as staff quarters), and an administration/OR/clinic building.<sup>33</sup> The physical plant soon outstripped the medical abilities of the first SF physicians, who had themselves just completed internship and were ill-prepared to treat the complicated combat wounds arriving in triage.

Interactions between the CIDG and US Army hospitals were crucial to the delivery of effective care, and SF physicians from the Command Surgeon on down went to extraordinary lengths to cultivate these relationships.<sup>34</sup> “Not that there was any sort of formal agreement between our unit and the hospital,” commented Captain Robert Drake, MSC, “it was handled primarily on a personal relationship between our physicians.”<sup>35</sup> Each CIDG hospital paired with a corresponding American one, and at Bien Hoa that meant the 93rd Evacuation and 3rd Surgical Hospital.<sup>36</sup> Army surgeons took the most complicated cases, including all neurosurgeries, into their own facilities, and used their own equipment and instruments; they then returned the patients to convalesce in the CIDG hospital’s ward.<sup>37</sup>

As years passed, the facilities improved and capabilities increased. With the arrival of Captain Eugene Edynak, MC, in 1967, the hospital at Bien Hoa acquired a trained surgeon, which vastly expanded the range of procedures they could perform to include thoracic and abdominal operations.<sup>38</sup> By 1970, the hospital included 160 beds, with ten classified as an Intensive-Care Unit, an elaborate laboratory, powerful x-ray equipment, and several full-fledged operating suites manned by residency trained surgeons. By 1968, the demand for beds had outstripped their availability, so the administration splintered off a separate convalescence center approximately forty kilometers from Bien Hoa at Long Hai. Here, a SF medic and a few local orderlies took charge of the forty to sixty patients who resided there until they healed or returned for further care.<sup>39</sup> This facility coupled with a prosthetic center that tried to replace amputated limbs and return some semblance of normalcy to the lives of disabled CIDG Soldiers.<sup>40</sup>

### EXPANSION OF THE CIDG HOSPITAL PROGRAM

Other regions followed the model of Bien Hoa. Captain Paul Ferguson, MC, Captain Brenton Burgoyne, MC, and Captain Homer House, MC, created facilities at Pleiku after spending a couple of months working in the original hospital down south in Bien Hoa. The Pleiku CIDG Hospital collaborated with the local American 71st Evacuation Hospital for complicated cases.<sup>41</sup> They also founded a small, satellite hospital in Ban Me Thuot

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in an effort to accommodate the large size of the military region. Staffed by a physician, it handled minor trauma, sickness, and convalescent care.<sup>42</sup>

In northern South Vietnam, the Special Forces established a CIDG hospital at Da Nang, where it partnered with American Naval medical facilities.<sup>43</sup> Captain Barry Zindel, the surgeon who founded the SF hospital here, had a unique methodology for acquiring the supplies and services of the Navy Seabees: in return for vasectomies, Seabees lent their assistance in building the physical plant.<sup>44</sup> Bartering like this again emphasizes the ad hoc nature of these efforts and the determination – and creativity – of the SF medical personnel supporting them. Compared to Bien Hoa and Pleiku, the hospital at Da Nang remained relatively small due to comparatively fewer CIDG camps and excellent cooperation with the Navy.

The Mekong Delta region never established a CIDG hospital. This absence partly reflected ethnic distribution: no Montagnards lived this far south, although the Nungs and Khmer Soldiers faced only slightly less discrimination. Closer to the capital in Saigon, the area had established a more robust medical infrastructure, and the Vietnamese hospital in Can Tho also did a more acceptable job treating the locals. SF doctors commandeered one of the wards to ensure their forces received equitable and adequate care, but in contrast to the other three military regions, the Mekong Delta had an effective hospital system already in place. The Special Forces preferred to prop up an extant system rather than try to create their own.<sup>45</sup>

Established to care for CIDG Soldiers, the CIDG hospital saw its patient load come include not only the armed fighters but also their families.<sup>46</sup> The US Army Special Forces built these hospitals for moral and morale reasons: they wanted to provide medical care for its fighters, and in doing so, hoped that more combatants would enlist. Including the families satisfied both of these intentions. Since the A-Team medics treated the quotidian cases, the staff at the hospitals saw only the most severely ill or wounded patients, evidenced by the preponderance of battle injuries in the patient load. Between 50% and 70% of CIDG admissions resulted from war wounds, compared to around 30% of the SF admissions.<sup>47</sup>

While the hospitals primarily existed to care for injured CIDG and their families, the institutions also served as ideal locations for practical on the job training, both for indigenous and SF medics. By 1966/1967 when the CIDG hospital program began, many of the SF medics rotating into Vietnam came straight from school in the

United States, and while most received excellent education, they lacked practical experience. Spending two weeks in a CIDG hospital at the start of their tour allowed them to gain this necessary experience before transferring to a village in the hinterlands. Other medics sought some continuing medical education in the middle of their tour, either brushing up on skills or increasing their surgical acumen.<sup>48</sup> Indigenous medics also trained there. Ranging between six to ten weeks, the instruction given to the natives usually augmented that already received in an A-Camp.<sup>49</sup> For both groups, the location served as an ideal school. An extensive and diverse patient population presented a variety of medical and surgical ailments, while full-fledged physicians and experienced paramedical personnel provided a wealth of medical knowledge for the students.

By the mid to late 1960s, the CIDG hospital system provided extensive care. At its peak, it boasted 420 beds and nine dedicated physicians, along with dozens of SF medics and scores of indigenous personnel serving as X-technicians, laboratory assistants, and such.<sup>50</sup> With nearly 60,000 CIDG Soldiers and their dependents, the facilities could not provide definitive care to each but did service over 16,000 patients through the course of the war.<sup>51</sup> Evolving from glorified clinics into fully functional hospitals, they featuring fully-equipped operating rooms with surgeons to staff them, intensive care units, and even rehabilitation departments. They provided a level of care otherwise unavailable to the Montagnard population, who recognized the value and quality of these institutions. When one CIDG sergeant with femur fractured from a gunshot wound was mistakenly deposited in the South Vietnamese army hospital in Saigon realized the mistake, he literally hopped out of the bed and hailed a taxicab so as to receive care at the Bien Hoa CIDG hospital.<sup>52</sup>

As important as the medical value was the morale these establishments inspired. Before their existence, Montagnards suffered from the nonfeasance and malfeasance of Vietnamese doctors; the tribesmen knew they received substandard medical care, and this knowledge affected their willingness to fight. After the construction of the hospitals, “the morale went up 300%,” according to SF medic John McCray.<sup>53</sup> Other medics and officers echoed this sentiment. Richard Miller, a field officer with the Special Forces in Vietnam in 1968 credited the CIDG hospitals as “one of the finest projects undertaken by the USASF that I have witnessed during my tour...the effect that the facility has on the morale of the CIDG is most significant...the facility at Bien Hoa has reduced the fatality and disability rate significantly. It is felt that the maintenance of this facility, with qualified

surgical support, is essential to the morale of CIDG in III CTZ.”<sup>54</sup> These establishments also improved the SF medic’s morale; by working there, they took away a well-deserved sense of accomplishment and a skill set unobtainable through their normal training. More importantly, medics in the CIDG camps finally could evacuate their patients with the confidence that they would receive good care.

With Operation Keystone Robin in 1970-1971, SF lost responsibility for the CIDG militias, and most A-Teams departed. The CIDG hospitals remained after the last camps shut down,<sup>55</sup> but as the CIDG program soon dissolved under South Vietnamese leadership, the Special Forces decided to close all the hospitals by 12 January 1972.<sup>56</sup> The institutions proved successful in multiple facets. The clearly provided significantly better care to the Montagnards than previously existed, reducing the morbidity and mortality of the fighting force. They served as effective training locales for both American and Montagnard medics before they deployed into the field. And they markedly elevated the morale of the CIDG units, whose Soldiers for the first time could expect competent, compassionate medical care when sick or injured. Simultaneously improving both health outcomes and the counterinsurgency objective of winning the hearts and minds, CIDG Hospitals represented a creative solution to a nuanced problem that ultimately fulfilled the mission to preserve the fighting force.

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# 2017

## Spurgeon Neel Annual Award Runners-up

The Army Medical Department Museum Foundation sponsors the Spurgeon Neel Annual Award competition for the best original essay that best exemplifies the history, legacy, and tradition of the US Army Medical Department. The following essays by CPT Denis Alfin and MAJ Michael Mobbs (US Army) were selected as runners-up for the 2015 competition.

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### Ambiguous Duty: Red Cross Nurses and the First World War

CPT Denis L. Alfin, USA

In the fall of 1918, just outside the small French hamlet of Grandpré, American Red Cross (hereinafter referred to as the Red Cross\*) nurse Estelle Davis struggled to stay awake. Death and misery surrounded her as she toiled in an American Expeditionary Force (AEF) surgical hospital. Just two years before, three hundred thousand French and German Soldiers had perished at Verdun, only a few miles to her southeast, and now Estelle's countrymen entered the fray. Her hospital served the 89th Infantry Division, a unit which had recently undergone a baptism by fire at the Battle of Saint-Mihiel. Estelle now supported the unit during the final Meuse-Argonne offensive. Men came into the hospital with grave wounds while Estelle and her Army counterparts worked feverishly to save as many as possible. The beds filled up quickly in the small hospital, leaving no room for long term recuperation. Only terminal patients could remain, and they rarely stayed long either. After surgery, the staff quickly shuttled patients to the hospital trains in order to evacuate them to hospitals equipped for long term care. Men with freshly amputated legs hobbled to the nearby train station in order to evacuate further west, away from the front. The hospital carried little in the way of drugs, except for the morphine administered on a regular basis which often did little more than dull the pain of the expectant. Estelle witnessed Army doctors make do by utilizing ancient, but effective techniques, such as filling festering wounds with maggots to eat away at rotting flesh. Like so many other American field and evacuation hospitals in France during the war, they were understaffed and often ill-equipped to deal with the brutality of industrial warfare.

Estelle's very presence was symptomatic of an Army ill prepared for war. Prior to the war, the federal government had designated the Red Cross Nursing Service as the Army Nursing Corps' reserve force. When the United States became an official belligerent, the Army enlisted thousands of nurses into the Army Nursing Corps from the Red Cross. But some nurses like Estelle remained—administratively at least—under the purview of the Red Cross. When they deployed to France, they were meant to serve in the safety of Paris and other relatively safe areas, working in canteens or convalescence hospitals. Despite this, Estelle found herself working as a civilian in an Army field hospital, as close to the front as any women could be in the war. The Army had ordered the Red Cross to send nurses to the front as if the organization fell under the military's command—and in a very real way this was true. Today, the idea of a militarized Red Cross might seem odd. The modern organization focuses primarily on humanitarianism, and maintains a guarded distance from the American military, limiting its interaction to providing support services to service members during family emergencies.<sup>1</sup> However, this was not always the case. Estelle's story raises numerous questions about the nature of both the Red Cross and the Army during the early twentieth century. How did it come to be that the Army relied upon the Red Cross for its nursing reserve? How did Red Cross recruitment compare to military recruitment? As the situation at the front deteriorated, the Army called upon the Red Cross to send forth even its civilian nurses. How did these women respond to militarization? This essay seeks to untangle the complicated relationship between the Red Cross and

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\*For simplicity, I refer to the American Red Cross as the Red Cross throughout this essay. References to the International Committee of the Red Cross (ICRC) or other countries Red Cross branches are explicitly specified.

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the United States Army, and by so doing, uncover the critical role these civilian nurses played in the war effort.

#### HISTORIOGRAPHY

During the First World War, the American Red Cross functioned as an extension of the United States military. Recently, historians have debated the significance of the Red Cross' militarization in relationship to the organization's long history. The late John Hutchinson argued that the American Red Cross had long been complicit in the militarization of civilians, and when its leaders rejected neutrality, it was simply the logical result of a long process.<sup>2</sup> More recently, Marian Jones criticized Hutchinson's depiction as reductionist, asserting that the Red Cross' nationalistic turn during the war was an aberration, and she focuses on the large amounts of humanitarian relief work done by the organization during and after the war. Jones dismisses the military reserve function of the Red Cross nursing service as insignificant compared to its broader humanitarian functions.<sup>3</sup> Although keeping this debate in mind, I largely sidestep the issue of neutrality. Understanding how the Red Cross interpreted neutrality during this time period requires in-depth analysis of the Geneva Conventions of 1864 and 1906, and American society—all well beyond the scope of this essay. However, it is important to keep this debate in mind, particularly when analyzing Red Cross recruitment material. As the war escalated and the Red Cross ramped up its nurse recruitment campaign, the organization operated along an ambiguous ideological boundary between humanitarianism and militarism. But before we look at recruitment, it is important to understand how the relationship between the Red Cross and the military was born.

#### CREATING THE RESERVE

During the Spanish-American War in 1898, the United States Army suffered enormous systemic failures trying to enlist various state militias into active service. Volunteers languished in mobilization camps stateside, overburdening a weak federal Army bureaucracy. Thousands perished in filthy and disorganized camps as soldiers lacked a basic understanding of field sanitation and the camps retained few trained medical personnel. The few doctors and nurses present offered advice on how to improve conditions in the camps, but that often fell on the deaf ears of officers that either did not care or did not have the resources to make any tangible improvements.<sup>4</sup> The Army Medical Department took these lessons to heart and worked with war department leaders to revise a variety of policies: Army medical authorities received authority to dictate camp sanitation procedures, medical supply lines became standardized, and female nurses became a standard component of Army field

hospitals.<sup>5</sup> However, the incorporation of nurses did not mean that they would become a regular part of the peacetime Army. War department leaders had little appetite for excess personnel in the peacetime Army, and nurses would have to be recruited only when necessary.

With this new demand for nurses, the Army Medical Department became yet another thorn in the side for military planners. President McKinley and United States Army leaders recognized that there needed to be major institutional changes to the manner in which the Army organized in times of war. McKinley tasked secretary of war Elihu Root with this mission. The military's newly minted civilian leader determined that the source of the problem lay in a poorly defined relationship between the state militias and the federal military. Although states had long resisted federal oversight of their militaries, Root and congressional leaders negotiated with them to reach deals in the Militia Act of 1903 and a subsequent major amendment in 1908. The crux of the new legislation stipulated that the federal government would provide increased funding to the state militias, while the states would subordinate their militaries to the president in times of war. Before this legislation, states had often balked at orders to leave the borders of the continental United States, some even refusing to leave the confines of their state. With this new legislation, they submitted deployments outside the boundaries of the United States. States would resist and grumble for many years over what they saw as federal meddling in state affairs, but they would never regain absolute control of their reserve and militias.<sup>6</sup>

It was in this historical moment that congress and the War Department designated the Red Cross as a reserve for the Army Nursing Corps. President Taft signed the legislation into law on April 24, 1912. The law's wording was relatively vague as to who would be called up and what kind of services they would provide. In fact, the bill referred to anyone called up as "civilian employees."<sup>7</sup> Based off a reading of various correspondence however, it was understood by the leadership of the main parties involved—the war department, the Army Nursing Corps, and the Red Cross—that the new policy designated Red Cross nurses as a military reserve force.<sup>8</sup> The Red Cross took up this new role with great enthusiasm, as it had in fact been designed by one of its own leaders, Jane A. Delano.

Jane Delano graduated from Bellevue Nursing School in 1886, and served with the Red Cross during the Spanish-American War. She loomed large in the American nursing world, serving as the president of the American Nursing Association, and the president of the Board of

Directors for the American Journal of Nursing. In 1909 United States Army Surgeon General George H. Torney called on Delano to serve as the Superintendent of the Army Nurse Corps.<sup>9</sup> Later that same year, the Red Cross created a new division, the Red Cross Nursing Service, and tapped Delano to be its director. Delano now led some of the most influential American civilian and military nursing administrations. Delano had long been disappointed with the inability of the Army to quickly enroll nurses into wartime service. She saw an opportunity to leverage the Red Cross' powerful administrative capabilities into a nursing reserve. Delano recommended that the Red Cross and the Army create a reserve system with two main components.<sup>10</sup> First, enrollment into the Red Cross nursing service would also mean enrollment as a reservist for the Army Nursing Corps. Second, the Red Cross designated entire hospitals along with their medical staffs as deployable medical reserve units. Red Cross and Army medical leaders designated some hospitals to fall under Army control and support combat units while others would remain under the Red Cross to perform civilian relief work.<sup>11</sup>

The plan for separate hospitals worked better in theory than in practice. As the war developed, many Red Cross hospitals would fall under the de facto command of the Army. A report written after the war explains how the Army used the Red Cross to set up "camouflage" AEF hospitals. The French government restricted where the AEF could set up military hospitals throughout the country. They also required that American military hospitals receive a specific permit from the central government, bogging down the effort to rapidly set up hospitals. As a supposedly civilian institution, the Red Cross had much more leeway when choosing a location to set up its hospitals. The AEF and the Red Cross then set up military hospitals in multiple locations, including one at Beauvais near the Cantigny front. These were Red Cross hospitals in name only, and operated with a full contingent of Army medical staff. The report concluded that such methods should be avoided in the future, as a "matter of principle."<sup>12</sup> There would be many gaps between Red Cross pre-war plans and the realities of operations during the war. Turning to the nursing recruitment campaign of 1917, we can see that there was still much ambiguity in the role of the Red Cross during the early days of the war.

#### RECRUITMENT

As the Red Cross ramped up its nursing recruitment campaign during the early days of the war, it wove together a narrative of professionalism, humanitarianism, and militarism. Red Cross leaders were still searching for their place in the war effort. They had their support

for the American military clear, but still maintained their ideological grounding in humanitarianism. In August 1917, the Red Cross released a bulletin that stated, "When war was declared between the United States and Germany, the neutrality of the American Red Cross of course ended automatically."<sup>13</sup> In the same breath that they rejected neutrality, the Red Cross acknowledged that they would treat all wounded Soldiers, stating "The Red Cross knows no such thing as the nationality of a wounded man."<sup>14</sup> The Red Cross needed to find a way to express these ideals in a cohesive recruitment campaign that would convince American women of the 1910s to enroll.

Christopher Capozzola argued that the First World War saw the rise of an American society based around "coercive volunteerism." At its worst, it silenced, sometimes violently, the voices of pacifists and others who objected to the war.<sup>15</sup> Capozzola discusses women's involvement in this process at length, but primarily focused on how they functioned in the home front economy as workers. Exploring the Red Cross nursing service's recruitment campaign during the early years of the war presents another angle into the ways in which women were brought into the war effort.

Prior to World War I, nurses generally operated within the domestic sphere of American life. Women often worked privately in patients' homes for little pay. The American public had only just begun to view nursing as a legitimate profession.<sup>16</sup> In a 1917 recruiting pamphlet, the Red Cross emphasized the professional opportunities that came with Red Cross service. American Red Cross leadership saw the war's potential to grow the profession for the long term. The brochure proclaimed the ability for a Red Cross nurse to earn an "assured livelihood" and demonstrate her capability as a genuine medical professional. Red Cross leaders envisioned the upcoming war as a potential way to cultivate new leaders in American nursing, and highlighted the possibilities for nurses to attain advanced positions of leadership within the Red Cross and other medical organizations. At the same time, the brochure also attempted to rally women with a nationalist message, arguing that it was their duty to join the Red Cross, comparing time in nursing school as an honorable duty akin to that of the drafted men training in Army camps. Alongside this call to patriotism, the brochure emphasized the domestic and humanitarian work that Red Cross nurses would be doing, such as prenatal care and in-home care for the sick and elderly. Beyond the implications of patriotic obligation, the brochure had no details regarding the duties of a Red Cross nurse at war.<sup>17</sup>

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American Red Cross recruitment posters simultaneously emphasized humanitarianism and militarism. One 1917 recruitment poster stated that “the Red Cross serves *humanity*,” emphasizing the universal nature of the organizations healing mission. Another poster calling for “Five Thousand [nurses] By June” depicted a Red Cross nurse in the foreground, with a military encampment behind her.<sup>18</sup> The American flag stands tall above the military side of the camp, and the Red Cross flag on the other. In this portrayal, the Red Cross was depicted as an extension of the military. If the Red Cross served humanity at large, then the United States military at least would be its first, if not primary customer. While various recruitment campaigns muddled the nature of service with the Red Cross, they all shared a similar theme of American patriotism. One rather blunt poster depicted a Red Cross nurse soothing a wounded American Soldier, while in the background—ostensibly somewhere far from the nurse’s location—Soldiers charge across “No Man’s Land,” bayonets fixed, carrying the American flag into battle, all the while a nurse sits at a desk ready to enlist women.<sup>19</sup> If soothing the pain of the sick and injured was the Red Cross nurse’s primary duty, assisting in the prosecution of the American war effort held near equal importance. The posters implied that the nurses would be far from the fighting, somewhere that they could tend to the wounded safely. As we see from the stories of Estelle and other nurses I discuss later, this would not be the case. The Red Cross advertisement campaign continually reinforced a humanitarian mission that laid within a nationalist framework. In its posters, the Red Cross affirmed its place as an American organization that dedicated itself to supporting the American military. Yet still, it did so by stressing the humanitarian work its nurses would do to support the mission, and not necessarily focusing on the employment of nurses near the front. Moving from an interpretive lens, if we turn to the early days of the war we uncover very tangible problems stemming from the relationship between the Army and the Red Cross.

#### BUREAUCRATIC LIMITATIONS

The ambiguous relationship between the Red Cross and the Army created logistical problems from the onset of the war. The Army took no responsibility for providing equipment to American nurses during the war, even those that were officially part of the Army Nursing Corps. The Office of the Quartermaster cited the original plan crafted by Red Cross and Army leaders prior to the war, which placed the obligation to outfit nurses solely on the Red Cross. Although the policy was no doubt shortsighted, it should also be understood in the context of the era. Prior to the war, there were only a few hundred active nurses. During peacetime, nurses were not considered an organic element of modern militaries,

and leaders from all organizations believed the logistics plan sufficient. Yet even as the first nurses began deploying to Europe, the Red Cross could not keep up with equipment requirements of a field environment.<sup>20</sup>

During the early days of American involvement in the war, the Red Cross struggled to properly outfit nurses for the kind of field duty they found themselves performing. Nurses received a single outfit consisting of a blue dress, overcoat, cape, and a few varieties of hats. Their winter issue consisted of a single blanket. The uniform issue did not include the white dress they were required to wear, and the nurses had to purchase the required white uniform out of their own pockets.<sup>21</sup> The whites would prove to be ill suited to the muddy conditions of field duty, and they eventually switched to a grey crepe uniform.<sup>22</sup> Over time, the Red Cross fleshed out its supply chain (with little help from the Army) and nurses received a more adequate collection of field equipment. The responsibility fell on Marie B. Rhodes, head of the Red Cross outfitting department. She leveraged assistance from the English and French chapters of the Red Cross, successfully procuring much needed equipment for nurses all over France. By the spring of 1919, Rhodes had spent over three million francs to outfit Red Cross nurses in Europe. Aside from uniforms, Army and Red Cross nurses also lacked a general military equipment issue. The allied armies generally issued their Soldiers “camp kits,” standard layouts that included a cot, blankets, cups, silverware, and other essentials of field life. Nurses received no such issue in the beginning, and had to rely on the generosity of French and British Red Cross supplies. The Army would eventually rectify the nursing equipment oversight, but not until December, 1918, a month after the war ended.<sup>23</sup> This equipment shortage was just one of many dilemmas Red Cross Nurses would face as the war developed. As conditions worsened on the front, the Army demanded the service of all spare nurses—military or not.

#### DURING THE WAR

The Red Cross attempted to retain a division of duties between those nurses that were sworn into the Army, and those that remained with the Red Cross. In March of 1917, just prior to the United States officially becoming a belligerent, the Army Nursing Corps maintained just 403 nurses on active duty, while the Red Cross listed nearly 8,000 nurses on their rolls.<sup>24</sup> Many of these nurses would fulfill their reserve obligation and be inducted into the Army Nursing Corps, but the Red Cross also sent hundreds of nurses, like Estelle, who retained their affiliation with the Red Cross. Red Cross and military leaders intended for these women to serve in the rear echelons during the war, far from the front lines. A

regulation from the 1916 United States Army's *Manual for the Medical Department* stated:

Except in cases of great emergency, Red Cross personnel serving with the land forces will not be assigned to duty at the front, but will be employed in hospitals in the service of the interior, at the base, on hospital ships and along lines of communications of the military forces of the United States.<sup>25</sup>

However, as casualties soared and the medical situation in eastern France deteriorated, the plans for a separation of duties came to an end. In the spring of 1918, the fighting became so intense that AEF leadership urgently requested a surge of medical personnel to the front.<sup>26</sup> The Red Cross responded by ordering their nurses in Paris to serve near the front in field and surgical hospitals.

Writing about her experience being militarized, one unnamed nurse described how as the German spring offensive of 1918 kicked off, her cohort of Red Cross nurses was sent to the Army hospital at Beauvais. The hospital was poorly equipped, and was little more than an old French schoolhouse with beds. There they worked under threat of constant German air raids. Fearing that German warplanes might spot the hospital, the hospital commander banned the use of lamps and matches. During one particularly bad raid, she wrestled to calm men suffering from "shell shock," all in pitch black conditions. She referred to her service there as being "loaned to the military," but in fact she was essentially operating as a military nurse. Without any kind of military training, she was working under the command of an Army doctor, subjecting herself to as much danger as any woman in the Army Nursing Corps.

Anna Johnson was another such nurse. The Red Cross sent her from Paris to Evacuation Hospital No. 9 at Vaubecourt, just outside of Verdun. There, Johnson and other Red Cross nurses worked in dreadful conditions alongside Army medical personnel. They worked in torrential downpours that would turn the entire base hospital into what she called a "sea of yellow clay mud." Johnson and other nurses at Vaubecourt worked excruciatingly long hours, and often felt that their efforts to save the injured were in vain. Patients streamed into the hospital from the front, but oftentimes the medical staff could do little but try and ease their suffering.<sup>27</sup> Johnson had few qualms about working so close to the front lines. She was among many Red Cross nurses that were exceedingly proud to support her countrymen. As the AEF continued to demand more medical personnel near the front, countless Red Cross nurses volunteered for the duty. Like so many men, these women often viewed service near the front as the epitome of patriotic duty.

Writing about the situation after the war, Julia Stimson wrote that when the call went out for service near the front, there always were more volunteers than the Red Cross could afford to send. She believed that "No special credit should be given [to] the nurses who achieved it," because "a hundred were anxious to take her place."<sup>28</sup>

#### FORCED MILITARIZATION

Despite Stimson's claim, there were some nurses who did not share her patriotic sentiments. The official history of the Army Nurse Corps in the Great War sheds some light on the story of these women. Julia Stimson, Superintendent of the Army Nurse Corps wrote the history in 1927, wherein she downplayed some of the issues that arose from the convoluted relationship between the Red Cross and the Army. However, in doing so she confirmed one important fact: The Red Cross (or the Army depending on your point of view) had forced some nurses into service with the Army Nurse Corps when these women had been expressly promised that they would remain under the purview of the Red Cross, and would serve in a civilian capacity. Prior to American involvement in the war, many of these women adamantly expressed that they had no desire to serve in the military. The Red Cross leadership soothed their fears that no such change in status would occur. In her book, Stimson acknowledged the forced enlistment, but explains that Army Nurse Corps and Red Cross officials in France were unaware of any such previous agreements with the Red Cross staff in the United States. She explained that ultimately, the rapidly filling military hospitals warranted such extreme measures, explaining:

The office in Paris had no means of knowing this fact or of the institution of this policy on the part of the Red Cross in Washington, but even if it had been known it is not probable that any other action could have been taken than that which was taken when these nurses were called from their civilian work and placed upon active duty with the Army.<sup>29</sup>

A memo written to Stimson just a month after the armistice further expands upon the issue of forced enlistments. Clara Noyes, the Director of the Field Nursing Service Bureau, lamented that she and her aides had personally assured a great number of women that they would not be enlisted into the Army. She wrote:

There was no nurse who left this country who did not have a formal communication from me saying that it was definitely understood that she was accepting service under the Red Cross and that transfer to the Army would not be permitted.<sup>30</sup>

Noyes did not feel particularly sympathetic to those women who did not wish to serve in the Army, calling

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them “unpatriotic” later on in the same letter. She and other Red Cross and Army leaders believed that the circumstances of the war in 1918 necessitated the measures taken, but still lamented that they had broken trust with so many women.

The problem of forced enlistments continued to fester after the armistice. Women enlisted into Army Nurse Corps, including those who had never wished to serve in the military, remained in the Army for some time. This only exacerbated the moral conundrum for the nurses that had never intended to be a part of the military. They had joined the Red Cross with the promise of humanitarian work, and now found themselves still languishing in the AEF, months after hostilities had ended. Some nurses officially protested the situation, stating that they believed the Army was irrevocably militarizing the Red Cross, and destroying the concept of neutrality on which the organization had been founded.<sup>31</sup> The crisis did not last too long however, as over the following few months base hospital units rapidly demobilized and the Army released most nurses from service. Those nurses who wished to remain in active service could apply directly to the superintendent of the Army Nurse Corps. However, either due to lack of interest, or more likely bureaucratic impediments, only forty-eight nurses had gone through this process by mid 1919.<sup>32</sup>

#### CONCLUSION

Problems stemming from the complicated relationship between the Red Cross and the Army continued to appear long after the armistice. Red Cross and Army Nursing Corps leaders lobbied successfully for Army nurses to receive rank in 1920, and then again for equal retirement benefits in 1926. However, the legislation regarding retirement only applied to “active nurses” and not those who had entered the Army Nursing Corps as “reserve nurses” through the Red Cross, even if they had continued to serve on active duty after the war. One such nurse, Edith Thorsen, attempted to retire from active duty in 1930 but was denied retirement by the Army comptroller on the basis that she was not eligible for retirement as a “reserve nurse.” The case made its way to federal court in 1934 where the judges eventually decided unanimously in Edith’s favor.<sup>33</sup> Still, she had trudged through a legal battle over four years, spending precious time and money to fight the Army over a matter of semantics in the law’s interpretation. The fact that Edith had entered the Army through the Red Cross labelled her as a “reserve nurse” for the entirety of her career. Jane Delano’s plan had certainly succeeded in supplying nurses in a time of need, but it had a variety of unintended effects long after the war ended.

Searching for its place in the war effort, Red Cross leadership struggled to balance its role as a civilian humanitarian organization while simultaneously serving as a de facto wing of the American military. The recruitment campaign in 1917 and 1918 laced principles of militarism and humanitarianism in a way that found widespread appeal among American nurses. Those like Estelle Davis joined the Red Cross out of a deep-seated sense of patriotism. In an interview in 1982, she said that she “could not think of not being with my country at a time like that.”<sup>34</sup> Like many other nurses, Estelle did not find anything odd in the dynamics of her service with the Red Cross. She understood the Red Cross’ principles in the same way that the organization depicted them: service with the military was humanitarianism. Yet still there were others who felt that the Army had forced militarization upon them, even though the Red Cross had promised no such thing would happen. As we find ourselves deep in the centennial of the United States’ participation in the First World War, this essay tries to recover both the stories of nurses like Estelle and also those Red Cross nurses that believed in a different brand of humanitarianism. While others have documented the struggle of pacifists and other anti-war protestors,<sup>35</sup> these particular nurses have unfortunately been silenced by the historical record.<sup>36</sup>

In her book *Doughboys, the Great War, and the Remaking of America*, Jennifer Keene argues that the integration of citizen-Soldiers into the Army profoundly shaped both the Army bureaucracy and American society after the war. Keene points out that despite the coercive power of the military establishment at the time, citizen-Soldiers made their voices heard and significantly impacted the way the U.S. Army trained and fought.<sup>37</sup> In the same light, civilian Red Cross nurses represented a critical element of the American war effort. Through their valiant efforts in the face of danger, both Red Cross and regular Army nurses cemented the place of nurses as a permanent asset within the Army’s medical establishment.

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#### ACKNOWLEDGEMENT

This essay was made possible in part by the US Army Center of Military History through the Omar N. Bradley Historical Research Grant. There exists no conflict of interest.

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# The Figures of Experience: A Brief History of Risk and Planning Within the Army Medical Community

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The experience set forth herein is largely that of the World Wars. Weapons and methods of warfare have changed since that time and such changes have always been reflected in battle casualties. The experience of the American Civil War would have proved to be largely unreliable in 1917-1918; and it may well be that the experience of the World Wars will prove to be equally unreliable in future wars. But, even if such an experience is of no greater value than to serve as the basis of an educated guess, it is still better than no experience at all.<sup>1</sup>

To engage in war is to engage with risk. From the American Revolution onward, American military leaders strove to reduce the cost in human lives inflicted by war. For most of the 18th and 19th centuries, American military commanders — officers charged with applying human and material resources against an opponent — believed risk was seated in the unknowable and unpredictable nature of conflict. They perceived reduced casualty rates as the happy consequence of a sound military plan. However, around the start of the 20th century, there arose within the American military medical community a perception that casualty rates represented a risk that could be predicted and controlled. This shift in perception was predicated on two events: the vast amounts of casualty statistics that only entered the printed record following the American Civil War, and the invention of tabulating and sorting machines that could reveal the patterns within this data. Within the recorded statistics of casualties, some medical officers believed, there existed a mathematical logic that could be deciphered to quantify risk in future engagements. Reduced casualty rates, then, were not a consequence but the actual goal of proper medical planning based in a mathematical approach to controlling risk. Spurred by the American experience during World War I, these events led to the publication of *Field Manual 8-55 Medical Field Manual*, which represented the culmination of the US Army's institutional experience with casualties in war, and a revolution in thinking about risk within the US Army.<sup>2</sup>

The collection of casualty data following the Civil War represented a first step towards risk prediction and

mitigation on the battle field. This collection of statistics became the foundation of Army medical planning in the beginning of the 20th century and represented a deliberate choice to represent casualties as a martial risk that could be understood and, perhaps, controlled. Military medical officers recognized that in order to ensure sufficient medical staff and supplies prior to an expected battle, they had to predict the number of battle casualties in advance. This was a problem that military thinkers on both sides of the Atlantic examined closely as Europe, and shortly thereafter, America entered into an unprecedented world war.<sup>3</sup>

America's entry into World War I heralded a massive mobilization and deployment effort that revealed the inadequacies of military medical planning and casualty prediction. By November 11, 1918, America sent over 2,000,000 service members to the European continent, the first American expeditionary force of this scale.<sup>4</sup> It soon became the opinion of World War I medical planners that the experiences of the American Civil War, preceding US conflicts, and observations of other wars were of little value.<sup>5</sup> New technologies, weaponized gas, and trench warfare represented new risks which rendered past experience completely obsolete in the eyes of the Army medical community. While military medical planners recognized the new challenges — mainly, that “the field army could not rely on evacuation to the United States in a few days” — articulating the medical equipment and personnel necessary for proper medical care without a relevant planning method proved problematic.<sup>6</sup> As a result, casualty predictions were sporadic and inaccurate, and the medical planning based on these predictions were wholly inadequate. The Meuse-Argonne offensive, for example, laid bare the consequences of inadequate casualty prediction and medical planning. Though military commanders understood the importance of providing adequate medical care, “they knew that transportation was so urgently needed for many other things that they did not feel that it should be provided for unnecessary hospital equipment or personnel.”<sup>7</sup> Because there was no suitable method of predicting battle casualties, only 18,000 hospital beds were planned to support an American force of 600,000.<sup>8</sup>

American battle casualties exceeded 18,000 in the first few days alone, requiring the evacuation of over 10,000 Soldiers, delaying lifesaving surgery and increasing morbidity and mortality rates.<sup>9</sup> The failure of military medical planners to predict casualties in advance of a combat operation represented a medical risk effecting tens of thousands of service members.

The military medical community knew it had to do better. This task fell to the Army's Medical Statistics Section, and to one officer in particular, Major Albert Gallatin Love. Love, a career Army Medical Officer, was assigned to the Medical Records section of the Surgeon General's Office in Washington, DC in 1910.<sup>10</sup> This position familiarized Love with the administration of the Surgeon General's Office and the methods of collecting and organizing the medical statistics of the Army. As Love would later write, this experience gave him the "unusual opportunity to become indoctrinated with the spirit that animated the office with the desire to improve the organization and administration of the medical service so that it could better preserve the health of the Army."<sup>11</sup> Love had a passion for medical statistics and for serving the Army's medical needs, traits that would set him apart in the eyes of his superiors. Love's assignment paved the way for his return to the Surgeon General's Office in 1917 as a newly promoted major, when he assumed charge of the Medical Records Section, Division of Sanitation.<sup>12</sup>

Major General William Gorgas, then Surgeon General of the Army, recognized that the poor performance of the Army's medical service in World War I highlighted the need to upgrade the Medical Department's record keeping and revisit the use of statistics to aid medical planning. Gorgas charged Love with overhauling the Medical Records Section and updating the records keeping process. As head of the Medical Records Section, and later as chief of the Medical Statistical Division, Love oversaw the collection and analysis of all the Army's medical statistics. Not only was Love responsible for the statistical tabulation of casualty data from the front, his department also worked with the Provost Marshall's office to tabulate the medical statistics of Selective Service registrants.<sup>13</sup> A proficient operator could process 1,500 cards a day, and by the end of the war, Love's section had processed the statistics of over 2.5 million records.<sup>14</sup> From 1921 until 1927, the Statistical Division under Love's leadership compiled over 2,000 pages of data, representing the complete statistical record of the anthropological, medical, and casualty data of World War I. Using a computerized tabulating machine and statistical analysis, Love generated mathematical models that accomplished three significant things: a

predictive model for hospital admission and discharge rates; a model predicting discharged patients that could return to battle; and a model that could predict combat casualties prior to an engagement.

Titled "War Casualties: Their Relation to Medical Service and Replacements," Love's study was published in *The Army Medical Bulletin Number 24* in 1931. Writing in the Forward, Colonel C. R. Reynolds, commandant of the Medical Service Field School, noted that Love's study furnished "the soundest basis for war planning," but offered the following warning:

"These data must be subjected to careful analysis, taking into consideration all evident and conceivable factors creating or influencing them. Upon the figures of experience thus obtained, and comparing the past with present and future conditions, must be based our estimates of the losses to be expected and of medical service requirements in future military operations."<sup>15</sup>

Reynolds continued, writing that Love's study was "valuable in determining more accurately than by previous methods the hospitalization requirements in any given situation... [and] will also be of value in studies relating to personnel procurement and replacement."<sup>16</sup> Love also expressed lofty goals for his study, presenting what he described as "a system for estimating, on the basis of our casualty experience in past wars, the requirements for medical service including hospitalization and evacuation of front line casualties."<sup>17</sup> Love presented his system in a simple table that provided a "summary of the daily casualty rates... to be used as a basis for estimating the requirement for medical personnel and equipment" in future conflicts (Figure 1).<sup>18</sup>

The data in this table gave a method for estimating the medical needs prior to an engagement while also estimating likely replacement needs.<sup>19</sup> For example, an infantry regiment comprising 1,000 Soldiers engaged in "severe combat" could expect a battle casualty rate of 15%, or 150 combat casualties. Medical officers in World War I, attempting to predict casualties for units in combat, fell victim to their inability to predict casualties and provide consistent and sufficient medical care. Love's method appeared to mitigate this risk, as medical officers could now plan to have the appropriate amount of personnel, supplies, litters, hospital beds, etc, on hand prior to an engagement. It appeared that Love had exceeded expectations and the demands identified by Army planners in France a decade prior.

As early as 1936, it seemed clear to senior military leaders that another war was near at hand, and Love's model was considered an important contribution to military

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	Total Casualties	Killed in action	Wounded by:	
			Gunshot missiles	Gasses
Inf. Regiment	15.0	2.40	9.60	3.00
Inf. Division	6.0	.96	3.84	1.20
Army Corps	3.0	.48	1.92	.60
Field Army	1.5	.24	.96	.30

Figure 1. Love presented a full explanation of where he drew his data and how he applied statistical analysis. This table represented “casualty rates per 100(%) of unit strength suggested as a basis for estimating the necessary medical relief on severe combat days, as determined by the American Expeditionary Forces experience.”

planning necessary for this next conflict.<sup>20</sup> By 1941, mere months before the attack on Pearl Harbor, the Army instituted Love’s method as doctrine, the “fundamental principles by which the military forces or elements thereof guide their actions in support of national objectives.”<sup>21</sup> Published on March 5, 1941, as *FM 8-55, Medical Field Manual, Reference Data*, Love’s model became not merely a suggestion, but the institutionally sanctioned method for conducting objective military medical planning. As *FM 8-55* acknowledged, even if the planning method “was of no greater value than to serve as the basis of an educated guess, it [was] still better than no experience at all.”<sup>22</sup> This “educated guess” was all the Army had as World War II progressed and the Allies turned their eyes to the shores of Normandy and began planning the largest amphibious operation in military history.

British and American medical officers assigned to plan the cross-Channel invasion agreed that they needed a common basis for estimating casualties, but were hampered by the fact that in 1941 and early 1942 the size of the Allied assault force was not yet known, nor was there previous experience with amphibious assaults of the scale proposed.<sup>23</sup> The British planners offered generic predictions, virtually meaningless absent the invasion plan itself: 2% casualties in the embarkation area, 25% casualties during the assault itself, 10% casualties per month during subsequent fighting, with an estimated 22,500 total casualties on the first day of an invasion.<sup>24</sup> Meanwhile, American medical officers advocated for the casualty estimation standards outlined in *Army Medical Bulletin No. 24* and *FM 8-55*.<sup>25</sup> Commenting on these documents in June of 1942, Colonel Paul R. Hawley (an American medical officer assigned to Britain in the fall of 1941, where he remained throughout the invasion planning) wrote that “insofar as battle casualties are concerned, these data are the most comprehensive in the world. The experience of [World War II] may indicate the necessity of revising these tables; but, until

such necessity is demonstrated, US estimates will follow this experience closely.”<sup>26</sup>

The first fully formed invasion plan, written by the British officer Lieutenant General Fredrick E. Morgan, acting as Chief of Staff Supreme Allied Commander (COSAC), called for three assault divisions and four detached brigades on the first day.<sup>27</sup> Morgan’s plan called for an invasion force of roughly 78,000 Soldiers, and this figure was the prime consideration for all other martial resources, especially landing craft.<sup>28</sup> In fact, when Morgan presented his plan to the Combined Chiefs in July 1943, he advised that “in proportion as additional shipping, landing craft, and transport aircraft can be made available, so the chances of success in the operation will be increased.”<sup>29</sup> Although the primary consideration for Morgan’s request of additional landing craft was the size of the invasion force, it also hinged on the casualty forecast devised by COSSAC planners. In his report to the Combined Chiefs of Staff, Morgan asserted that “unless suitable ships and/or crafts are earmarked and adapted where necessary for [the evacuation of wounded], adequate provisions for medical evacuation will not be possible.”<sup>30</sup> While Morgan admitted that “no definite plan at present exists for the evacuation of wounded during the assault,” the casualty estimate forecasted a total of 19,500 wounded in the first 48 hours of the invasion.<sup>31</sup> This figure accounted for 25% of the planned invasion force, indicating that Morgan and the COSSAC medical staff used the generic estimation method established by British planners the previous year. In fact, the chief medical officer advising COSSAC was a British doctor named Lieutenant Colonel G. M. Denning, and his small informal section also included a Royal Navy medical officer. It is possible that these men applied the British method of casualty estimation to Morgan’s original invasion plan.<sup>32</sup>

While these initial numbers derive from the British method, COSSAC documents make it clear that the

American doctrine for casualty estimation became the favored method as early as mid-August of 1943. A table titled "casualty estimates" produced by the COSSAC medical staff and dated August 12, 1943 placed total casualties for the first day of the invasion at 26,223.<sup>33</sup> Written in pencil at the top of the casualty table a planner noted that the data was derived using "Gen Love's Scheme of Estimation of Casualties."<sup>34</sup> In addition to the British officers serving on the COSSAC medical staff, an American medical officer named Lieutenant Colonel Thomas J. Hartford also advised the medical support planning for OVERLORD.<sup>35</sup> Hartford was on loan from the medical section of the American command structure set up to oversee American forces in Britain, and would have been aware of *FM 8-55* and *Army Medical Bulletin No. 24*.<sup>36</sup> Whether or not Hartford had direct influence on the casualty estimation is unknown, but an unsigned memo dated September 25, 1943 and produced by the COSSAC operations staff outlined a new method for estimating casualties.<sup>37</sup> The memo stated that given that the "battle casualty rate will depend upon the strength, composition and organization of the forces involved and the type and severity of the action anticipated," a new method would be used to "determine the severity of losses."<sup>38</sup> This new method, adopted merely two months after Morgan submitted his report, mirrored that process outlined in *FM 8-55* (Figure 2).<sup>39</sup>

Love's model forecasted significantly more casualties than the figures cited by Morgan to the Combined Chiefs of Staff in July, and this new estimate served to amplify Morgan's request for additional transportation craft. As a result, in order to meet the demands of the cross-Channel invasion, American war production increased its output of landing craft by 25% at the direction of the US Chiefs of Staff.<sup>40</sup> Even with this increase, the COSSAC staff faced the fact that landing craft was "the most critical item of equipment for the world-wide strategical

program. Every operation contemplated [was] a landing of one sort or another," and the plan for OVERLORD was in competition for this scarce resource.<sup>41</sup> A comparison of expected casualties to available evacuation craft illustrated the point of these urgent requests for more landing vessels. An April 1944 study showed that after the first wave of landing craft delivered the initial invasion force, the US wounded would reach 4,600. Meanwhile, the second wave of landing craft, scheduled to arrive within hours on the next tide, was supposed to evacuate these casualties after depositing fresh troops. The only problem was that after the second wave landing craft were reconfigured to accommodate litters, they could only evacuate a total of 1,950, leaving an excess of 2,650 casualties on the beach. The study showed that this excess of wounded would continue to compound from D Day through D+14, when the number of available landing craft for casualty evacuation would finally catch up with demand.<sup>42</sup>

Though casualty estimates drove much of the discussion over landing craft resources, the significance of these estimates reached well beyond landing craft alone. SHAEF planners used the casualty estimates to request hundreds of thousands of hospital beds, tens of thousands of bags for patients' personal effects, dozens of hospital trains capable of transporting thousands of casualties at a time throughout Britain.<sup>43</sup> Medical supplies included hundreds of blankets, a hundred liters of blood, splint sets, cases of dressings, and boxes of plasma for every landing craft.<sup>44</sup> Military litters were converted to fit into civilian ambulances, while hundreds of medical officers and thousands of corpsmen were assigned to support the invasion and the expected number of casualties.<sup>45</sup> Meanwhile, commanders used the casualty estimates to plan for replacements needed to keep the invasion moving forward.<sup>46</sup>

BATTLE CASUALTY RATE			
Type of formation	"Light" battle day	"Severe" day	"Maximum" day
Brigade or regiment	2.5	15	25
Division	1	8	15
Corps	.5	3	5
Army	.35	1	2.5

Figure 2. Comparing the rates listed in this table to the rates listed in *FM 8-55* make it clear that COSSAC planners adopted the American doctrine of casualty estimation. There are only minor points of variation between the above table and that printed in *FM 8-55*. For example, the "Light" battle day" column above is a direct copy of the "Average for all days in line" column listed in *FM 8-55*. While *FM 8-55* lists a range for "Severe battle day" (ie, 12-15, 6-8, 2-3, and .7-1.5) the table above adopts the high end of these ranges. Finally, *FM 8-55* lists 35% for Brigades and 12% for Divisions on "Maximum battle days." There is no accounting for the adjustments made in the table above, but while the reader may conclude the adjustment to 25% for a brigade or regiment listed above is evidence of the influence of the British method, it should be noted that the British method applied a 25% casualty rate to all types of formations, without variation.

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In the aftermath of the invasion, Love's casualty prediction model proved less than accurate. While the realities of a chaotic battlefield prevent an exact count, historians generally place Allied casualties closer to 10,000 — far less than the number predicted by Love's model. Nevertheless, after World War II, *FM 8-55* was updated to include new elements of warfare that were not present in the World War I data Love used.<sup>47</sup> Statistics from armored, amphibious, and airborne operations, as well as casualty data from the Korean and Vietnam wars were incorporated into future planning manuals.<sup>48</sup> These updated manuals stated that Love's method was "designed for rough, quick estimates only and not as a substitute for factors carefully chosen to fit the specific assumptions and conditions of a particular operation plan."<sup>49</sup> The updated publications advise planners that experience "clearly indicates that the estimation of probable casualty rates in advance is not a simple matter that can be reduced to a general formula," while providing updated formulas based on Love's original work.<sup>50</sup> Nearly 100 years later, Albert Love's legacy and influence on Army medical planning lives on.

of the Civil War came from Prussian officers and their examination of battle casualties in the Franco-Prussian War. In general, there was a fascination among American officers of how the Prussians organized their staff and planning structure, and of their approach to war fighting. It is no exaggeration to state that the structure of the American Expeditionary Force in World War I was based on the Prussian model.

ENDNOTES

1. *Field Manual 8--55 Medical Field Manual Reference Data* (Washington, DC: United States Government Printing Office, 1941), 46.
2. For this paper I will use the modern military definition of casualties: "Any person who is lost to the organization by having been declared dead, duty status – whereabouts unknown, missing, ill, or injured" (JP 1-02, 2010). Medical planning, especially by World War II, attempted to predict not only casualties resulting from battle, but also rates of sickness and disease. The word "casualties" was ubiquitous and did not always make a distinction between casualties occurred in direct combat with an opposing force versus casualties caused by illness or accidents. As much as possible I will try to use the term "battle casualty" to specifically refer to killed, wounded, or missing as a direct result of combat operations. Otherwise I will use the more general term "casualty" to refer to military service members who become sick, or otherwise sustain an injury or are killed by causes not related to direct combat with an enemy force.
3. D. M. Giangreco, "Casualty Projections for the U.S. Invasions of Japan, 1945-1946: Planning and Policy Implications," *The Journal of Military History* 61, no. 3 (1997): 527. As Giangreco points out, as does Paul Straub (a medical officer in the Philippine War and Medal of Honor recipient who was the first to develop a casualty prediction model in America), much of the methodology applied to the statistics

4. Marvin A. Kreidberg and Merton G. Henry, *History of Military Mobilization in the United States Army: 1775-1945* (Washington, DC: Department of the Army, 1955), 336.
5. Love, Albert Gallatin, Eugene L. Hamilton, and Ida Levin Hellman, *Tabulating Equipment and Army Medical Statistics*, (Washington, DC: U.S. Government Printing Office, 1958), 87.
6. Sanders Marble, "How Many Hospitals to Deploy?," *The AMEDD Historian* 17 (2017): 3.
7. Love, et al, 87.
8. Marble, 3.
9. Ibid.
10. Rohlader, Esther E., "A Concise Biography of Brigadier General Albert Gallatin Love, M.C.," (Washington, D.C.: General Reference and research Branch, Historical Unit, US AMEDS, Forest Glen Section, Walter Reed Army Medical Center, 1964), 1-8.
11. Love, et al, 31.
12. Rohlader.
13. Ibid., 73. There is a separate history behind Love's project to analyze Selective Service data. The Provost Marshall intended that the data analysis aid future wars by providing estimates for uniforms, equipment, etc., based on the measurements of the "average" draftee. Additionally, this data was used to gather eugenics data on draftees and much of the data published by Love and the Medical Statistics Division presents data on race, geography, health, etc.
14. Ibid., 64 and 74.
15. Love, v.
16. Ibid.
17. Ibid., vii.
18. Ibid., 122.
19. Included in Love's monograph was a lengthy discussion of what percentage of casualties who would return to the front based on their wound or injury. This information is not presented in the table above, but medical planners would account for this as they allocated medical supplies and personnel into their

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- plan. For example, of the 12.6% expected wounded in an infantry regiment, some percentage of that would quickly recover and be discharged from a care facility and be returned to the front.
20. Patton, George, letter to George C. Marshall, 26 August 1936, private collection of Ellene Winn, Baltimore, MD, 1 January 2017. On the occasion of Marshall's promotion to brigadier general, Patton wrote that "as things look now we seem to be about to have some sort of a new war, dont [sic] forget me if we do." At the time Patton was a lieutenant colonel assigned to a military intelligence unit in Hawaii.
  21. Joint Publication 1-02 Dictionary of Military and Associated Terms (Washington, DC: United States Government Printing Office, 2010), 71.
  22. Field Manual 8-55, 46.
  23. Sanford V. Larkey, Administrative and Logistical History of the Medical Service Communication Zone, European Theater of Operations, Record Group 498, Entry 54a, Stack Area 290, Row 57, Compartment 18, Shelf 4, Box 164 (College Park, M.D.: National Archives of the United States), 59.
  24. *Ibid.*, 60.
  25. Blanche B. Armfield, Medical Department, United States Army, Organization and Administration in World War II (Washington, D.C.: Office of the Surgeon General, Department of the Army, 1963), 305.
  26. Paul Hawley, "M.P.S. (42) 9, War Cabinet, BOLE-RO Combined Committee (London) Provision of Medical Services SubCommittee," June 14, 1942. Record Group 112, Entry 31 (ETO), Stack Area 390, Row 17, Compartment 5, Shelf 2, Box 309 (College Park, M.D.: National Archives of the United States), 1.
  27. "War Cabinet, Chiefs of Staff Committee, Operation 'Overlord' Report and Appreciation with Appendices," July 30, 1943, File: Committee Report COSSAC Planning Report (Dwight D. Eisenhower Library, Walter Bedell Smith Papers).
  28. *Ibid.*, 85.
  29. *Ibid.*, ii.
  30. *Ibid.*, 106.
  31. *Ibid.*
  32. Graham A. Cosmas and Albert E. Cowdrey, The Medical Department: Medical Services in the European Theater of Operations (United States Army in World War II: The Technical Services) (Washington D.C.: Center of Military History), 153. From the primary historical record available to me at this time, it is impossible for me to definitively state what methodology was used to forecast casualties when Morgan presented his plan to the Combined Chiefs of Staff in July of 1943. I draw my conclusion by comparing the number of casualties Morgan presented (19,500) against the proposed assault force (~78,000). The resulting percentage is 25%. It is clear that if COSSAC planners in July, 1943 were using Love's method, then the appropriate planning factor for Division casualties would have been 15%. Thus, I conclude the 25% method was a carryover from the ROUNDUP staff that were reassigned to support COSSAC.
  33. "Operation 'Overlord,' Casualty Estimates (Excl of Air Force)" August 12, 1943, Evelyn A Sutton Papers. The Museum of Military Medicine Archives, Keogh Barracks, U.K.
  34. *Ibid.*
  35. Cosmas and Cowdrey, 153.
  36. *Ibid.*
  37. "Estimate of Casualties," September 25, 1943, Entry 1, Evelyn A Sutton Papers, The Museum of Military Medicine Archives, Keogh Barracks, U.K.. For the same reasons cited previously, I cannot definitively state that Hartford was the driving force behind the revision in planning method. From the record it is clear that the method of estimating casualties changed between July and August of 1943, and that the subsequent casualty prediction changed as well. The record also makes explicitly clear that the method used to forecast casualties was the method outlined by Love. As Hartford was the senior American medical planner on the COSSAC staff, it is reasonable to infer that he was a part of this change.
  38. *Ibid.*
  39. *Ibid.*, 1a.
  40. "Minutes of COSSAC Held on Saturday, 28th August 1943, Report on 'QUADRANT,'" August 30, 1943, Record Group 331, Entry 3, Box 122 (College Park, M.D.: National Archives of the United States).
  41. *Ibid.* During this meeting the staff conference discussed comments made during the QUADRANT Conference, and this quote was among the items discussed, originally stated at the QUADRANT Conference.
  42. "Table - Estimated Casualties," April 8, 1944, File SHAEF 370-05 MED, Record Group 331, Entry 65, Stack Area 290, Row 7, Compartment 34, Shelf 4, Box 3 (College Park, M.D.: National Archives of the United States).
  43. "Conference with General Hawley," April 18, 1951, Page 4, File HD 000.71 Interviews Shooock-Beasley-Fitzpatrick-Gorby-Hawley-Kenner McGee Mason Welch, Record Group 112, Entry 1013 (Center

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- of Military History Refiles, Stack Area 390 Row 18, Compartment 19, Shelf 02, Box 1 (College Park, M.D.: National Archives of the United States), and “Receipt and Shipment of Bags, Patient’s Effects,” June 1, 1944, File 370.05 Planning-Evacation-ETO, Record Group 112, Entry 31 (ETO), Stack Area 390 Row 17, Compartment 5, Shelf 3, Box 311 (College Park, M.D.: National Archives of the United States), and “Page 13 - Reception and Distribution of Casualties in U.K. - Operation OVERLORD, Appendix A,” May 25, 1944, File Evacuation, Record Group 112, Entry 31 (ETO), Stack Area 390 Row 17, Compartment 5, Shelf 3, Box 312 (College Park, M.D.: National Archives of the United States).
44. “Initial Evacuation of Casualties from Far to Near Shore: Army/Navy Responsibilities,” May 19, 1944, File 370.05 Planning-Evacation-ETO, Record Group 112, Entry 31 (ETO), Stack Area 390 Row 17, Compartment 5, Shelf 3, Box 311 (College Park, M.D.: National Archives of the United States)
45. F. J. Horne, “LSTs Special Fittings for Casualty Evacuation, page 1,” October 28, 1943, File COS-SAC, Record Group 112, Entry 31(ETO), Stack Area 390, Row 17, Compartment 4, Shelf 6, Box 282 (College Park, M.D.: National Archives of the United States).
46. “Operation ‘Overlord,’ Casualty Estimates (Excl of Air Force)” August 12, 1943, Evelyn A Sutton Papers. The Museum of Military Medicine Archives. Keogh Barracks, U.K.
47. Giangreco, 528.
48. *Ibid.*, 529.
49. *Field Manual 101-10-1/2: Staff Officers’ Field Manual, Organizational, Technical, Data Planning Factors (Volume 2)* (Washington, D.C: Headquarters, Department of the Army, 1987), 4-7. See Giangreco, p 540, “Early in the Pacific war, medical and campaign planners built their casualty estimates as best they could using tables constructed from the U.S. Army’s World War I experience when factored with projected troop strength, operational plans, and intelligence estimates of Japanese capabilities, terrain, and relative firepower. By the invasion of the Philippines, planners at various echelons in MacArthur’s headquarters were able to realistically replace the World War I baseline figures with data compiled from the hard-won battles on and around New Guinea.” Giancrego does not provide any reference or analysis to back up this claim, but the idea of incremental updates based on timely data is an accepted fundamental of sound forecasting. If Giangreco is correct, it would seem inexcusable that planners in the European Theater did not do the same thing because they too, like their counterparts in the Pacific Theater, had access to all the casualty experience of the British.
50. *Ibid.*, 5-1.

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