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The Role of Birds in Arboviral Disease Surveillance in Harris County and the City of Houston, Texas

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Abstract

Avian arboviral surveillance is an integral part of any disease-based integrated mosquito control program. The Harris County Public Health Mosquito and Vector Control Division has performed arboviral surveillance in the wild birds of Harris County and the City of Houston since 1965. Blood samples from live trapped birds were tested for arboviral antibodies to West Nile virus (WNV), St. Louis encephalitis, Eastern equine encephalitis, and Western equine encephalitis. A dead bird surveillance program was created in 2002 with the arrival of WNV in Harris County. Since implementation, the program has detected considerable variability in viral activity with annual WNV seroprevelance rates ranging from 2.9% to 17.7%, while the percentage of positive dead birds has ranged from 0.3% to 57.2%. In 2015, 1,345 live birds were sampled and 253 dead birds were tested, with WNV incidence rates of 16.5% and 5.9%, respectively.

The Avian Section of Harris County Public Health (HCPH) Mosquito and Vector Control Division (MVCD) is responsible for monitoring avian populations in Harris County and the City of Houston, Texas, for West Nile virus (WNV), St. Louis encephalitis (SLE), and other mosquito-borne diseases. Since birds are the main hosts for many of these viruses, they are included in mosquito surveillance activities to provide a complete picture of the enzootic cycle rather than simply monitoring mosquitoes alone. While infected birds are only viremic for a few days,[1] surviving birds produce detectible levels of virus specific antibodies for a much longer period, up to several years.[2,3] This persistence allows for a more long-term "big picture" surveillance effort that can identify the presence of a virus in a new area even if no virus is currently circulating.

In 1999, the first sign that WNV had reached the United States was due to large numbers of dead birds, primarily crows, in New York City.[4] Subsequently, wildlife and disease ecologists noted continued large die-offs of crows and Blue Jays as the virus spread across the continent.[5] Harris County Public Health MVCD created a Dead Bird Hotline for residents to report dead birds. The first detection of WNV in the Houston area was from a dead Blue Jay in 2002.

Since WNV has been established in Harris County, avian monitoring helps explain the complex ecological interactions responsible for outbreaks. For humans to be infected, the virus must first amplify in the mosquito-avian transmission cycle. Avian antibodies, like human ones, are important to immune responses and make surviving, seropositive birds essentially resistant to reinfection.[6] In standard epidemiological susceptible-infected-removed (SIR) models, large numbers of immune individuals reduce the viral transmission rate below a sustainable level, bringing outbreaks to an end.[7] Although more research is needed, there is evidence that some arboviruses, such as WNV and SLE, follow multiyear SIR cycles. A 40-year history of SLE seroprevelances in Harris County demonstrated a consistent cyclical pattern while a multiyear field study in Chicago found that the best predictor of WNV activity in a given year was the proportion of seropositive birds at the end of the previous year.[2] Similarly, in Los Angeles, a preseason seropositive rate of less than 10% was associated with WNV outbreaks.[8] This study provides the results from the 2015 MVCD avian surveillance activities.

Collection Methods

Live bird trapping was conducted daily throughout the year, except when prevented by inclement weather. Trapping locations were located in Houston or Harris County parks and varied on a rotational basis. Sites that were chosen for live bird trapping provided the most uniform coverage available, ie, included historic and current WNV and SLE "hot spots" and provided a high degree of trapping success. In 2015, 27 sites were trapped, with 20 monitored biweekly from June to December when

seasonal help was available, and on a monthly rotation during the rest of the year (Figure 1).

During bird trapping, an average of 3 to 5 Japanese mist nets were set up before sunrise and maintained until 9:30 am. The nets were 2.6 m in height and 6 or 12 m in length, and attached to poles that were hammered into the ground with mallets. Nets were positioned in areas that were expected to attract birds or serve as flyways and baited with commercial bird seed at least one day prior to trapping. Specific characteristics indicating favorable areas included open spaces interspersed with layered vegetation, backyard bird feeders, open trash cans, and picnic tables. Nets were checked at least every 15 minutes with optimum trapping occurring when all nets were observed from one central location. After trapped birds were removed from the mist nets, they were placed in a holding bag, transported to a mobile laboratory station and processed on-site.

During processing, we recorded the bird's species, sex (if possible), age, and whether or not it was previously banded, as required by the Bird Banding Laboratory. We used the National Geographic Society *Field Guide to Birds of North America*[9] along with *The Sibley Field Guide to Birds of Eastern North America*[10] for species identification, and Pyle's *Identification Guide to North American Birds*[11] for ageing, sexing, and determining the correct band size. The age of a bird is of particular interest for WNV and SLE surveillance because positive hatch year birds indicate exposures that occurred within the current season. After we collected all pertinent information, we drew blood from the bird's jugular vein or brachial vein if it was a dove, because the jugular vein is frequently obscured by engorged skin in doves. We collected either 0.1 cc of blood for smaller (sparrow-sized) birds or 0.2 cc for larger birds (ie, Blue Jays) and added it to a corresponding amount of 0.4 cc or 0.8 cc diluent of 0.9% normal buffered saline in a 13 mm by

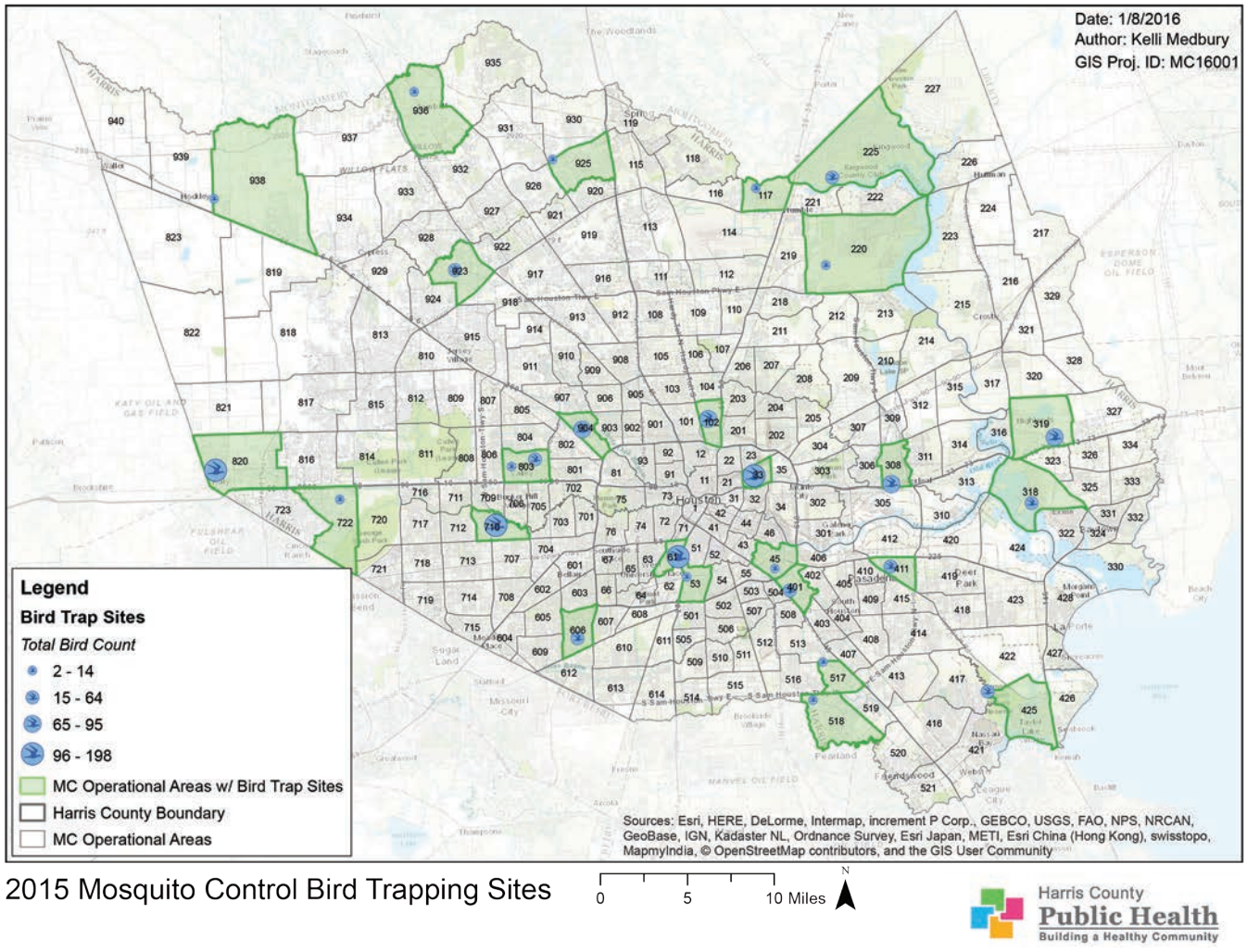

Figure 1. Map of Harris County and the City of Houston, Texas, showing bird trap sites in 2015.

100 mm glass culture tube. Once blood collection was complete, the bird was banded and released.

The culture tubes were labeled with a unique identifying number and kept in a refrigerated cooler while in the field. Once the diluted blood samples arrived at the MVCD Avian Laboratory, they were placed in a refrigerated (4°C) centrifuge which ran for 15 minutes at 2,500 rpm. The sera were extracted with disposable transfer pipettes into similarly labeled 2 ml cryovials, which were kept frozen until they were transferred to the University of Texas Medical Branch at Galveston (UTMB-G) or the MVCD Virology Laboratory for testing.

Most dead birds were reported to the Dead Bird Hotline, while a smaller number was reported to the HCPH website (https://secure.hcphes.org/MC/DeadBirdReport.html). Residents may also bring dead birds to the MVCD Avian Laboratory. Information collected on all birds included the contact information (name, address, and phone number) of the caller, location, condition of the bird, and time and date the bird was found. If the bird was found within 24 hours of the call and appeared reasonably fresh, the caller was instructed to double bag the bird and place it in the freezer or a bag of ice to preserve it until retrieved by a MVCD technician. In years with large numbers of dead bird calls, 18 additional drop off centers located throughout Harris County were activated. Once the bird was collected, it was identified and its condition evaluated. If the carcass was fresh and contained no maggots or ants, it was submitted for WNV/SLE testing and assigned a unique identifying number.

Testing Procedures

Since 2002, the UTMB-G has been conducting the initial screening of live bird samples and confirmatory testing of dead birds. Sera samples were screened with the hemagluttination inhibition (HI) test, which detected the presence of viral antibodies, but could not differentiate between antibody subtypes. Since some subtypes, such as IgG, can persist for long periods of time, this test cannot be used to distinguish currently infected birds from those that were infected in previous years. West Nile virus and SLE are also structurally similar, causing HI antigens to cross-react. If the titer levels for one virus were 4 times or more titers higher than the other, the virus with the higher titer was considered the causative agent.[12] If the causative virus was identified, the sample was considered positive solely for the causative virus. Samples in which the causative virus could not be determined were listed as WNV/SLE and analyzed separately.

To determine if the birds were recently infected, the MVCD Virology Laboratory preformed a second test, ie, the IgM antibody capture method, on all HI positive samples. This test does not cross-react and detects only IgM antibodies which are only produced during the initial stages of infection, and persist for about a month. A limitation of the IgM test is its potential for reacting to a naturally occurring antigen in the blood of some individual birds, resulting in a false positive rate of approximately 10%. Given these limitations, the most definitive results were from combining these tests. For example, a bird that tested both HI and IgM positive was definitely exposed and, most likely, that exposure was within the last month, indicating recent viral activity.

Dead birds were initially screened at the MVCD Avian Laboratory with vector and rapid analyte measurement platform (RAMP) test kits, both of which can also be used for mosquitoes. The vector test is a quick dipstick test that detects SLE and Eastern equine encephalitis (EEE) in addition to WNV. While the RAMP test takes longer, it is more accurate and provides quantitative results. For both tests, the bird's throat was swabbed with a cotton-tipped applicator. For vector tests, the applicator was swirled in a 0.6 ml vial containing 600 µl of grinding solution for 10 seconds. A test strip was placed in the vial and left to mature for 15 minutes. Each virus had its own specific detection zone on the test strip. For RAMP testing, a separate applicator was swirled in a 1.5 ml vial containing 1 ml of RAMP buffer solution for 10 seconds. Then, 120 µl of the supernatant was transferred to a smaller 0.6 ml vial where it was mixed with a florescent dye. Finally, 70 µl was transferred to the sample well of the test cartridge and dried for 90 minutes. The dry cartridge was placed into a RAMP reader which provided a quantitative ratio in a range of less than 10 to more than 640. Results of 50 or greater were considered positive.

After testing, dead birds were labeled with weatherproof tags, double-bagged and placed in an ultra-low (-62°C) freezer until they were transferred to UTMB-G. Information on the tags included date, location, species, and MVCD Avian Laboratory number. If more than 25 birds were collected during the week, only those that tested positive or species with historically high rates of WNV were transported to UTMB-G for tissue culture and WNV confirmation. During tissue culture, a small portion of brain was removed from each dead bird and was homogenized in approximately 2.0 ml of phosphate-buffered saline, pH 7.4, containing 10% fetal bovine serum. After centrifugation at 10,000 rpm for 10 minutes, the supernatant was filtered (0.22 µm) and 200 µL was inoculated into a flask culture of Vero cells. Vero cultures were maintained at 37°C for 14 days and observed daily for viral cytopathic effect (CPE). If CPE

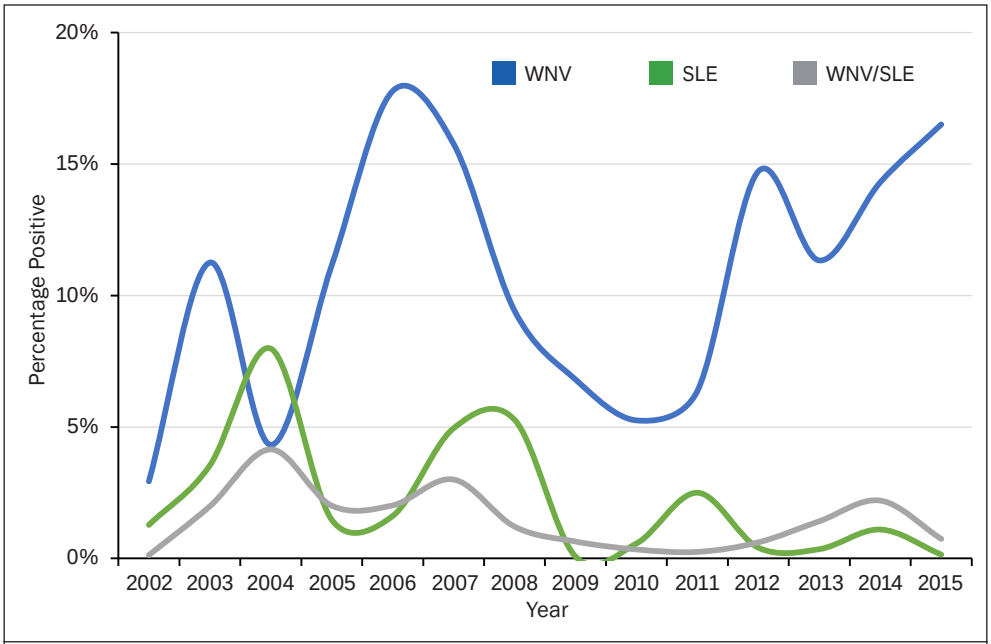

Figure 2. Percentage of live bird sera samples collected from Harris County and the City of Houston that tested HI positive for WNV, SLE, and WNV/SLE since 2002.

was observed, the culture was confirmed as positive for WNV by complement-fixation test or by the vector test antigen assay done on the culture medium. Due to funding limitations, an alternative confirmation method consisting of a RAMP result of 100 or greater, corresponding to a UTMB-G confirmation rate of 90%, was implemented by MVCD Avian Laboratory from October 14, 2015 to December 31, 2015.

The capture and handling of wild birds was done in accordance with the US Federal Migratory Bird Treaty Act[13] and required both federal and state permits. This work was conducted under Scientific Collecting Permit MB730179-0 from the US Fish and Wildlife Service, Federal Bird Banding Permit 09415 from the US Geological Survey, and Scientific Research Permit SPR-0816-179 from the Texas Parks and Wildlife Department.

Results

The MVCD Avian technicians collected a total of 1,345 sera samples and received 358 dead bird calls from 40 species of live bird species and 39 species of dead birds in 2015 (Tables 1 and 2). Sera collections increased over 2014, improving upon the 50% increase over the number of samples collected in 2013 and surpassing all previous years since 2009. Of the dead birds called in, 319 (89%) were collected and 253 (79%) were fit for testing. Fifteen dead birds (5.9% of those tested) were confirmed positive for WNV, while 222 (16.5%) sera samples were HI positive for WNV, 2 (0.15%) were HI positive for SLE and 10 (0.7%) were HI positive for either WNV or SLE. Additionally, 2 samples each were HI positive for EEE and Western equine encephalitis (WEE). Of the samples that were HI positive, 25 (10.6%) were IgM positive for WNV and 4 (1.6%) IgM positive for SLE. This represented about 1.8% and 0.3% of all samples. The percentage of HI positive samples for WNV increased slightly over 2014, maintaining the generally elevated numbers recorded since 2012 (Figure 2), while the percentage of IgM positives decreased by half or more (Figure 3). The percentage of positive dead birds also decreased from 2014 (Figure 4).

The percentage of WNV positives fluctuated throughout the year (Figure 5). The period of greatest activity was from July to September, 2015. There were no IgM positive samples in January. Between 5% and 9% of samples were IgM positive from February to June, 2015. This increased to 11% in July before peaking in August at 28%. The first positive dead bird was found on July 2, 2015. More followed, with all other positive dead birds, with one exception, occurring in July and August. The percentage of HI WNV positives peaked in September at 25%. The IgM WNV positives also remained high in September at above 20% before falling below 10% in October. There were no IgM positives in November and December. The last positive dead bird was found on November 6, 2015. The percentage of HI WNV positive samples was the only metric to remain elevated during the winter months, remaining at around 15% for January, November, and December 2015. This is not surprising given the persistence of WNV antibodies and the high levels of WNV activity in 2012 and 2014.

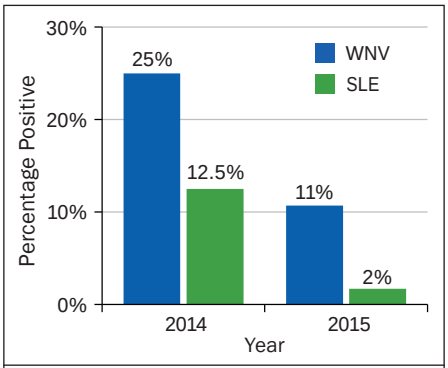

Figure 3. Percentages of live bird seropositive samples collected from Harris County and the City of Houston, Texas that tested IgM positive for WNV and SLE in 2014 and 2015.

Table 1. Number of live bird sera samples collected from Harris County and the City of Houston, Texas, that tested HI and IgM positive at the University of Texas Medical Branch, Galveston and the Mosquito and Vector Control Division in 2015.

Common Name	Scientific Name	Sera	HI WNV	HI SLE	HI WNV/SLE	HI EEE	HI WEE	IgM WNV	IgM SLE
House Sparrow	*Passer domesticus*	628	86	1	6	1	0	11	2
Blue Jay	*Cyanocitta cristata*	158	46	0	1	0	0	10	2
Northern Cardinal	*Cardinalis cardinalis*	117	54	0	1	0	0	2	0
Northern Mockingbird	*Mimus polyglottos*	66	11	0	0	0	2	2	0
Mourning Dove	*Zenaida macroura*	63	9	0	0	0	0	0	0
Myrtle Warbler	*Dendroica coronata*	48	0	0	0	0	0	0	0
White-winged Dove	*Zenaida asiatica*	37	3	0	1	0	0	0	0
Red-winged Blackbird	*Agelaius phoeniceus*	34	0	1	0	0	0	0	0
European Starling	*Sturnus vulgaris*	20	4	0	0	0	0	0	0
Common Grackle	*Quiscalus quiscula*	18	0	0	0	0	0	0	0
Great-tailed Grackle	*Quiscalus mexicanus*	18	2	0	0	0	0	0	0
Savannah Sparrow	*Passerculus sandwichensis*	17	0	0	0	0	0	0	0
Brown-headed Cowbird	*Molothrus ater*	15	1	0	0	0	0	0	0
Red-bellied Woodpecker	*Melanerpes carolinus*	13	1	0	0	0	0	0	0
Chipping Sparrow	*Spizella passerina*	12	0	0	0	0	0	0	0
American Robin	*Turdus migratorius*	11	2	0	0	0	0	0	0
Nutmeg Manakin	*Lonchura punctulata*	8	1	0	0	0	0	0	0
Carolina Wren	*Thryothorus ludovicianus*	8	0	0	0	0	0	0	0
Tufted Titmouse	*Baeolophus bicolor*	7	0	0	0	0	0	0	0
House Finch	*Carpodacus mexicanus*	5	1	0	0	0	0	0	0
White-throated Sparrow	*Zonotrichia albicollis*	5	0	0	0	0	0	0	0
Hermit Thrush	*Catharus guttatus*	4	0	0	0	0	0	0	0
Inca Dove	*Scardafella inca*	4	0	0	0	0	0	0	0
Yellow-bellied Sapsucker	*Sphyrapicus varius*	4	0	0	0	0	0	0	0
Downy Woodpecker	*Picoides pubescens*	3	0	0	0	0	0	0	0
Red-headed Woodpecker	*Melanerpes erythrocephalus*	3	0	0	0	0	0	0	0
Bronzed Cowbird	*Molothrus aeneus*	2	0	0	0	0	0	0	0
Brown Thrasher	*Toxostoma rufum*	2	1	0	0	0	0	0	0
Eurasian Collared Dove	*Streptopelia decaocto*	2	0	0	0	0	0	0	0
Loggerhead Shrike	*Lanius ludovicianus*	2	0	0	0	0	0	0	0
Rock Dove	*Columba livia*	2	0	0	0	0	0	0	0
Carolina Chickadee	*Parus carolinensis*	1	0	0	0	0	0	0	0
Eastern Bluebird	*Sialia sialis*	1	0	0	0	0	0	0	0
Eastern Phoebe	*Sayornis phoebe*	1	0	0	0	0	0	0	0
Gray Catbird	*Dumetella carolinensis*	1	0	0	1	0	0	0	0
Lincoln's Sparrow	*Melospiza lincolnii*	1	0	0	0	0	0	0	0
Orange-crowned Warbler	*Vermivora celata*	1	0	0	0	0	0	0	0
Purple Martin	*Progne subis*	1	0	0	0	1	0	0	0
Rose-breasted Grosbeak	*Pheucticus ludovicianus*	1	0	0	0	0	0	0	0
White-eyed Vireo	*Vireo griseus*	1	0	0	0	0	0	0	0
(40 Bird Species)	Totals	1,345	222	2	10	2	2	25	4

HI indicates hemagluttination inhibition. SLE indicates St Louis encephalitis. WEE indicates Western equine encephalitis.
WNV indicates West Nile virus. EEE indicates Eastern equine encephalitis. IgM indicates immunoglobulin M

Both HI and IgM results for WNV differed by age. After-hatch year birds had a HI seropositive rate more than double that of hatch year birds, while hatch year birds that were HI positive for WNV were twice as likely to be IgM positive (Table 3). This is consistent with after-hatch year birds maintaining protective immunity after being exposed to WNV within the previous 2 to 3 years. When disease prevalence in hatch year and after-hatch year birds was plotted by month, the differences were more pronounced (Figure 6). After-hatch year birds accounted for the majority of all positives until July, when hatch year IgM prevalence increased dramatically. After-hatch

Table 2. Number of dead birds collected from Harris County and the City of Houston, Texas, that were tested and confirmed positive at the University of Texas Medical Branch, Galveston, and the Mosquito and Vector Control Division in 2015.

Common Name	Scientific Name	Tested	Vector Test Positive	RAMP Positive	Confirmed Positive	Percentage Confirmed
White-winged Dove	*Zenaida asiatica*	61	0	0	0	0.0%
Blue Jay	*Cyanocitta cristata*	30	9	9	11	36.7%
European Starling	*Sturnus vulgaris*	22	0	1	0	0.0%
Blue-winged Teal	*Anas discors*	19	0	0	0	0.0%
House Sparrow	*Passer domesticus*	18	0	0	0	0.0%
Great-tailed Grackle	*Quiscalus mexicanus*	17	1	1	1*	5.9%
Mourning Dove	*Zenaida macroura*	14	0	0	0	0.0%
Northern Mockingbird	*Mimus polyglottos*	8	0	1	1	12.5%
American Robin	*Turdus migratorius*	7	1	0	0	0.0%
Cedar Waxwing	*Bombycilla cedrorum*	6	0	0	0	0.0%
Common Grackle	*Quiscalus quiscula*	6	0	0	0	0.0%
Rock Dove	*Columba livia*	4	0	0	0	0.0%
Yellow-crowned Night-heron	*Nycticorax violacea*	4	0	1	0	0.0%
Cooper's Hawk	*Accipter cooperii*	3	0	0	1	33.3%
Eastern Screech-owl	*Otus asio*	3	0	0	0	0.0%
Northern Cardinal	*Cardinalis cardinalis*	3	0	1	1	33.3%
Brown-headed Cowbird	*Molothrus ater*	2	0	1	0	0.0%
Carolina Wren	*Thryothorus ludovicianus*	2	0	0	0	0.0%
Eurasian Collared Dove	*Streptopelia decaocto*	2	0	0	0	0.0%
Hermit Thrush	*Catharus guttatus*	2	0	0	0	0.0%
Purple Martin	*Progne subis*	2	0	0	0	0.0%
American Coot	*Fulica americana*	1	0	0	0	0.0%
American Crow	*Corvus brachyrhynchos*	1	0	0	0	0.0%
Baltimore Oriole	*Icterus galbula galbula*	1	0	0	0	0.0%
Brown Pelican	*Pelecanus occidentalis*	1	0	0	0	0.0%
Chipping Sparrow	*Spizella passerina*	1	0	0	0	0.0%
Laughing Gull	*Larus atricilla*	1	0	0	0	0.0%
Lesser Snow Goose	*Chen caerulescens*	1	0	0	0	0.0%
Mallard	*Anus platyrynchos*	1	0	0	0	0.0%
Myrtle Warbler	*Dendroica coronata*	1	0	0	0	0.0%
Orange-crowned Warbler	*Vermivora celata*	1	0	0	0	0.0%
Ovenbird	*Seiurus aurocapillus*	1	0	0	0	0.0%
Red-eyed Vireo	*Vireo olivaceus*	1	0	0	0	0.0%
Red-winged Blackbird	*Agelaius phoeniceus*	1	0	0	0	0.0%
Sora	*Porzana carolina*	1	0	0	0	0.0%
Tennessee Warbler	*Vermivora peregrina*	1	0	0	0	0.0%
Veery	*Catharus fuscescens*	1	0	0	0	0.0%
White-faced Ibis	*Plegadis chihi*	1	0	0	0	0.0%
Yellow-breasted Chat	*Icteria virens*	1	0	0	0	0.0%
(39 Bird Species) Totals		253	11	14	15	5.9%

*Includes bird confirmed in MVCD Avian Laboratory. RAMP indicates rapid analyte measurement platform.

year IgM rates did not undergo a similar increase until August. Hatch year HI rates were generally low, peaking at 21.5% in September, about 15% less than after-hatch year HI rates for the same month. There were no hatch year birds in January and February, and low sample sizes prevented the analysis of hatch year seropositive rates in March and April, which had one HI positive each.

Each species had a seropositive rate markedly different from the average. Northern Cardinals had the highest HI incidence rate of 46.1%, followed by Blue Jays at 29.1% (Figure 7). Of the HI positive samples, the majority were from House Sparrows (39%), Northern Cardinals (24%), and Blue Jays (21%) (Figure 8). The highest IgM incidence rates were in Blue Jays (21.3%), Mockingbirds (18.2%), and House Sparrows (11.8%), while Blue Jays and House Sparrows comprised the bulk of the IgM positive samples (84.0%). No HI or IgM positives were found in Myrtle Warblers, Red-Winged Blackbirds, Common Grackles, and Savanah Sparrows. Myrtle Warblers and Savanah Sparrows are migratory birds that only reside in the Houston area during

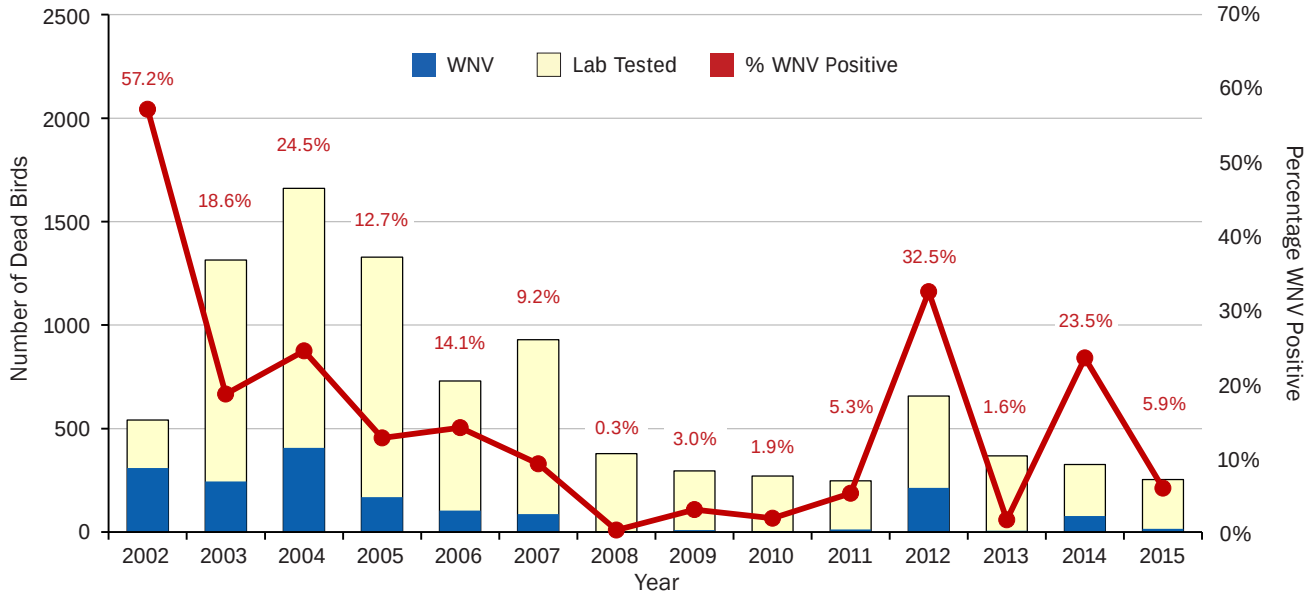

Figure 4. Number and percentage of dead birds collected from Harris County and the City of Houston, Texas, that were tested and confirmed WNV positive since 2002.

the winter months. House Sparrows were by far the most frequently tested species, caught 4 times as much as Blue Jays, the next most commonly caught species, and comprised 46.7% of all samples tested. The next 4 most commonly tested species were Blue Jays, Northern Cardinals, Northern Mockingbirds, and Mourning Doves, comprising 30.0% of all samples tested. Dead Blue Jays were also positive frequently, accounting for 73.3% of positive dead birds. All other dead positive bird species (Cooper's Hawk, Northern Cardinal, Northern Mockingbird, and Great-tailed Grackle) had one positive sample each (Figure 9).

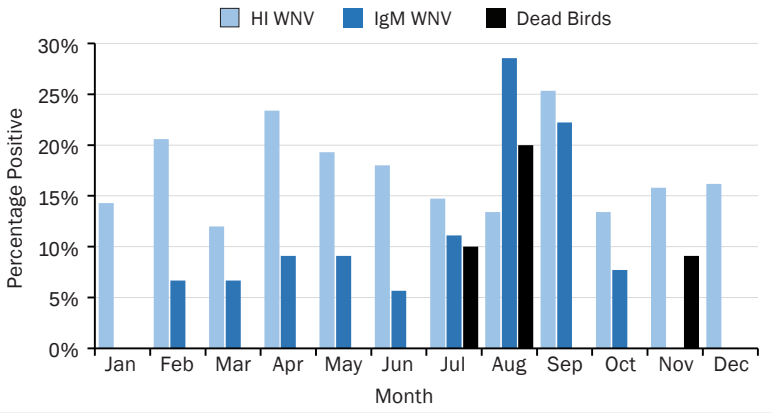

Figure 5. Monthly WNV activity in live and dead birds collected from Harris County and the City of Houston, Texas, in 2015.

Positives were not uniformly distributed across Harris County. Parts of the Harris County that had high prevalence rates were the Spring-Cypress area in the northwest, Kingwood in the north, and western Houston. Figure 10 shows the distribution of HI positive results within Harris County for all viruses in

Table 3. Number and percentage of live birds collected in Harris County and the City of Houston, Texas, that tested HI and IgM positive in 2015.

2015	Total Sera N	HI WNV (%N)	HI SLE (%N)	HI WNV/SLE (%N)	HI EEE (%N)	HI WEE (%N)	IgM WNV+ (%N)	IgM SLE+ (%N)
All Birds	1,345	222 (16.5%)	2 (0.1%)	10 (0.9%)	2 (0.1%)	2 (0.1%)	25 (10.7%)	4 (1.7%)
Age								
After Hatch Year	777	177 (22.8%)	1 (0.1%)	7 (0.9%)	1 (0.1%)	0 (0.0%)	16 (8.7%)	3 (1.6%)
Hatch Year	568	45 (7.9%)	1 (0.2%)	3 (0.5%)	1 (0.2%)	2 (0.3%)	9 (18.3%)	1 (2.0%)
Sex								
Male	395	91 (23.0%)	1 (0.2%)	3 (0.7%)	0 (0.0%)	0 (0.0%)	7 (7.3%)	0 (0%)
Female	234	42 (17.8%)	1 (0.4%)	2 (0.8%)	1 (0.4%)	0 (0.0%)	0 (0.0%)	1 (2.2%)
Unknown	716	89 (12.4%)	0 (0.0%)	5 (0.7%)	1 (0.1%)	2 (0.2%)	18 (19.3%)	3 (3.2%)

HI indicates hemagluttination inhibition. SLE indicates St Louis encephalitis. WEE indicates Western equine encephalitis.
WNV indicates West Nile virus. EEE indicates Eastern equine encephalitis. IgM indicates immunoglobulin M.

2015. The prevalence rate for WNV ranged from 0.0% to 28.6%, and areas with the highest percentages were in the center and southern part of Harris County. Only 6 samples tested positive for SLE, EEE, and WEE. Areas 411 and 425 tested positive for SLE, while EEE was detected in areas 820 and 904, and WEE was detected in 710 and 923, which was the first time it was detected since 2011.

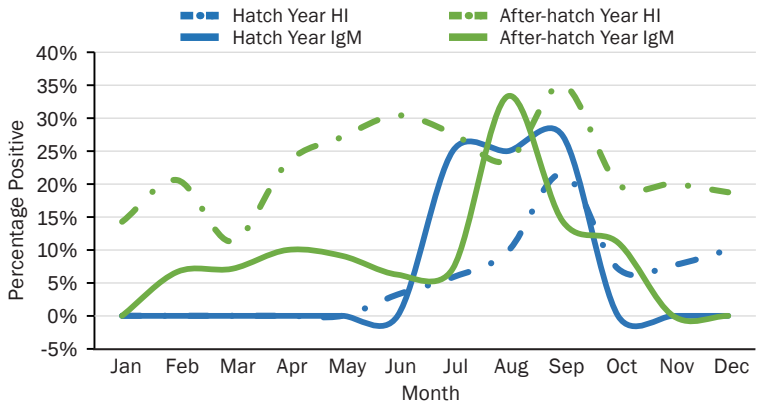

Figure 6. Monthly WNV activity in live hatch year and after-hatch year birds collected from Harris County and the City of Houston, Texas, in 2015.

Figure 11 shows the distribution of IgM results for WNV in Harris County. The percentage of IgM positive samples is the percentage of HI samples testing IgM positive and indicates the proportion of HI positives in each area that is due to a recent infection. The percentage of IgM positives in each area ranged from 0.0% to 36.4%. The areas with the highest percentage of IgM positives were 820, 225, and 606. Area 606 was the only one that had a high percentage of both IgM and HI positives.

Most dead birds were reported from the western loop and northwestern Harris County, near the towns of Spring and Cypress (Figure 12). Positive birds were clustered in Baytown and near the intersection of highways 610 and 290. Five more birds were confirmed from outlying areas northwest of the Sam Houston Beltway and one from the town of Humble.

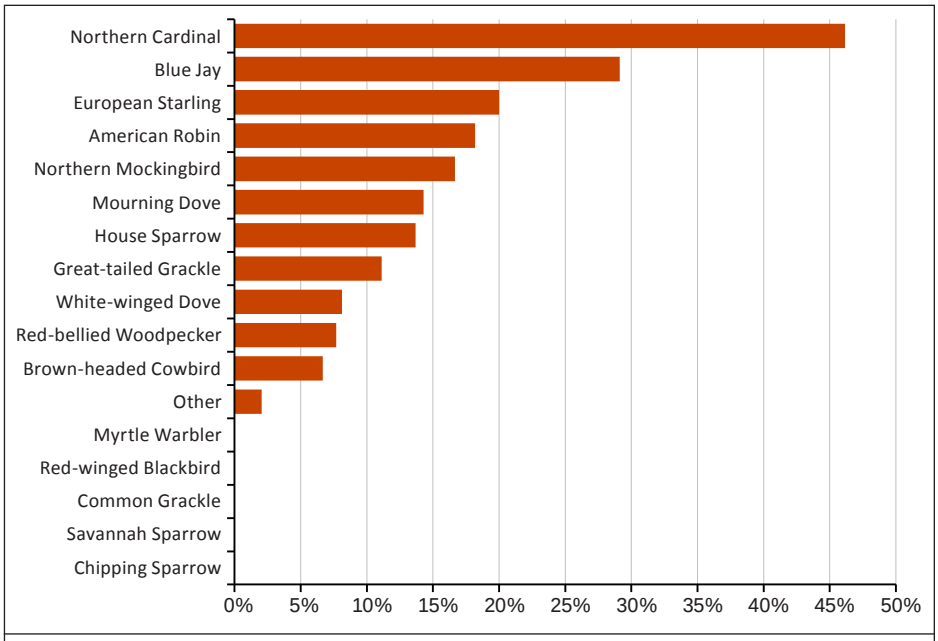

Figure 7. Percentages of live bird sera samples collected from Harris County and the City of Houston that tested HI positive for WNV in 2015.

Comparisons to Previous Years

In 2015, the percentage of positive confirmed dead birds was low, while the percentage of HI WNV positive live birds was high, relative to their historical occurrences (Figure 13). This could be explained by the high prevalence of antibodies in the avian population providing protective immunity against WNV resulting in the avian low death rate, which is reasonably well supported by the historical data. However, high antibody levels did not prevent a high death rate in 2014.

In 2002 when WNV was first detected in Harris County, birds were particularly susceptible as none had been previously exposed. Although SLE antibodies provide some protection against WNV,[6] they were at a historically low rate of 1.9%. Mortality was high, particularly for American Crows (68.4%) and Blue Jays (66.9%). It took 4 years for antibody levels to peak in 2006 at 17.7%, double the highest annual SLE rate from 1975 to 2001 of 8.4%. From 2006 to 2011, the mortality and seroprevalence rates decreased, until antibody levels reached 6% to 5% (Figure 13). In 2012, there was a sudden resurgence in WNV activity, with mortality rates reaching 2003-2004 levels. Antibody levels jumped to 14.7% and remained high throughout 2015. Recent mortality rates were inconsistent; 1.6% in 2013 and 23.5% in 2014.

Program Expansion

The objectives were to expand and improve the MVCD Avian Surveillance Program in 2016. Trapping sites were continually evaluated and subjected to change if catch rates decreased. Scouting for new trapping locations,

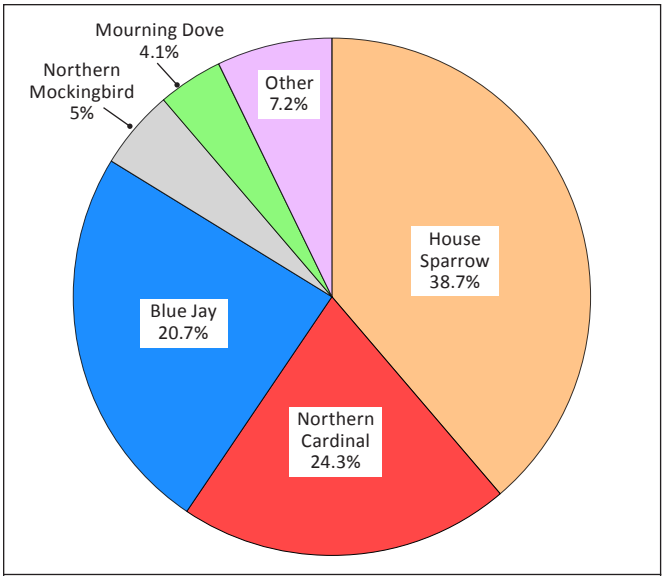

Figure 8. Species distribution of HI positive live bird samples collected from Harris County and the City of Houston, Texas, in 2015.

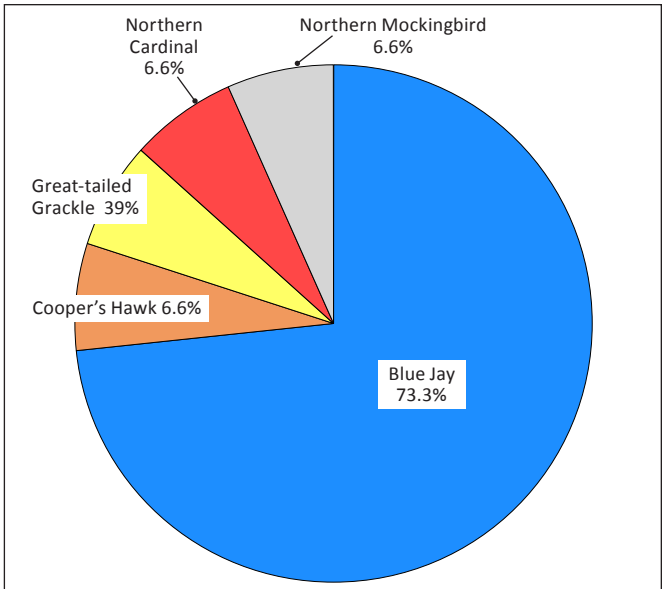

Figure 9. Species distribution of WNV dead birds collected from Harris County and the City of Houston, Texas, in 2015.

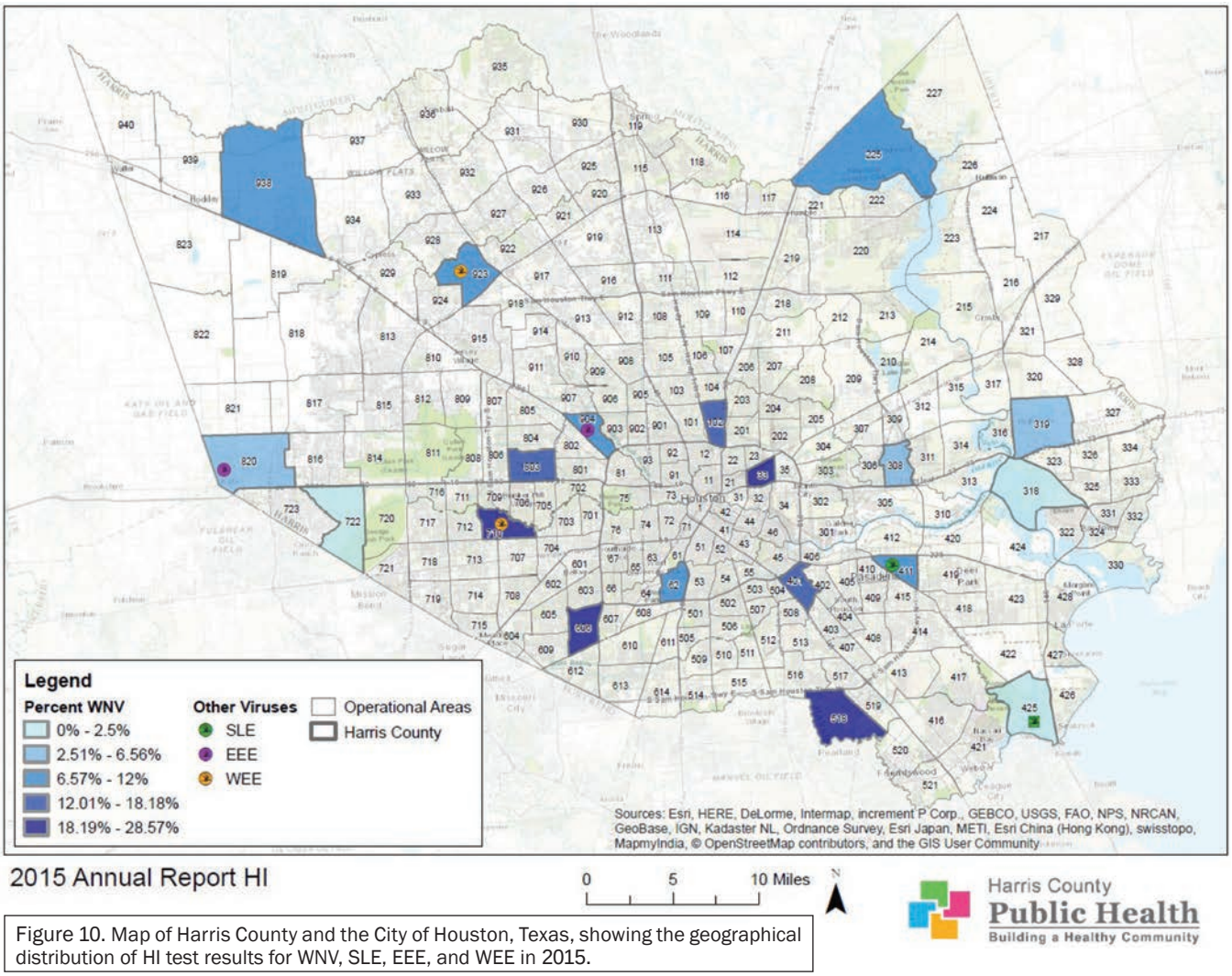

Figure 10. Map of Harris County and the City of Houston, Texas, showing the geographical distribution of HI test results for WNV, SLE, EEE, and WEE in 2015.

particularly in areas of high virus activity or low current coverage, were conducted as time permitted. Testing was expanded to include Highlands J virus (HJV), an alphavirus in the WEE complex. Although HJV is not thought to cause illness in humans, it can react with antigens for WEE similarly to WNV and SLE. Testing for both will allow for definitive identifications of WEE. Five sera samples have already tested HI positive for HJV in 2016.

Testing for non-arboviral viruses, such as Newcastle disease virus (NDV) and highly pathogenic avian influenza (HPAI) can also be considered. The UTMB-G has frequently recovered NDV, which is classified as a select agent by the US Department of Agriculture in pigeons and doves collected in Harris County and the City of Houston.[14] Although HPAI is not a vector-borne disease, it is a high-profile, economically devastating, and potentially life-threatening disease that uses avian species as the main host. In 2015, an HPAI outbreak occurred in at least 15 states (3 declaring states of emergency) resulting in the destruction of 49.5 million birds and at least $500 million spent in emergency funds.[15-17] The HPAI was also found in wild birds in Kansas and Missouri,[18] states that are part of the same migratory flyway that includes important wintering grounds in the Houston area. The HPAI may persist in wild birds and resurface from time to time in the United States as it has in Asia. Indeed, the 2015 outbreak was linked to incidences in British Columbia and the Pacific Northwest in 2014.[19] Another incidence was confirmed on January 15, 2016, in Indiana where 43,000 birds were destroyed as part of the containment effort.[20]

We plan to test for Avian Flu in 2017. Test kits are available from several commercial manufactures for use by veterinarians. Further testing by UTMB-G will be needed to confirm any positive samples as highly pathogenic.

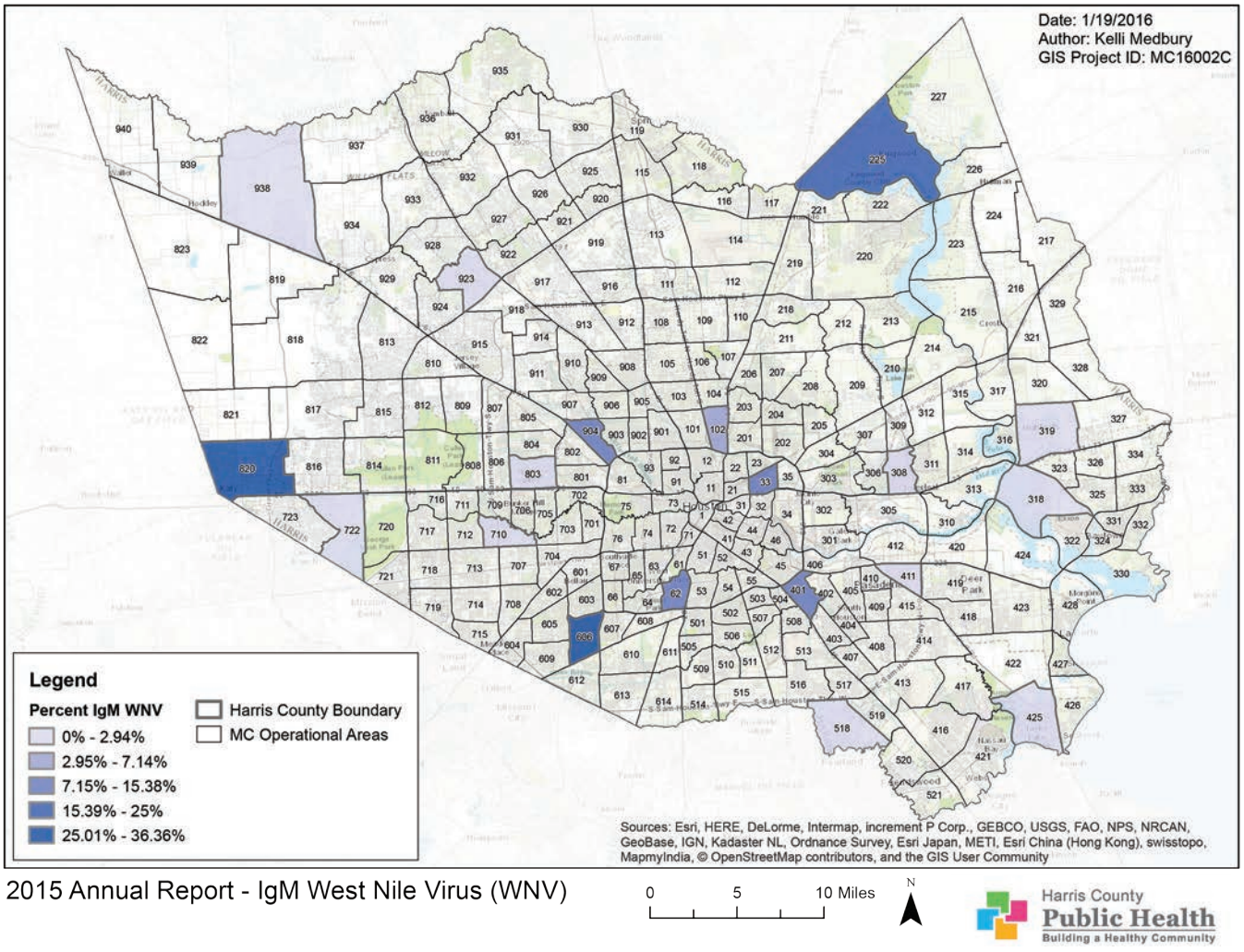

Figure 11. Map of Harris County and the City of Houston, Texas, showing the geographical distribution of IgM test results for WNV in 2015.

CONCLUSION

Avian surveillance is an integral part of disease-based integrated mosquito and vector management programs. Overall, an average level of Avian WNV activity was indicated in 2015, and low levels of EEE and SLE in Harris County and the City of Houston. However, WEE was last detected in 2011. Most infections were in residential birds. If viral activity conforms to SIR models, Harris County should be entering a low point in the WNV cycle. Initial results showed WNV activity was low in 2016, with only one confirmed dead bird. Low levels of viral activity will eventually diminish avian arboviral protection, inviting another outbreak. Continued surveillance is needed to determine when this will likely occur.

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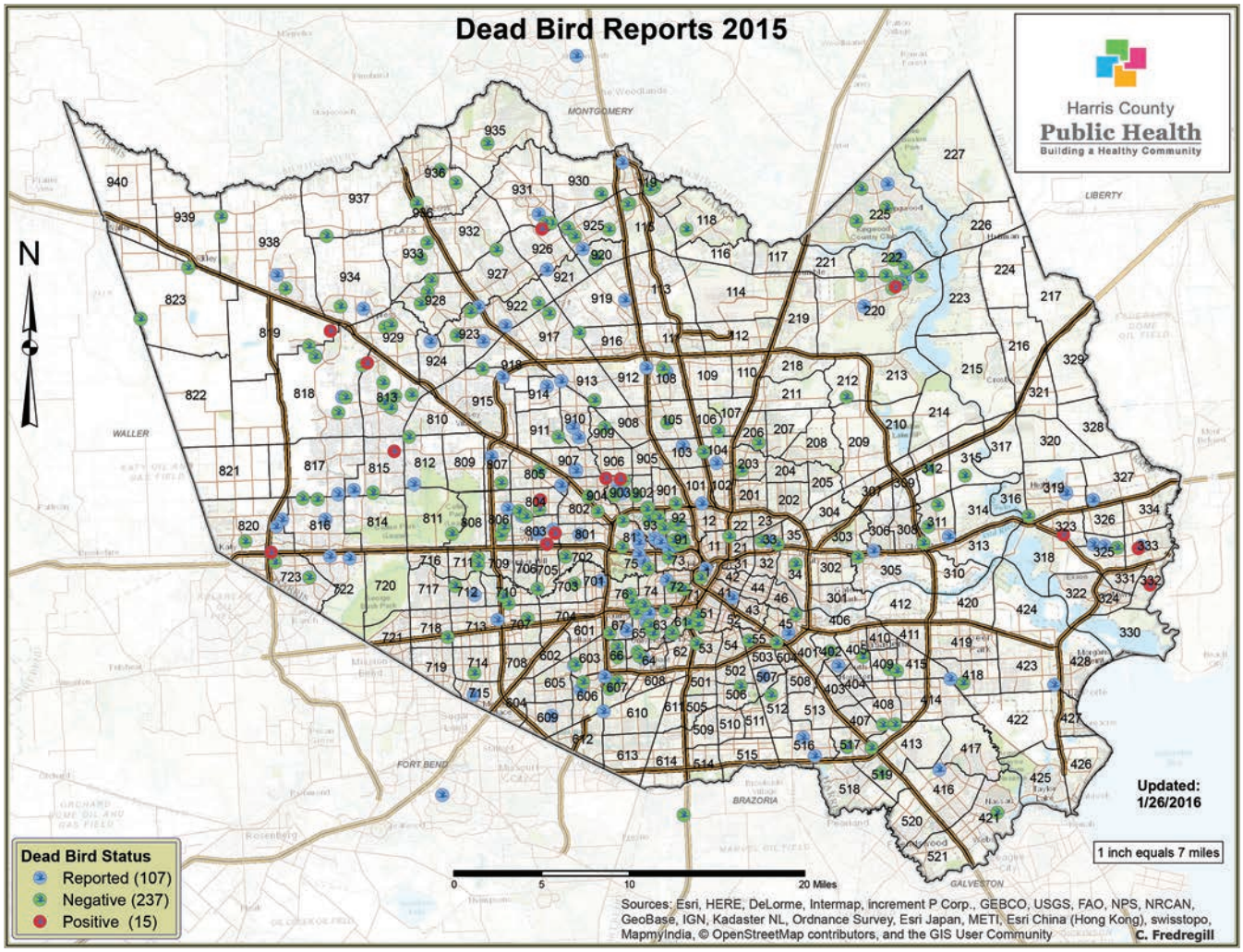

Figure 12. Map of Harris County and the City of Houston, Texas, showing the geographical distribution of dead birds reported, tested, and confirmed WNV positive in 2015.

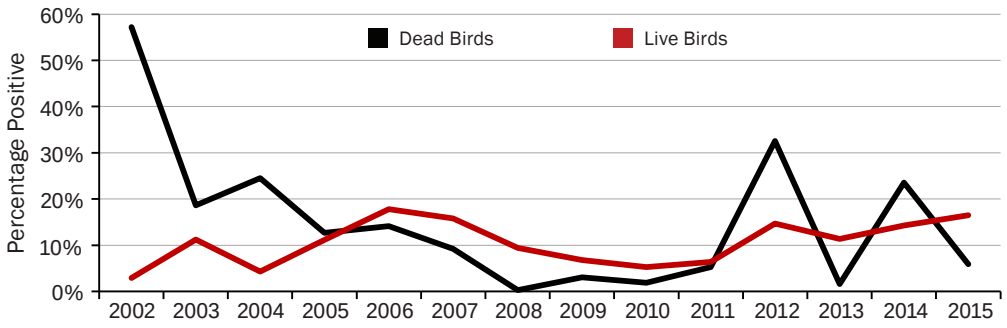

Figure 13. Percentage of dead birds confirmed positive and live birds that tested HI positive for WNV from Harris County and the City of Houston, Texas, since 2002.

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Laboratory and Semi-field Evaluations of Two (Transfluthrin) Spatial Repellent Devices Against *Aedes aegypti* (L.) (Diptera: Culicidae)

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Abstract

Two transfluthrin-based spatial repellent products (Raid Dual Action Insect Repellent and Home Freshener and Raid Shield (currently not commercially available), SC Johnson, Racine WI) were evaluated for spatial repellent effects against female *Aedes aegypti* (L.) mosquitoes under laboratory (wind tunnel) and semi-field (outdoor enclosure) conditions. The placement of either product in the wind tunnel significantly reduced host-seeking behaviors. The mean baseline (control) landing counts for the Raid Dual Action and Raid Shield were reduced by 95% and 74% respectively. Mean probing counts for the Raid Dual Action were reduced by 95%, while the probing counts for the Raid Shield were decreased by 69%. Baseline blood-feeding success was significantly reduced for both treatments: Raid Dual Action (100%) and Raid Shield (96%). Semi-field evaluations were conducted in outdoor enclosures at the Navy Entomology Center of Excellence, Jacksonville, Florida. A moderate reduction in mosquito entry into military style tents resulted when either product was placed near the tent opening. The Raid Shield reduced mosquito entry into tents by 88%, while the Dual Action decreased entry by 66%.

Aedes aegypti (L.), also known as the "yellow fever mosquito," is an important vector of viral diseases such as yellow fever, dengue, Chikungunya, and, most recently, Zika virus.[1,2] Despite current vector control efforts, the incidence and geographical expansion of these pathogens continue to increase at an alarming rate. For example, the incidence of dengue has increased 30 fold over the last 50 years.[3] The World Health Organization (WHO) estimates 50-100 million cases occur annually in over 100 endemic countries, putting almost half of the world's population at risk. More than 70% of the population at risk for dengue worldwide live in member states of the WHO southeast Asia and western Pacific region.[3] As a result, the Department of Defense (DoD) personnel and family members stationed in these regions are at risk.

Vector control remains the primary defense against vector-borne diseases. Standard preventive deterrence against vector-borne diseases incorporates integrated pest management (IPM) with personal protective measures (PPMs). The IPM approach to pest control integrates cultural, biological, physical, and chemical methods which minimize health and environmental risk. Often in situations when the threat of vector-borne disease transmission is high, direct suppression of vectors using pesticides is favored over nonchemical control methods. However, the repeated failure of conventional insecticides to control vector species in some areas continues to put Soldiers at risk. Furthermore, the failure to control vector populations may result in additional chemical applications that potentially contribute to other vector control problems such as pesticide resistance, unintended exposure to nontarget organisms, and environmental pollution.

The PPM standards mandated by the military include use of a topical repellent, N, N-diethyl-3-methylbenzamide (DEET) or 1-piperidinecarboxylic acid 2-(2-hydroxyethyl)-1-methylpro-pylester) (Picaridin), prop er wear of a permethrin-treated uniform, and the use of a bed net.[4] Additionally, malaria prophylaxis is prescribed when service members are deployed in endemic areas. However, compliance with PPM standards remains a problem among service members.[5-7] Sanders et al[8] reported that responders in a 2004 study of personnel returning from Iraq and Afghanistan indicated that a majority (51%) did not use DEET, even though most knew that it was readily available. A report on the use of PPM among confirmed malaria cases in 2012 revealed that compliance with 4 preventive measures (use of DEET, proper uniform wear, adherence to prophylaxis,

and postdeployment antirelapse therapy) was 0%.[9] Even when compliance with PPM is high, service members are still exposed to vectors during physical training or while off duty. Additional control methods are needed to augment the DoD's current vector control strategy.

Spatial Repellents

Recently, area (spatial) repellents have received interest as a novel system for vector control. Spatial repellents work by releasing chemicals into the air to reduce mosquito entry into treated spaces and to inhibit blood-feeding behavior. An additional benefit of this type of behavioral modification is a delayed or diminished development in the emergence of insecticide resistance.[10] Several types of commercial spatial repellent products have been reported in the literature: impregnated plastic or paper strips,[11] coils,[12-14] candles,[15-17] fan emanators,[18-21] heat-generating devices,[20-22] and microdispensers.[23]

Impregnated Plastic

Plastic or other substrates impregnated with fluorinated pyrethroids (metofluthrin and transfluthrin) can passively deliver spatial repellents without external energy input. Yayo et al[11] investigated the effect of Rambo Insecticide Paper (0.45% transfluthrin) on indoor resting densities and biting rates of 2 important malaria vectors (*Anopheles funestus* Giles and *An. gambiae s.l.*) at a peri-urban field site in Nigeria. A significant reduction in both indoor resting density (91%) and biting rate (86%) was reported. Passive emanators may be preferred by the DoD, as these devices would be most likely to meet logistical and safety requirements.

Mosquito Coils

Mosquito coils are the most commonly used household insecticidal product in the world.[12] Avicor et al[13] conducted a laboratory evaluation of 3 commercial coil products for protection efficacy against *An. gambiae* Giles in southern Ghana. All 3 coils (FISH M (0.005% metofluthrin)) 86%, Shieldtox (0.15% d-trans allethrin) 74%, and Tesco Value (0.3% allethrin) 72% showed high knockdown efficacy. Hill et al[12] demonstrated the effectiveness of using the Transfluthrin mosquito coil (Raid, SC Johnson, Racine, WI) in households to reduce malaria transmission. The Transfluthrin mosquito coil provided 77% protection against *Plasmodium falciparum* Welch. Msangi et al[14] assessed feeding inhibition and repellency of 3 pyrethroid brands (Kiboko, Total, and Risasi) of mosquito coils in experimental huts in northern Tanzania. Induced exophily was high for *Culex quinquefasciatus* Say (95%), but was low in *An. gambiae* (61%) for all products tested. Feeding inhibition was significantly reduced for both species: *Cx. quinquefasciatus* (97%) and *An. gambiae* (88%).

Fan Emanators

Fan emanators that vaporize metofluthrin are marketed as promising tools with long-lasting spatial protection against mosquitoes. Various studies have evaluated the efficacy of the Off! Clip-On device (SC Johnson). Xue et al[18] evaluated this device in the field in northeastern Florida, which proved to be 70% and 79% effective at repelling *Aedes albopictus* Skuse and *Ae. taeniorhynchus* (Wiedemann) from human test subjects. In a semi-field study in Israel, the device reduced biting on the arms of volunteers against *Ae. albopictus* by 96% and *Cx. pipiens* L. by 95%.[19] Lloyd et al[20] reported a 64% reduction in *Ae. albopictus* collected from Biogents Sentinel (BGS) traps located near the repellent device. The Off! Clip-On device has also been reported to have limited to no effect on certain species. In a semi-field study conducted by Dame et al,[21] the device failed to reduce the populations of *An. quadrimaculatus* Say and *Psorophora columbiae* (Dyar and Knab) collected in surrogate traps. Reduction rates for this study ranged from 8% to 16%.

Heat Generating Devices

The ThermaCell (Thermacell Repellents, Bedford, MA) spatial repellent device (active ingredient allethrin), is one of the most popular heat generating devices on the market. Collier et al[22] conducted a study in Louisiana using several spatial repellent devices (Dragon fly/Mosquito Cognito, ThermaCell, SC Johnson OFF! Mosquito Lantern) and systems to determine their effect on backyard mosquito population levels, in which the ThermaCell device was the most effective device in reducing mosquito populations (*Cx. salinarius* Coquillett, *Cx. quinquefasciatus*, and *Ae. vexans* (Meigen)). In a field study conducted in Jacksonville Florida,[20] the number of *Ae. albopictus* repelled by the ThermaCell was significantly higher than the other spatial repellents evaluated (Lentek Bite Shield and Bug Button Mosquito Eliminator).

The objectives for this study were (1) to evaluate the efficacy of two commercially available transfluthrin spatial repellents for inhibiting *Ae. aegypti* (L.) host-seeking behaviors (anemotaxis, landing, and blood feeding) under laboratory conditions, and (2) determine if these products were effective in reducing the number of host-seeking *Ae. aegypti* from entering a baited military-style tent in a controlled semi-field environment.

Materials and Methods

Mosquitoes

Aedes aegypti eggs (Liverpool and Orlando strain) were obtained from the US Department of Agriculture (USDA) Agricultural Research Service (ARS) Center

Figure 1. The SC Johnson spatial repellents tested in this study: (A) Raid Dual Action Insect Repellent and Home Freshener; (B) Raid Shield.

for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida. They were reared in an insectary using a 12:12 hour (light/dark) photoperiod at 26ºC and 35% to 50% relative humidity (RH). Larvae were fed ground tropical fish flakes (Tetra Werke, Melle, Germany). Adults were held in screened, 3.79 liter plastic buckets, and fed with a cotton pad moistened with 10% aqueous sucrose solution. Adult female mosquitoes were 5 to 10 days old and incubated in plastic buckets along with a water-moistened pad without sugar for 24 hours before they were used in bioassays.

Spatial Repellent Devices Evaluated

Two transfluthrin-based spatial repellent (SC Johnson Company) products: Raid Dual Action Insect Repellent and Home Freshener, and Raid Shield were evaluated in this study (Figure 1).

In 2012, SC Johnson, Cornell University's Center for Sustainable Global Enterprise, and the Bill and Melinda Gates Foundation created the WOW, which is a business concept that creates access to pest control products that can help prevent malaria in at-risk populations at the "base of the pyramid," as well as home-cleaning and personal care products valued by rural consumers. The Raid Dual Action Insect Repellent and Home Freshener is one of 6 products developed from the WOW program in Ghana. The product consists of a spray bottle (0.40% transfluthrin) and a colorful plastic poster decorated with a well-known Ghanaian adinkra symbol.

Raid Shield is a product developed for research purposes (not commercially available) by the SC Johnson Company. It is a clear plastic strip that is impregnated with transfluthrin (1%). Once the plastic strip is exposed to air, transfluthrin is slowly released into the immediate area.

Laboratory Evaluations (Wind Tunnel)

Laboratory evaluations were conducted in a 3.0 m x 61 cm x 60 cm wind tunnel (Figure 2A) at the USDA-ARS Invasive Insect Biocontrol and Behavior Laboratory in Beltsville, Maryland. With a few minor deviations, the wind tunnel was set up in accordance with Klun et al.[24] Air temperature in the chamber was controlled by a window air conditioner and baseboard electric heaters and maintained at 25ºC ± 4ºC in the tunnel. The RH inside the chamber was maintained at 25% to 35% using a misting humidifier. The upwind end of the tunnel was connected to the air conditioning chamber by a cowling that housed a 61 cm diameter fan driven by a rheostat-controlled Dayton Electric Model 2Z846A motor that pushed conditioned air through an air filter into the tunnel. Air flow within the tunnel was monitored using an air flow gauge, while temperature and relative humidity was recorded using a HOBO data logger (Onset Computer Corp, Bourne, MA).

The wind tunnel was fitted with a 6-well mosquito feeding reservoir (Figure 2B) that was positioned at the upwind end of the tunnel parallel to tunnel airflow. The reservoir was placed on a stand made of 0.5 cm thick Plexiglas with a 33.8 cm x 15.4 cm base and a 29.8 cm x 7.0 cm (length x width) upper shelf with 1.2 cm high edges at each end of the shelf to secure the reservoir on the

Figure 2. (A) Wind tunnel at the Invasive Insect Biocontrol & Behavior Laboratory, USDA-ARS, Beltsville, MD. (B) Mosquito feeding reservoir.

shelf. Positioned on the shelf, the top surface of the reservoir was 18 cm above the tunnel floor, at the center of the tunnel, and 47 cm away from the upwind tunnel screen. In order to maintain a constant temperature (38ºC) for the blood contained in the reservoir, it was modified and connected to a water bath circulator (Lauda E100, Wobser GMBH and Co, Konigshofell, Germany). A breath-delivery tube (2.5 m x 22 mm inner diameter; Tri-Anim, Sylmar, CA) ran from outside of the upwind end of the tunnel to breath exhaust position inside the tunnel 20 cm above the tunnel floor, at the tunnel center, and 20 cm from the upwind end of the tunnel.

Prior to each test, the upper surface of the feeding reservoir was coated with a thin layer of high-vacuum silicone grease (Dow Corning Corp, Midland, MI), and the wells were filled (approximately 6 ml capacity) with human blood from the Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, to which Adenosine triphosphate (ATP) was added on the day of testing to obtain a concentration of 10^{-3} mmol/L ATP. The filled cells were covered with an Edicol collagen membrane (G Street Fabrics, Rockville, MD). During treatment evaluations, the treatment device was suspended from the wind tunnel ceiling facing downwind and positioned 38 cm in front of the feeding reservoir.

Trials for each spatial repellent device were replicated 5 times. Each trial consisted of one control test followed by 5 consecutive treatment tests. The purpose of the control evaluation was to establish a baseline for mosquito activity under normal conditions (untreated) and to confirm the absence of pesticide contamination in the wind tunnel. A test began by placing a mosquito release canister containing 20 female *Ae. aegypti* (Liverpool-strain) inside the wind tunnel. Subsequently, human breath was expired from the breath-delivery tube for 5 seconds and the mosquitoes were released and allowed to freely fly inside the wind tunnel for 10 minutes. During this time period an observer recorded the frequency of probing and landing occurring on the mosquito feeding reservoir. Upon completion of the 10-minute period, the mosquitos were visually examined for evidence of knockdown from chemical exposure and feeding status (blood-fed or unfed). Mosquitoes were removed from the chamber using a vacuum aspirator.

Semi-field Evaluations (Enclosures)

Semi-field evaluations were conducted using 2 outdoor enclosures (Figure 3A), located at the Navy Entomology Center of Excellence in Jacksonville, Florida. Each enclosure consisted of a metal frame that measured 6.1 m wide x 10.7 m long x 3.4 m high. The enclosure was completely covered with mosquito screen and had a concrete base. Several potted plants were placed in the interior of each enclosure to mimic the natural outdoor habitat. A single military style tent (3-person) was placed at the far end of the enclosure, opposite the entrance. Each tent housed a BGS 2.0 trap baited with dry ice and BGS proprietary lure. Temperature, relative humidity, and wind speed were measured throughout the evaluation using a HOBO data logger.

Figure 3. (A) A semi-field enclosure used in this study. (B) A commercial tent used in this study.

Enclosure experiments were conducted simultaneously in the 2 enclosures. Prior to each trial, one of the enclosures was randomly selected as the treatment and the other as the control. Inside the treatment enclosure, the spatial repellent device was placed on the outside of the tent near the upper door opening (Figure 3B). Trials were conducted in the morning (8 AM to 11 AM) which corresponded with the peak host-seeking period of *Ae. aegypti*. Two hundred female *Ae. aegypti* (Orlando-strain) were simultaneously released into each enclosure and allowed to move freely for one hour. At the end of the trial, the mosquitoes inside the tents were collected from the BGS traps and vacuumed out using an aspirator.

Statistical Analysis

Laboratory (Wind Tunnel) Study

Statistical 2-way hypothesis tests were conducted at the 95% confidence level ($\alpha=0.05$) to assess differences in landing, probe, blood-fed, and knock down counts among the 2 products (Raid Dual Action and Raid Shield) and treatments (Control and treatment).

Semi-field Study

Statistical 3-way and 2-way hypothesis tests were conducted at the 95% confidence level ($\alpha=0.05$) to assess differences in mosquito counts (inside tent and BGS traps) between the 2 products (Raid Dual Action and Raid Shield), between the treatment and control, and between the 2 enclosures. Preliminary goodness-of-fit testing using the Kolmogorov-Smirnov test for normality[25] and the Bartlett test for homoscedacity (homogeneity of variances)[26,27] indicated that the data were non-normal and non-homoscedastic. Thus, the nonparametric Kruskal-Wallis test[28] was used instead of the parametric ANOVA test for the statistical analysis. Post hoc Tukey multiple-comparison,[29] Newman-Keuls, Duncan multiple-range,[30,31] and Scheffe multiple-contrast[32] tests were evaluated based on an optimization analysis, and the optimal model was used to identify the specific pairwise combinations that are significantly different from each other and contribute to the overall variance source. Interaction effects of air temperature and humidity on mosquito counts were evaluated by regression analysis. All statistical analysis was performed using Intel Visual Fortran Compiler XE 2013 (Intel Corporation, Santa Clara, CA).

RESULTS

Laboratory (Wind Tunnel)

Both products were effective in reducing host-seeking activity of *Ae. aegypti* in the wind tunnel. Figure 4A shows the treatment effect of both products on the mean landing counts of the mosquitoes. The mean baseline (control) landing counts for the Raid Dual Action and Raid Shield were reduced by 95% ($P<.006$) and 74% ($P<.006$) respectively. Mean probing counts for the Raid Dual Action were reduced by 95% ($P<.02$), while the probing counts for the Raid Shield were decreased by 69% ($P<.02$) (Figure 4B). Baseline blood-feeding success was significantly reduced ($P<.002$) for both treatments: Raid Dual Action (100%) and Raid Shield (96%) (Figure 4C). Figures 5 and 6 show the time analysis (50 minutes) for the mean counts of landing, probing, and blood feeding for each treatment. Maximum reduction rates occurred during the first 30 minutes. After 30 minutes, reduction rates dropped below 50%. Blood-feeding reduction rates remained above 85% for the duration of

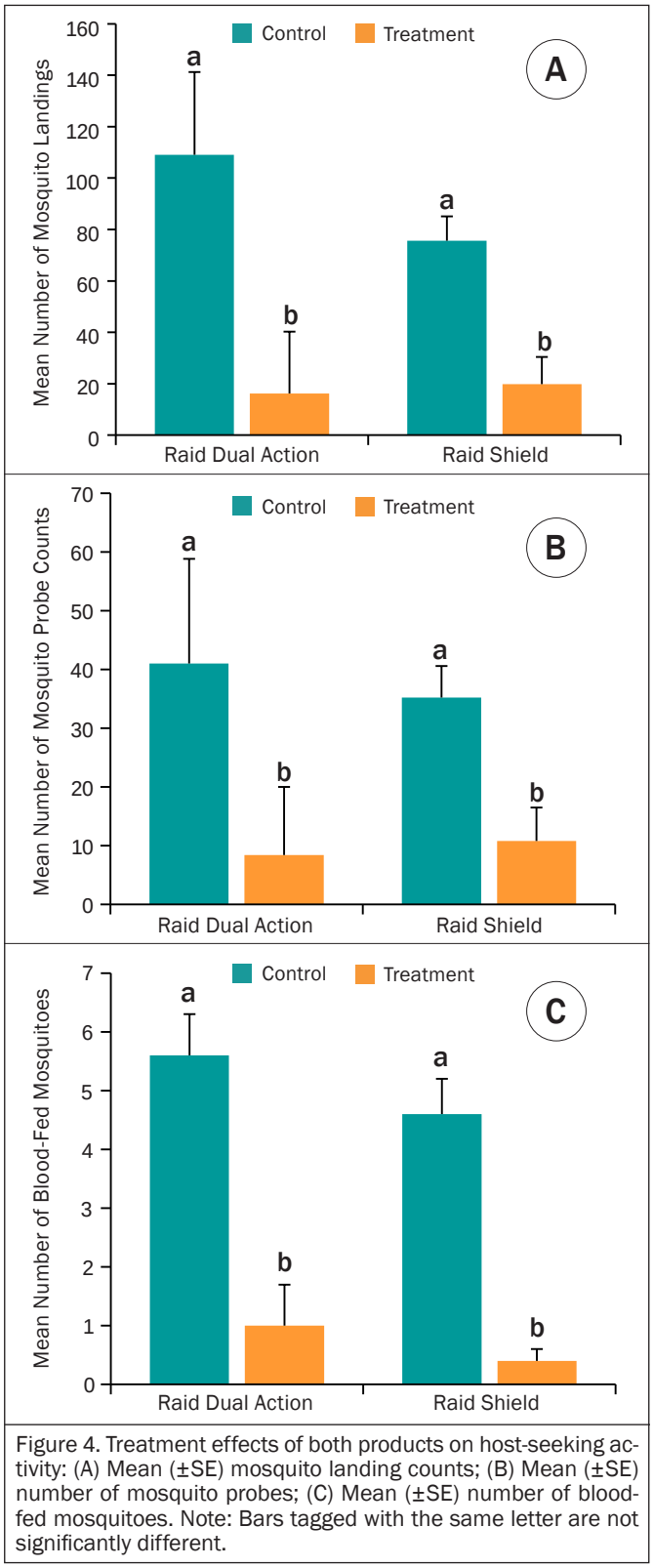
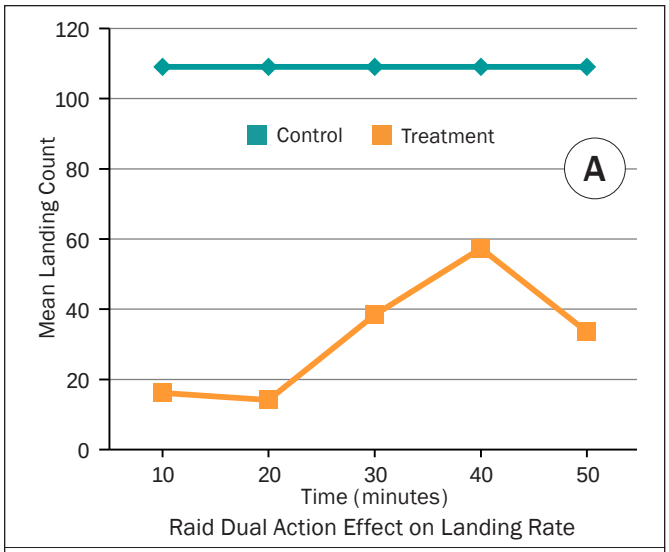
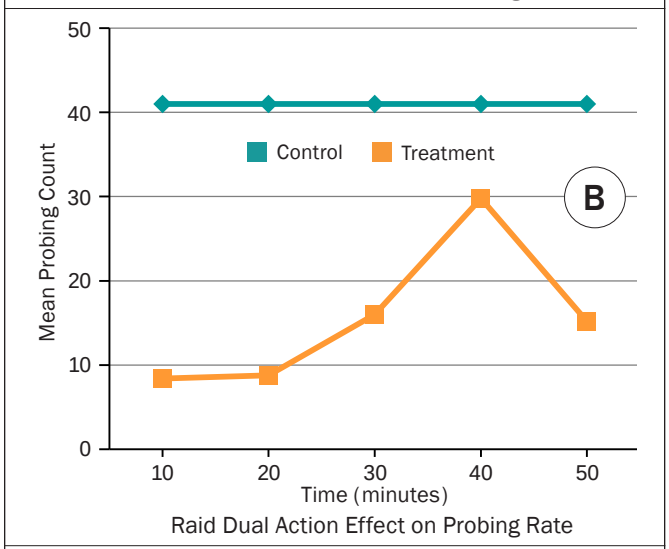


Figure 4. Treatment effects of both products on host-seeking activity: (A) Mean (±SE) mosquito landing counts; (B) Mean (±SE) number of mosquito probes; (C) Mean (±SE) number of blood-fed mosquitoes. Note: Bars tagged with the same letter are not significantly different.





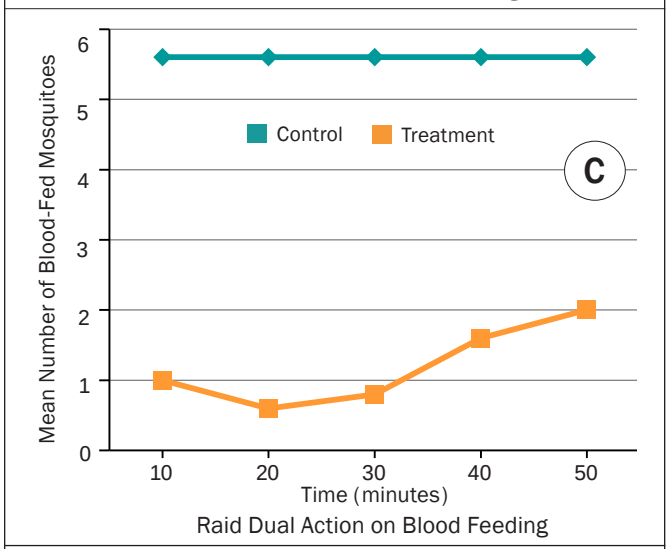
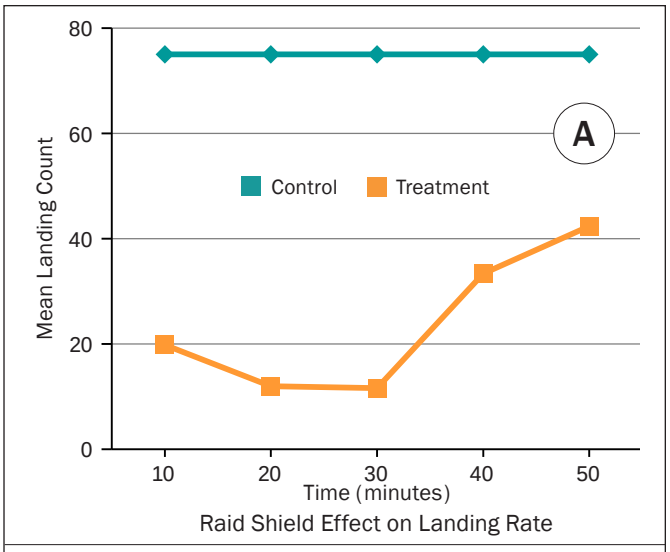
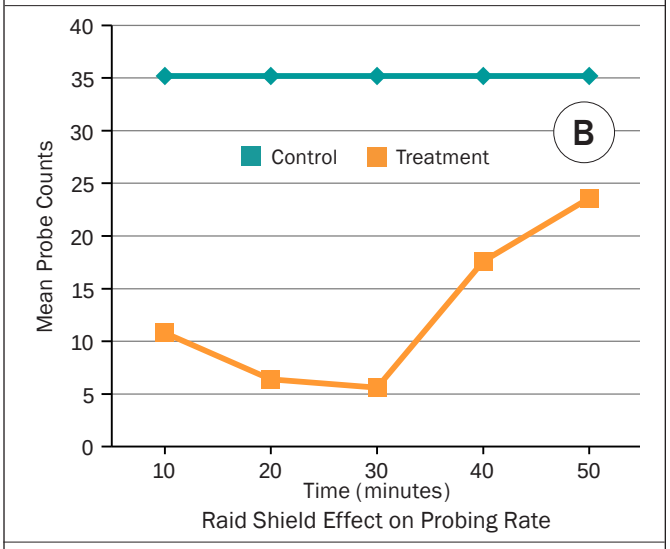


Figure 5. Time-series analysis of the Raid Dual Action effects on *Ae. aegypti* host-seeking activity: (A) Mean mosquito landing counts; (B) Mean number of mosquito probes; (C) Mean number of blood-fed mosquitoes.





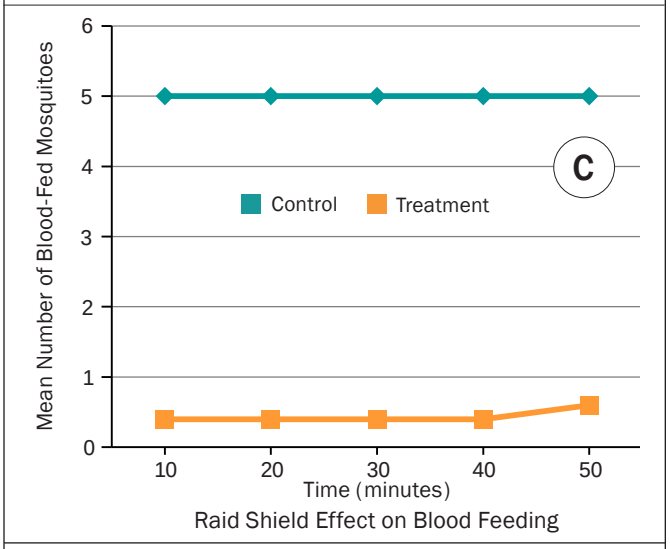


Figure 6. Time-series analysis of the Raid Shield effects on *Ae. aegypti* host-seeking activity: (A) Mean mosquito landing counts; (B) Mean number of mosquito probes; (C) Mean number of blood-fed mosquitoes.

the 50 minute test. Overall, knock-down effects for both products were minimal. Average knock-down was 1.08 mosquitoes, with a maximum count of 4 (20%).

Semi-field (Enclosure)

Weather conditions at NECE, Naval Air Station, Jacksonville, Florida were hot and humid. Temperature and relative humidity recorded inside the semi-field enclosures averaged 25.5ºC (max=29ºC and min=21ºC) and 86.8% (max=97% and min=66%). The prevailing wind direction was from the northeast with average daily wind speeds less than one kph. Regression analysis showed negative correlation of rep-averaged mosquito counts with temperature (C°) and humidity (%) (r=-0.4637, df=16, P=.3406). Collections of mosquitoes from treatment (with a spatial repellent device) tents were significantly lower than the control (without a spatial repellent device) tents (P<.0001; Figure 7). The Raid Shield reduced mosquito entry into tents by 88%, while the Dual Action only decreased entry by 66%.

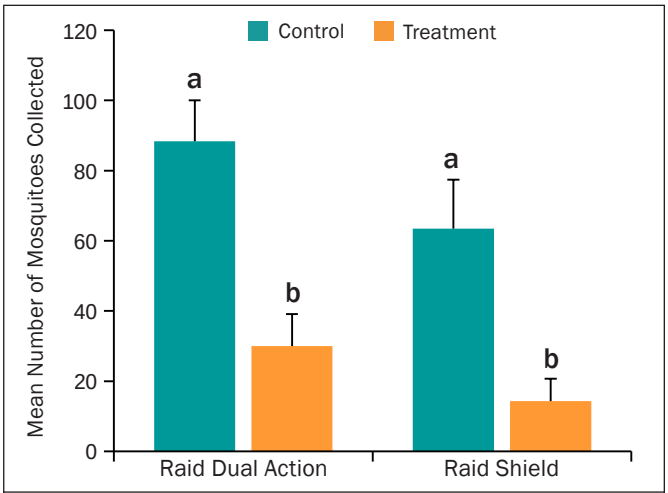


Figure 7. Mean (±SE) mosquito capture rate per tent for control and treatments. Note: Bars tagged with the same letter are not significantly different.

Comment

Various definitions and characterizations of spatial repellents have been reported in the literature. Dethier[33] defined spatial repellents as any stimulus constituting a vapor physical state which elicits an avoidance reaction. Gouck et al[34] expanded the definition to include any compound or agent that can produce repellency at a distance. Nolen et al[35] defined spatial repellents as "an inhibiting compound, dispensed into the atmosphere of three dimensional space which inhibits the ability of mosquitoes to locate and track a target such as a human or livestock." Results from our investigation indicated that both of the SC Johnson spatial repellent products functioned as spatial repellents. The active ingredient used in both products was the synthetic pyrethroid transfluthrin. Unlike many of the other common pyrethroid chemicals, transfluthrin has a high vapor pressure which enables the chemical to passively vaporize at ambient temperature. Other pyrethroids such as permethrin require an external energy source for volatilization. In order to be a practical tool for Soldiers, a spatial repellent device must be effective, nontoxic, simple to construct and operate, and it should not require frequent maintenance. The 2 products used in this study are lightweight, inexpensive, contained low amounts of pesticide, and are easy to set up. From a logistics standpoint, these devices are available in high quantity and can be easily integrated into military logistic transportation platforms. For example, more than a hundred of these devices could be carried in a Soldier's rucksack and the load effect would be minimal. The operation of these devices only consisted of placing it at the desired location and exposing the treated surface to the air (spray the product on the poster (Dual Action) or open the folded plastic (Raid Shield)). Compliance with mandated topical repellent use remains an issue in the military. Unlike topical repellents, these devices do not require reapplication by the user. Furthermore, the protection from the device benefits more than one person at a time.

Our laboratory (wind tunnel) evaluations of the SC Johnson spatial repellent products showed significant reductions in host-seeking behaviors (landing, probing and blood-feeding) of *Ae. aegypti*. However, reduction rates significantly decreased after 30 minutes. Previous studies using transfluthrin showed high efficacy with longer periods of effectiveness. Ogoma et al[36] evaluated transfluthrin treated fabric (hessian cloth) against laboratory-reared *An. arabiensis* Patton. A single hessian strip (4.0 m x 0.3 m) treated with 10 ml of transfluthrin reduced the mosquito attack rate on human volunteers by 99% and consistently conferred greater than 90% protective efficacy for a period of 6 months. Andrés et al[37] evaluated transfluthrin dispensed from modified mosquito landing boxes against *An. arabiensis* in a semi-field system. The protective transfluthrin bubble provided 68.9% protection to human volunteers, and continued to show knockdown effects up to 3 weeks in postlaboratory testing. Govella et al[38] tested transfluthrin treated strips in the field against *An. gambiae*. The strips conferred 99% protection and remained effective for over 3 months. One major limitation of our study was control of the amount of active ingredient used. Material Safety Data Sheets for both products showed that the amount of transfluthrin was less than 60 mg (less than 2% concentration). In comparison with previous studies, these amounts were relatively low. Additionally, it is important to note that the 2 products are marketed for indoor use.

Knockdown is the incapacitation of arthropods as a result of contact with a sublethal dose of pesticide. In this study, knockdown effects from transfluthrin exposure were low for both products. However, the relative knockdown of mosquitoes was consistently higher for the Dual Action device. This is particularly interesting due to the fact that the Dual Action device contained less transfluthrin. In the wind tunnel, mosquitoes often made direct contact with the Dual Action device. This was not observed with the Raid Shield device. The Dual Action product was designed with a multipurpose function of repelling insects and serving as a home freshener.

Our semi-field experiment demonstrated a tactical utility for spatial repellents. The BGS traps are commonly used by mosquito vector control districts to conduct surveillance and have been proven to be highly effective for collecting *Ae. aegypti* and *Ae. albopictus.* In this study, BGS traps baited with CO_2 were placed in military style tents and served as surrogates for human-bait collectors. Mosquito entry into military style tents was moderately reduced when using the spatial repellent devices. As expected due to environmental conditions, there was a significant variation in the amount of reduction. However, mosquito levels in treated tents remained well below that of control tents. This result suggests that the transfluthrin spatial repellents used in this study may have functioned as attraction inhibitors. A more likely explanation is that this behavior involved excito-repellency. For example, the mosquitoes could have detected and followed the CO_2 source inside the tent, but due to increased transfluthrin exposure, some of them quickly egressed from the tent to seek fresh air. Other studies suggest that placement of the device inside certain structures enhances the spatial repellent effect. Kawada et al[39] evaluated metofluthrin-impregnated plastic strips against *Ae. aegypti* in houses located in My Tho City, Vietnam. Multiple regression analysis of environmental factors indicated that both an increase in average room temperature and a decrease in area of openings in rooms that were treated with metofluthrin-impregnated strips positively affected its spatial repellency. Transfluthrin exposure could have also negatively affected other key mosquito behaviors such as blood-feeding and ovipositon. Unfortunately, the experimental design for the semi-field experiment did not include evaluations of these behaviors. Therefore, the effect of the transfluthrin on the mosquitoes that entered the experimental tents remains unknown.

This study suggests that spatial repellents have great potential for enhancing existing vector control and PPM efforts. However, sole reliance on this technology is not practical. Although spatial repellents have shown high efficacy rates in the field reaching levels of 99% protection, changing environmental conditions (wind, precipitation, temperature, and humidity) may present a major challenge for sustaining high efficacy rates. Traditional military approaches to mosquito control are based on the use of insecticides for area-wide abatement and PPM (topical repellents, treated uniforms, and bed nets). However, there is much concern among entomologists that these approaches may be severely restricted in the future due to insecticide resistance. Over the past few decades, there has been an overreliance on the use of pyrethroid insecticides for mosquito control. Global malaria eradication programs depend almost exclusively on pyrethroid-based indoor residual spraying and long-lasting insecticidal nets (LLINs). Although this strategy has been viewed as having an important role in the recent reductions in global malaria mortality, major malaria vectors have developed resistance to this class of insecticides and the resistance alleles are very common in mosquito populations throughout the world. One way to overcome resistance is to rotate different classes of insecticides into a control program. Unfortunately, the acquisition of other classes of insecticides is often difficult and their cost is significantly higher than pyrethroids. Spatial repellents such as transfluthrin are readily available and are relatively inexpensive. A potential means of slowing the evolution of pyrethroid resistance may be the integration of spatial repellents into the program strategy.[40] For example, the incorporation of a spatial repellent with LLINs would result in a 3-tier line of defense: (1) the spatial repellent, (2) contact pyrethroid, and (3) physical barrier (net). The spatial repellent would provide protection outside of the LLIN and contribute to reducing selection for resistance. Additional studies that explore the effects of integrating spatial repellents into DoD field vector control activities are warranted.

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Georgia's Collaborative Approach to Expanding Mosquito Surveillance in Response to Zika Virus: A Case Study

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Abstract

Zika virus (ZIKV) was declared an international public health emergency by the World Health Organization on February 1, 2016. Due to the known and estimated range of the ZIKV mosquito vectors, southern and central US states faced increased risk of ZIKV transmission. With the state of Georgia hosting the world's busiest international airport, a climate that supports the ZIKV vectors, and limited surveillance (13 counties) and response capacity, the Department of Public Health (DPH) was challenged to respond and prevent ZIKV transmission. This case study describes and evaluates the state's surveillance capacity before and after the declaration of ZIKV as a public health emergency.

Method: We analyzed surveillance data from the DPH to compare the geographical distribution of counties conducting surveillance, total number, and overall percentage of mosquito species trapped in 2015 to 2016. Counties conducting surveillance before and after the identification of the ZIKV risk were mapped using ArcMap 10.4.1. Using SAS (version 9.2) (SAS Institute, Inc, Cary, NC), we performed the independent 2 sample *t* test to test for differences in prevalence in both years, and a χ^2 analysis to test for differences between numbers of species across the 13 counties. In addition, weighted frequency counts of mosquitoes were used to test (χ^2) an association between major mosquito vector species and 7 urban counties. Lastly, using data from 2012-2016, a time-trend analysis was conducted to evaluate temporal trends in species prevalence.

Results: From 2015 to 2016, surveillance increased from 13 to 57 (338% increase) counties geographically dispersed across Georgia. A total of 76,052 mosquitoes were trapped and identified in 2015 compared to 144,731 (90.3% increase) in 2016. Significant differences between species ($P<.001$) and significant associations ($P<.0001$) between 7 urban counties and major mosquito vectors were found. Significant differences in prevalence were found between several species and year highlighting species-year temporal trends.

Conclusions: The DPH collaborative response to ZIKV allowed a rapid increase in its surveillance footprint. Existing and new partnerships were developed with the 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.

Zika virus (ZIKV), a mosquito-borne disease, was declared an international public health emergency by the World Health Organization (WHO) on February 1, 2016 due to confirmed reports of various adverse health effects from multiple countries.[1] The state of Georgia Department of Public Health (DPH) lacked sufficient monitoring and surveillance capacity to properly assess the risk of ZIKV transmission, as well as trained staff to respond to potential local outbreaks of the disease. This limited capacity was a result of federal budget reductions that affected funding earmarked for mosquito monitoring and surveillance programs. Once a strong program that had a large number of county health departments conducting mosquito surveillance and several trained staff, the program had been reduced to one state level position and a few counties conducting surveillance. This case study describes and evaluates Georgia's rapid response from the perspective of those challenges.

Overview

Zika virus is a mosquito-borne *Flavivirus* (family Flaviviridae) first discovered in 1947 in the Zika forest of Uganda from captive monkeys used in yellow-fever surveillance and later isolated in humans in 1952.[2] Unlike West Nile virus (WNV) that circulates between birds and mosquitoes with humans serving as dead-end hosts,[3] ZIKV can be transmitted from human-to-human via the bite of an infected *Aedes* spp mosquito.[1] From the 1960s through the 1980s, the disease was found in mosquitoes from several Asian countries with few human cases. It eventually traveled east, resulting in a large outbreak on Yap Island in 2007 and additional outbreaks

in French Polynesia in 2013.[2] In 2015, Brazil reported unusual cases of rash and ultimately associated a high number of babies born with microcephaly and cases of guilliain-barré syndrome to ZIKV, resulting in the international public health emergency order.[2]

Research and analysis of previous outbreaks indicated that the primary vector responsible for transmitting ZIKV is the *Aedes* spp mosquito with the urban dwelling *Ae. aegypti* (L.) the likely vector in the Americas.[4] A daytime biter of humans, this mosquito prefers tropical to somewhat temperate climates and lays eggs in containers around urban areas.[4] A secondary ZIKV vector is *Ae. albopictus* (Skuse), the Asian tiger mosquito. This mosquito also lay eggs in containers, but feeds on humans and other animals, thus lowering the risk of transmission.[4] However, *Ae. albopictus* can survive in cooler areas, increasing its potential distribution across the United States. The Centers for Disease Control and Prevention (CDC), using several limited data sources, developed 2 maps (Figure 1) that estimate the potential geographic range of *Ae. aegypti* and *Ae. albopictus.*[4]

The potential distribution of these 2 vectors placed several states in the southern to central half of the United States on alert for potential transmission of ZIKV and raised the alarm for public health officials in Georgia. Unfortunately, the maps demonstrate the lack of surveillance across the country, as it only estimates the potential range of the 2 ZIKV vectors. This data gap is a result of public health budget cuts and lack of priority placed on mosquito surveillance in the last decade. Consequently, this negatively affected the state's ability to critically assess the actual risks of ZIKV transmission for its citizens.

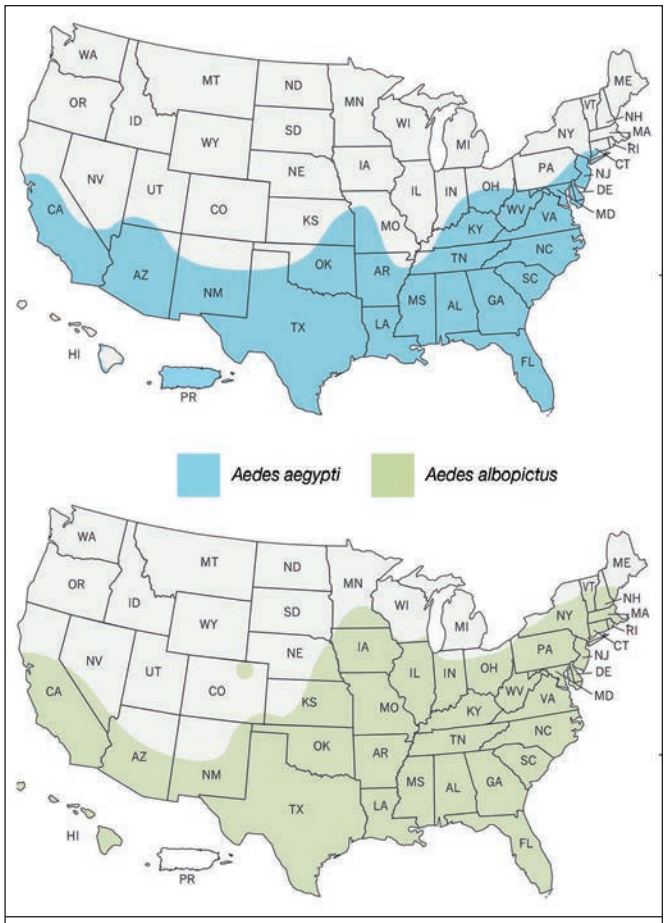

Figure 1. Estimated range of *Aedes albopictus* and *Aedes aegypti* in the United States, 2016. The maps represent the best estimate by CDC of the potential range of the named mosquitoes. Maps courtesy of the Centers for Disease Control and Prevention.[4]

National Funding Trends

Understanding national funding trends is important to explaining the gaps in surveillance across the United States. The primary mission of public health and mosquito control programs is to inform, prevent, and protect the public from injury and disease. This mission is achieved through disease and vector surveillance programs that provide critical data used to quickly respond and control threats. Mosquito surveillance, coupled with clinical (human and animal) surveillance programs, are critically important because they can detect the abundance and distribution of vectors, monitor for viral diseases, aid in quantifying human risks, and predict changes in the dynamics of disease transmission.[6-8] Unfortunately, funding and support of surveillance programs have decreased over time, leaving a patchwork of jurisdictions conducting surveillance and resulting in significant gaps in vector data.[9]

West Nile virus (WNV), the last major new and exotic arboviral outbreak in the United States, generated significant media attention and heightened the public's fear.[10-12] This outbreak highlighted a general lack of capacity for public health and mosquito control agencies to conduct human and vector surveillance and rapidly respond to disease events.[13] To improve detection, monitoring, and control capacity for WNV (which eventually spread to 48 states), the CDC provided significant funding to all 50 states and 6 major cities through its Epidemiology and Laboratory Capacity (ELC) grant.[13] This funding was eventually expanded to cover over 20 mosquito-borne and tick-borne diseases and allowed states and jurisdictions to increase human and vector surveillance, mosquito trapping and identification, viral testing, avian surveillance systems; and develop response plans.[14]

In 2002, approximately $34.7 million was provided to states to fund these important programs, but by 2014, this had been reduced by approximately 75% to $9.2

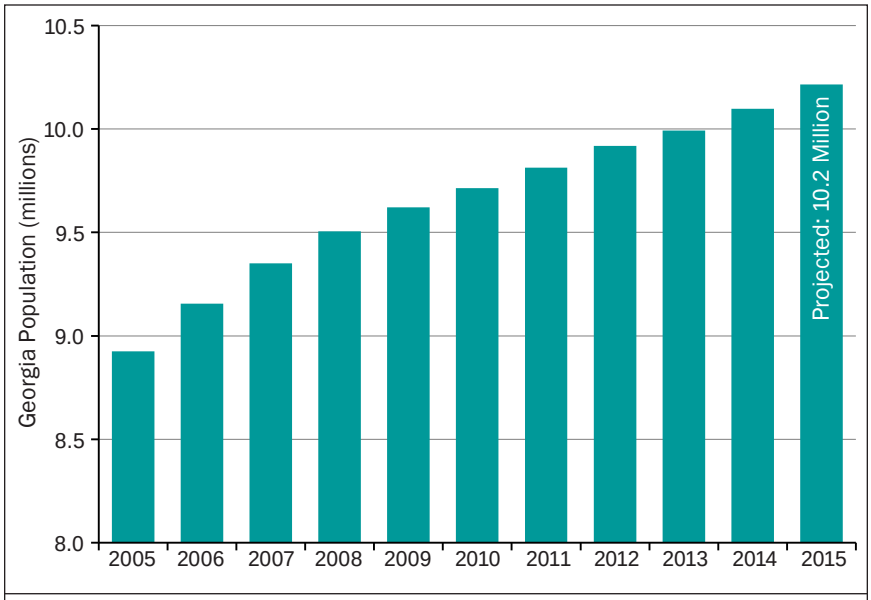

Figure 2. Population of the state of Georgia by year, 2005-2015.

million.[14] A CDC report based on a follow-up survey conducted by the Council of State and Territorial Epidemiologists indicated that since 2005, 22% of funded jurisdictions eliminated active human surveillance, 13% eliminated mosquito surveillance, 70% eliminated mosquito trapping, and 64% stopped avian surveillance.[13] Since 2005, the number of counties conducting routine mosquito surveillance in Georgia was reduced by 78%, from 60 to 13 counties, and all public health funding for arboviral testing and avian surveillance was eliminated (R. Kelly, unpublished data*). This report concluded that the state's ability to rapidly detect and respond to emerging disease threats is "compromised,"[13] thus placing states, including Georgia, at a disadvantage in responding to vector-borne diseases.

Georgia Preparedness

Georgia, like many states, faces several challenges to monitoring and responding to emerging arboviral diseases, with ZIKV highlighting this issue. In addition to the CDC estimating that the range of ZIKV vectors spans the entire state, several additional issues raise the risk of ZIKV transmission. First, Georgia is home to the world's busiest international airport, thus hosting visitors and tourists from ZIKV endemic countries. Additionally, many Georgia citizens travel to tourist destinations that have active ZIKV circulating. This raised the risk that a traveler could become infected and return to Georgia, thus spreading ZIKV to local mosquitoes. Second, Georgia lacks statewide surveillance and vector control which are limited to just 13 counties conducting surveillance and 6 counties providing comprehensive mosquito control services. Third, previous surveillance has determined that *Ae. albopictus* is present in every county,*[15] but with limited surveillance, the true distribution of *Ae. aegypti* is unknown having only been recently found in 2 counties. *Ae. aegypti* had been a common species in Georgia until the introduction of *Ae. albopictus* in the 1990s. This lack of surveillance data prevents accurate quantification of human risks. Lastly, due to the federal funding cuts, the DPH has limited capacity to conduct comprehensive surveillance and mapping of vector abundance and evaluate insecticide resistance, and limited ability to provide emergency vector control.

These challenges are compounded by the fact that Georgia's population is over 10 million people and growing (Figure 2), and has the largest number of counties (159), second only to the state of Texas.[16] These counties range from urban to suburban, with approximately 108 (68%) of the counties classified as rural (less than 35,000 population).[17] With the population of the state steadily increasing, and a public health program with limited resources, more people are potentially at risk of exposure to emerging vector-borne diseases. However, faced with these challenges, the DPH used the expertise of its State Entomologist and the environmental health program to evaluate its weaknesses and implement a rapid, collaborative response to the threat of ZIKV.

Purpose

The purpose of this case study was to describe and compare the DPH's mosquito surveillance capacity in 2015 before ZIKV was declared a public health emergency to the 2016 surveillance capacity following that declaration. Statistical comparisons were made between years on the number of counties conducting surveillance and differences in prevalence and species, in addition to a time trend analysis of mosquito species distribution. These data were used to evaluate the state's rapid response to the threat of ZIKV and the risk of autochthonous vector transmission based on the presence of the ZIKV vectors.

Methods

Mosquito surveillance trapping data provided by the DPH and surveillance data collected in collaboration

*Unpublished arboviral summary statistics report prepared quarterly and annually by author R. Kelly and distributed internally within DPH, public health departments, MC agencies, and academic partners.

with DPH were analyzed. The geographical distribution of counties conducting surveillance, total number, and overall percentage of mosquito species collected in 2015 were compared to 2016 data. The distribution of counties conducting surveillance was mapped using ArcMap 10.4.1 (Esri, Inc, Redlands, CA).

Statistical comparisons were made for the 13 counties conducting mosquito surveillance between 2015 and 2016 to test for differences in response before and after the ZIKV emergency declaration. To test differences in prevalence (number of mosquitoes trapped/100,000 population) in both years, the overall number of mosquitoes was compared using the independent 2 sample *t* test. A χ^2 analysis was performed to test for statistical differences between numbers of species trapped across all 13 counties. In addition, weighted frequency counts of mosquitoes were used to conduct a test of association (χ^2) between the major vector species of mosquitoes and the geographic county in 7 urban counties. The counties included in this χ^2 analysis were Chatham, Fulton, Glynn, Liberty, Lowndes, Muscogee, and Richmond. Lastly, a time-trend analysis was conducted using surveillance data from 2012-2016 to test differences in species prevalence over time. Statistical significance was established at the significance level 0.05.

Results

In 2015, prior to the declaration that ZIKV represented a public health emergency, Georgia had 13 counties conducting surveillance, with the DPH medical entomologist (one full time equivalent/statewide) providing routine surveillance in 4 of those counties. In 2016 (March-December), the DPH expanded surveillance to 57 counties (338% increase) geographically dispersed in urban and rural areas (Figure 3).

This rapid expansion of surveillance was a result of hiring new staff and collaborating with the local health departments (LHDs) and the military. Table 1 shows that 76,052 mosquitoes were trapped and identified in 2015 compared to 144,731 mosquitoes trapped in 2016, representing a 90% increase. Forty-four mosquito species were identified in both years with *Culex quinquefasciatus* (Say), Georgia's primary WNV vector, representing the highest percentage of mosquitoes trapped in both years (79.45% and 62.53% respectively). In reference to ZIKV vectors, *Ae. aegypti* represented only 0.108% and 0.018% respectively of the total mosquitoes trapped each year, and were found in Muscogee County only, home to Fort Benning. *Aedes albopictus* represented only 1.50% and 3.703% of the total mosquitoes trapped respectively each year, reported from 11/13 (84%) counties in 2015 and 42/57 (74%) counties in 2016.

Overall Mosquito Prevalence

To test differences in overall prevalence (number of mosquitoes/100,000 population) of mosquitoes trapped in both years from the 13 counties that have historically conducted surveillance, the number of mosquitoes was compared using the independent 2 sample *t* test. The value of the number of mosquitoes was normalized

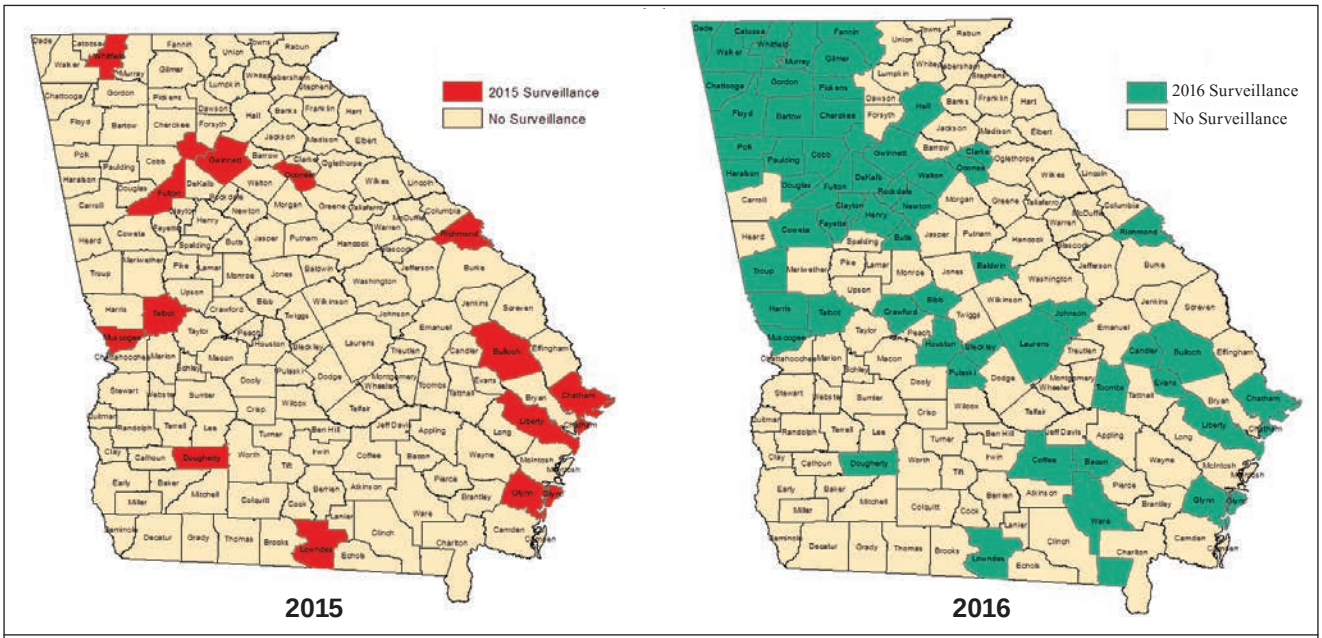

Figure 3. Georgia counties conducting mosquito surveillance in collaboration with the Georgia Department of Public Health in 2015 (13 counties) and 2016 (57 counties).

Table 1. Mosquito Species Collected, 2015-2016.

Species*	2015 Totals	13 Counties Percentages	2016 Totals	57 Counties Percentages
Ae. aegypti	82	0.108%	26	0.018%
Ae. albopictus	1,141	1.500%	5,360	3.703%
Ae. cinereus	0	0.000%	4	0.003%
Ae. japonicus	0	0.000%	1	0.001%
Ae. vexans	162	0.213%	6,536	4.516%
Aedes/Ochlerotatus spp.	6	0.008%	120	0.083%
An. barberi	0	0.000%	1	0.001%
An. crucians	25	0.033%	1,873	1.294%
An. punctipennis	26	0.034%	486	0.336%
An. quadrimaculatus	61	0.080%	265	0.183%
Anopheles spp.	5	0.007%	134	0.093%
Cq. perturbans	1,265	1.663%	5,969	4.124%
Cs. inornata	130	0.171%	14	0.010%
Cs. melanura	906	1.191%	996	0.688%
Culex spp.	4,996	6.569%	10,830	7.483%
Cx. coronator	262	0.345%	604	0.417%
Cx. erraticus	300	0.394%	2425	1.676%
Cx. nigripalpus	5,657	7.438%	11,071	7.649%
Cx. peccator	0	0.000%	12	0.008%
Cx. quinquefasciatus	60,423	79.450%	90,505	62.533%
Cx. restuans	100	0.131%	389	0.269%
Cx. salinarius	350	0.460%	2,746	1.897%
Cx. territans	1	0.001%	33	0.023%
Ma. titillans	0	0.000%	98	0.068%
Oc. japonicus	8	0.011%	52	0.036%
Oc. atlanticus	1	0.001%	757	0.523%
Oc. canadensis	0	0.000%	117	0.081%
Oc. fulvus pallens	0	0.000%	1	0.001%
Oc. infirmatus	2	0.003%	45	0.031%
Oc. mitchellae	0	0.000%	9	0.006%
Oc. sticticus	0	0.000%	31	0.021%
Oc.taeniorhynchus	0	0.000%	5	0.003%
Oc. triseriatus	25	0.033%	78	0.054%
Or. signifera	3	0.004%	23	0.016%
Ps. ciliata	0	0.000%	25	0.017%
Ps. cyanscens	2	0.003%	30	0.021%
Ps. columbiae	88	0.116%	332	0.229%
Ps. ferox	10	0.013%	106	0.073%
Ps. howardii	3	0.004%	34	0.023%
Psorophora spp.	6	0.008%	0	0.000%
Tx. rutilus	1	0.001%	52	0.036%
Ur. iowii	0	0.000%	13	0.009%
Ur.sapphrina	2	0.003%	115	0.079%
Unknown	3	0.004%	2,408	1.664%
Total	76,052		144,731	

*Data for distribution of all species statistically significant ($P \leq .05$).

using the log transformation and the normality was confirmed with Kolmogorov-Smirnoff test. While the total number of mosquitoes trapped increased from 2015 to 2016 for the 13 counties, this analysis shows there was no statistically significant differences ($P=.7901$) in the overall prevalence of mosquitoes trapped before and after ZIKV was declared an international public health emergency (Table 2A).

Table 2A. The 2-sample *t* test for difference in mosquito prevalence between 2015 and 2016.

Year	Method	Mean	95% CI
2015		6.34	4.79, 7.88
2016		6.63	4.86, 8.39
Diff (1-2)	Pooled	-0.29	-2.51, 1.93
Diff (1-2)	Satterthwaite	-0.29	-2.51, 1.94

Table 2B. The significance of the *t* test assuming equal and unequal variances.

Method	Variances	*df*	*t* value	*P* value
Pooled	Equal	24	-0.27	.7901
Satterthwaite	Unequal	23.589	-0.27	.7902

Table 2C. Results for the test of equality of variances between the 2 samples.

Method	Num *df*	Den *df*	F-value	*P* value
Folded F	12	12	1.30	.65

Table 2B shows the significance of the *t* test assuming equal and unequal variances. Table 2C shows the results for the test of equality of variances between the 2 samples

Species Analysis

To test for differences in the prevalence of individual mosquito species trapped in 2015 versus 2016 for the 13 counties, a χ^2 analysis was performed. Results show significant differences ($P<.001$) in the distribution of all mosquito species trapped in those years (Table 1). However, while there were significant differences in prevalence, this does not tell us the behaviors associated with individual species.

By combining both years (2015, 2016) of mosquito data, a secondary χ^2 analysis was conducted to test for an association between Georgia's primary arboviral vector species and urban counties with a large population of people at risk. Adjusting for inconsistent or missing data, weighted frequency counts of mosquitoes were used in the analysis. While the previous *t* test results (Table 2) indicate that overall difference in prevalence of mosquitoes is not significant between 2015 and 2016, there

Table 3A. Weighted frequency counts and corresponding weighted row percentages of important mosquito species stratified by county.

	Chatham	Fulton	Glynn	Liberty	Lowndes	Muscogee	Richmond	Total
Ae. aegypti	2 1.67%	2 1.67%	2 1.67%	2 1.67%	2 1.67%	108 90.00%	2 1.67%	120
Ae. albopictus	26 0.70%	871 23.46%	72 1.94%	53 1.43%	177 4.77%	653 17.59%	1,861 50.12%	3,713
Ae. vexans	2 0.07%	94 3.23%	2 0.07%	2 0.07%	2 0.07%	101 3.47%	2,704 93.02%	2,907
Cs. melanura	196 10.36%	2 0.11%	2 0.11%	2 0.11%	1,686 89.11%	2 0.11%	2 0.11%	1,892
Culex spp.	13,985 99.30%	28 0.20%	2 0.01%	2 0.01%	2 0.01%	17 0.12%	48 0.34%	14,084
Cx. nigripalpus	1,417 8.56%	2 0.01%	529 3.20%	2 0.01%	13,556 81.90%	2 0.01%	1,044 6.31%	16,552
Cx. quinquefasciatus	58,184 42.09%	8,472 6.13%	41,910 30.32%	85 0.06%	23,776 17.20%	778 0.56%	5,031 3.64%	138,236
Total	73,812	9,471	42,519	148	39,201	1,661	10,692	177,504

Table 3B. Chi-square value with corresponding *P* value (α=0.05).

Statistic	*df*	Value	*P* value
χ^2	36	143,393	<.0001

is a significant association (*P*<.0001) between urban counties and major vector species as shown in Table 3. However, while χ^2 tests can provide information on distribution of species between years, it does not provide detailed information on behavior of individual species, trends, and patterns over time.

Time Trend Species Analysis

A time-trend species analysis using generalized linear models (Proc GLM in SAS 9.2) was conducted on mosquito data from 2012 to 2016 to evaluate temporal trends in prevalence of mosquitoes. Statistically significant differences were observed over the 5-year period in species prevalence (*P*<.0001). While time (in years) was not significantly associated with change in overall prevalence, (*P*<.1219) its differential effect on species specific change in prevalence was significant (interaction term – *P*<.0001) (Table 4).

Looking more closely at the specific species estimates in Table 5, it can be seen that certain species had significantly different prevalence compared to the reference group, ie, *Oc. sticticus* (Meigen), throughout the 5-year period. This reference group was chosen due to low numbers of species trapped. These species were *Ae. albopictus* (S.) (*P*<.0001), *Ae. vexans* (Meigen) (*P*=.0240), *Cq. perturbans* (Walker) (*P*<.0001), *Cs. melanura* (Coquillett) (*P*<.0001), *Culex* spp. (*P*<.0001), *Cx. coronator* (Dyer and Knab) (*P*=.0463), *Cx. erraticus* (Dyer and Knab) (*P*<.0001), *Cx. nigripalpus* (Theobald) (*P*<.0001), *Cx. quinquefasciatus* (Say) (*P*<.0001), *Cx. restuans* (Theobald) (*P*=.0004), *Cx. salinarius* (Coquillett) (*P*<.0001) and *Oc. triseriatus* (Say) (*P*<.0001).

Table 4. Time trend analysis showing the type III sums of squares and corresponding *P* values (α=0.05) for the variables species, year, and the interaction term.

	df	Type III SS	Mean Square	F-value	*P* value
Species	41	1589.943667	38.779114	14.25	<.0001
Year	1	6.521391	6.521391	2.40	.1219
Year species	41	318.828534	7.776306	2.86	<.0001

For associated species-year trends in Table 5, *Cs. melanura* (*P*<.0042), *Culex* spp. (*P*<.0013), *Cx. erraticus* (*P* <.0271), *Cx. nigripalpus* (*P*<.0074), *Cx. restuans* (*P*<.0433) and *Cx. salinarius* (*P*<.0266) were found to have a significant difference in change of prevalence with each year compared to the reference species *Oc. sticticus.*

Comment

The public health entomology program, which includes mosquito surveillance, falls under the DPH Environmental Health (EH) Section. This program is managed by a medical entomologist who holds graduate degrees in public health and entomology and who was originally hired under Epidemiology and Laboratory Capacity funding in 2002 to manage the WNV outbreak. The state medical entomologist provides technical assistance and consultation to LHDs, mosquito control agencies and the general public and was largely responsible for overseeing Georgia's rapid response to ZIKV. In 2015, surveillance and identification of mosquitoes was limited to just 13 counties in Georgia. With this limited data, DPH provided quarterly surveillance reports to partner agencies for planning and risk assessment purposes.

When ZIKV was declared an international public health emergency,[1] the DPH recognized its weaknesses and formed a core team of professionals to develop and lead a response. This diverse team was comprised of EH, medical entomology, medical epidemiology, communications, emergency preparedness, and leadership. An assessment was conducted to ascertain agency strengths, weaknesses, and overall needs to develop a response. This evaluation assessed geographical gaps in mosquito data and surveillance staff needs, opportunities for new or expanded partnerships that could enhance a response and facilitate data sharing, training of existing staff, and funding needs. Leadership made the ZIKV response a priority and identified existing grant funding, which significantly contributed to a rapid response. The assessment guided DPH in utilizing those funds to hire additional surveillance staff and assign them regionally across the state, updated existing surveillance and response plans originally written for WNV, provided rapid training to new and existing staff across the state in surveillance and response, purchased equipment, and expanded collaborations with the military to share data and respond to this threat.

Table 5. The results of the PROC GLM (SAS 9.2) procedure showing changes in the log of mosquito prevalence by year and the differential trends in log of mosquito prevalence by year.*

Parameter	Estimate	Standard Error	*t* value	*P* value
Intercept	0.18	0.61	0.29	.76
Ae. albopictus	5.34	1.20	4.44	<.0001
Ae. vexans	1.84	0.81	2.26	.02
Cq. perturbans	2.84	0.71	4.00	<.0001
Cs. melanura	3.03	0.72	4.21	<.0001
Culex spp.	4.02	0.75	5.33	<.0001
Cx. coronator	1.50	0.75	1.99	.0463
Cx. erraticus	4.00	0.79	5.01	<.0001
Cx. nigripalpus	4.07	0.68	5.91	<.0001
Cx. quinquefasciatus	6.79	0.66	10.16	<.0001
Cx. restuans	2.48	0.69	3.55	.0004
Cx. salinarius	2.97	0.69	4.30	<.0001
Oc. triseriatus	1.62	0.70	2.29	.02
Oc. sticticus	Ref			
year species *Cs. melanura*	-1.29	0.45	-2.87	.004
year species *Culex* spp.	-1.51	0.46	-3.23	.001
year species *Cx. erraticus*	-1.10	0.50	-2.21	.02
year species *Cx. nigripalpus*	-1.15	0.43	-2.68	.007
year species *Cx. restuans*	-0.88	0.43	-2.02	.04
year species *Cx. salinarius*	-0.95	0.43	-2.22	.02
year species *Oc. sticticus*	Ref			

*Only results significant at $\alpha = 0.05$ are shown.

As the results in Table 1 indicate, DPH successfully and rapidly expanded its surveillance footprint by 338%, from 13 counties in 2015 to 57 counties in 2016, representing 36% of the state. While the overall number of counties conducting surveillance seems low, given there are 159 counties, it should be noted that this rapid expansion occurred in just 8 months (May-December) and all major urban population centers have active surveillance. In addition, the distribution of surveillance covered all regions of the state. This expansion led to the overall number of mosquitoes trapped increasing by 90.3%, from 2015 to 2016, allowing better decision making. Rapid expansion was achieved because the DPH leadership made the ZIKV response a top priority and streamlined the hiring and purchasing processes. Prior to hiring new staff (March-April), the environmental health program updated its WNV surveillance and response plan by tailoring it for ZIKV. In addition, a training curriculum, standard operating procedures, job description for surveillance staff, and regions were established. The DPH collaborated with regional public health departments to provide office and storage space for the new surveillance staff, a critical component for a successful program.

In April and May, 5 new surveillance staff members were hired and provided 2 weeks of just-in-time training on mosquito surveillance techniques and identification, with priority placed on ZIKV vectors, risk communication, data management, and vector control. Each new staff member was assigned a region to cover and provided with surveillance and response equipment (traps, larvicides, backpack sprayers, microscope, educational material). In addition, the state entomologist provided ongoing training and consultation throughout the year, and assisted new staff with establishing surveillance sites throughout their region. To assist the new regional staff, the DPH provided training, surveillance equipment, and funding to existing LHD environmental health staff across the state, efforts which were invaluable in expanding the surveillance footprint across Georgia.

Historically, Georgia's mosquito surveillance objectives were driven by the threat of WNV, and mosquito trapping was prioritized to capture the primary WNV vector, *Cx. quinquefasciatus.* This is highlighted in Table 1, with *Cx. quinquefasciatus* as the highest percentage of mosquitoes trapped overall in 2015 and 2016 at 79.4% (60,423) and 62.5% (90,505) respectively. However, the threat of ZIKV required surveillance staff to shift some focus away from the WNV vector and prioritize trapping in urban/suburban areas to target container-breeding *Ae. aegpyti* and *Ae. albopictus.* While overall counts and percentages of both ZIKV vectors were low in 2015 and 2016, it is important to note that this shift in surveillance focus is demonstrated in Table 1 by an

increase in *Ae. albopictus* trapped (1.5% to 3.7%) and a decrease in WNV vectors trapped (79.4% to 62.5%). *Aedes aegypti*, the primary ZIKV vector, was trapped in one county (Muscogee) for both years and overall numbers decreased from 82 mosquitoes in 2015 to 26 in 2016, representing an 83% decrease. While surveillance has been limited, *Ae. aegpyti* was only found in Muscogee County and Chatham County in the last decade, suggesting that *Ae. albopictus* has outcompeted this species given its propensity for surviving in surburban/urban areas.[18]

The next 4 highest percentages of mosquitoes trapped in 2016 were *Cx. nigripalpus*, *Culex* spp, *Ae. vexans*, and *Cq. perturbans*. The number of *Ae. vexans* exploded in 2016 to 6,583 mosquitoes compared to 2015 at 162. This increase came in the aftermath of Hurricane Matthew, which left behind ideal breeding conditions for this inland floodwater species, resulting in standing water in low-land and grassy areas of southeast Georgia. It's important to point out that the top 5 mosquitoes captured are major vectors of Eastern Equine Encephalitis (EEE), St. Louis Encephalitis (SLE), and WNV, either serving to amplifying the virus enzootically, or as a primary or bridge vector to humans.[19-21]

Statistical Analysis

To determine if there were any statistical differences or associations in the DPH surveillance program before and after the ZIKV emergency declaration, a series of statistical tests were run comparing data between the same 13 counties historically conducting surveillance. The *t* test examined the overall prevalence of mosquitoes per 100,000 population. While this test did not show statistically significant differences in prevalence, it should be noted that the overall number of mosquitoes trapped increased by 66%, from 76,052 to 126,584 mosquitoes in these 13 counties. This increase demonstrated the rapid expansion of surveillance in response to ZIKV.

A series of χ^2 analyses were conducted to test for any differences between both years for the same counties at the species level. Overall, there were significant differences found in several of the mosquito species trapped between years. Of importance to ZIKV, there was a statistically significant increase in *Ae. albopictus* from 2015 to 2016 and a statistically significant decrease in *Ae. aegypti* between those years. In addition, there was a statistically significant decrease in the number of *Cx. quinquefasciatus*. This was likely due to a shift in focus on targeting the ZIKV vectors which have different breeding habitats. However, Georgia experienced a dry late spring and summer with abnormally cooler nights, which could have affected both vector species.

When Georgia's primary disease vector species was statistically compared to large urban counties, a significant association between species and county was found. This is critically important because the majority of the state's population is found in these large urban counties, putting more people at risk for arboviral diseases and demonstrating the ongoing need for surveillance.

To determine if there were any temporal trends in the distribution of species over time, a time-trend analysis was conducted on mosquito data from 2012 through 2016. This analysis demonstrated that over a 5-year period, there were significant differences in prevalence for several important disease vector species in Georgia that transmit WNV (*Cx. quinquefasciatus*) , EEE (*Cs. melanura, Ae. vexans, Culex* spp), SLE (*Cx. nigripalpus)* and LaCrosse encephalitis (*Oc. triseriatus*), in addition to nuisance species. Of note is the significant prevalence of *Oc. triseriatus* over the 5-year period. This mosquito represents Georgia's primary vector for LaCrosse encephalitis and demonstrates potential risk, as it has been trapped consistently over the 5-year period. It can adapt to its surroundings and lay eggs in forest or urban container environments,[22] highlighting the critical importance of continued surveillance for this species and other major disease vectors. Knowing these temporal trends allows public health and mosquito control professionals to predict risks and better prepare.

Military Collaboration

While Georgia was able to expand its surveillance capacity rapidly with internal resources, it is important to point out the vital collaborations and contributions made by the military. These collaborations were essential because military personnel travel to ZIKV endemic countries and many of them live off base, necessitating a need to partner and share data. In past years when WNV was the focus of concern, the DPH Vector-borne and Zoonotic Section formed a cooperative agreement with the US Army Center for Health Promotion and Preventive Medicine (USACHPPM) to share mosquito surveillance and testing data collected within the State. Unfortunately, when USACHPPM-South moved its headquarters from Georgia to Fort Sam Houston, Texas, that collaboration was largely lost.

In 2016, as mosquito surveillance was being increased in Georgia, a connection with the military was sought again. Not having one central agency made this process somewhat difficult, as each base had to be approached individually, but ultimately several connections were made. As one example, Dobbins Air Reserve Base Bioenvironmental Engineers (BEE) partnered with the DPH as part of the Department of Defense initiative to

combat ZIKV and other mosquito-borne diseases. One of DPH's new vector surveillance coordinators, along with BEE personnel, laid traps at the Dobbins firing range, an area of frequent mosquito complaints. Mosquitoes collected were identified, and education, source reduction, and chemical control were used to reduce mosquito numbers in the area and reduce risk to military personnel. Follow up surveillance was done, and plans were made to continue this partnership in 2017. This important partnership was published by the US Air Force News Service as shown in Figure 4.[23]

Figure 4. Georgia Department of Public Health vector surveillance coordinator installing mosquito traps at the Dobbins Air Reserve Base.[23]

In addition, Fort Benning, Fort Stewart, Hunter Army Airfield, and Robins Air Force Base agreed to share mosquito surveillance and testing data with the DPH. This not only provides additional information to the DPH concerning disease risks, but also provides mosquito control both on and off the bases with the information they need to reduce mosquito populations and disease risk. It should be noted that Fort Gordon has had a long-standing collaboration with the Richmond County Mosquito Control Program, a division of the local health department.

Conclusion

The collaborative response to ZIKV allowed DPH to rapidly increase its surveillance footprint across the state and train new and existing staff on outbreak response. With these new monitoring and response capabilities, the DPH can make sound public health decisions regarding disease risks and quickly respond to local outbreaks of ZIKV or other vector borne diseases.

Enhanced Capabilities

The Georgia DPH was able to rapidly expand its surveillance capacity statewide because its leadership recognized the seriousness and potential impact of ZIKV on the state and prioritized vector-borne diseases and surveillance. This allowed the agency to maximize existing resources to expand surveillance capacity and reignite historic and develop new collaborations with various entities, most importantly the LHDs and the military. This expanded surveillance network provided a clearer picture of the types of mosquitoes potentially exposing the public to mosquito-borne diseases and allowed DPH to better quantify risks and provide public education. Statistical analysis of the data validates the need for ongoing expanded surveillance.

In evaluating the risk of ZIKV transmission, recent historic data for the primary vector of ZIKV, *Ae. aegypti,* was isolated to just 2 counties in Georgia. Expanded surveillance in 2016 confirmed a low presence of *Ae. aegypti* having been found in just one county, suggesting the primary vector for Zika has been displaced by *Ae. albopictus.* This also suggests a reduced risk of autochthonous transmission of Zika virus in Georgia due to the affinity of *Ae. Albopictus* for feeding on both humans and animals. However, this should be interpreted with caution due to unstandardized reporting techniques for each county, lack of systematic surveillance in every county, and a dry spring and summer that reduced overall number of mosquitoes trapped. The DPH is working with all counties to improve the quality of data reported, and will continue to assess the abundance and distribution of *Ae. aegypti* each year to evaluate risks. In addition, while DPH expanded surveillance to 57 counties which comprise approximately 36% of the state's 159 counties, the agency continues to work with the LHDs to establish additional surveillance sites with the goal of 100% of counties conducting mosquito surveillance. This will allow better interpretation of overall mosquito abundance and distribution across the state.

Public Health Implications

By increasing the number of counties involved in surveillance and knowing the specific species of mosquitoes throughout the state, the DPH can better predict and quantify the risks of disease transmission in specific

regions of Georgia. However, increasing and maintaining surveillance comes with its own challenges, with stable funding being the largest obstacle to a robust surveillance system. The DPH was able to prioritize existing funding to kick start a rapid response and was subsequently awarded grants from the CDC specifically for ZIKV, but these funding streams are temporary and allocated for a specific disease. It is critically important that funding for monitoring, surveillance, and response becomes a permanent federal funding source, and that it does not follow the same path as WNV funding reductions of the past. If so, the United States may not be prepared to respond to the next emerging arboviral disease, and the results could be disastrous for a population that has no natural immunity.

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A Location-specific Spreadsheet for Estimating Zika Risk and Timing for Zika Vector Surveillance, Using US Military Facilities as an Example

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ABSTRACT

Local Zika virus transmission in the United States involving one or both of the known vector species, *Aedes aegypti* and *Ae. albopictus*, is of major concern. To assist efforts to anticipate the risks of transmission, we developed an Excel spreadsheet tool that uses vector and virus temperature thresholds, remotely sensed maximum temperature, and habitat suitability models to answer the questions: "is Zika transmission likely here?" and "when should we conduct vector surveillance?" An example spreadsheet, updated regularly and freely available, uses near real-time and forecast temperature data to generate guidance, based on a novel four level Zika risk code, for 733 US military facilities in the 50 states, the District of Columbia, and the territories of Guam and Puerto Rico.

In 2016, Zika virus and congenital infections became nationally notifiable conditions in the United States.[1] A total of 2,382 confirmed and probable cases of Zika virus (ZIKAV) with illness onset were reported to ArboNET, the US national arboviral surveillance system managed by the Centers for Disease Control and Prevention and state health departments, from January 1 to July 31, 2016.[2] In July 2016, the first locally acquired case of ZIKAV disease from mosquitoes was confirmed in the state of Florida.[3] *Aedes* mosquitoes transmit ZIKAV, chikungunya virus (CHIKV), dengue virus (DENV), and yellow fever virus, among others. Although *Aedes albopictus* Skuse is thought to be a competent vector of ZIKAV,[4] *Ae. aegypti* (L.) has been implicated as the primary transmitter of the virus in human populations in the ongoing outbreak in the Americas.[5,6] This is likely the result of *Ae. aegypti* preferring to feed more frequently on humans,[7,8] and being highly peridomestic compared to *Ae. albopictus*, which can inhabit more rural environments.[9,10] The role of other mosquito species in ZIKAV transmission is either unknown, refers to species not present in the United States, or is controversial,[11] therefore only *Ae. aegypti* and *Ae. albopictus* are considered here.

In this study, we concentrated on US Department of Defense (DoD) facilities, but the approach could be used for any area of interest. Some military facilities have longstanding mosquito surveillance programs,[12] and Zika virus surveillance is being enhanced in the US military as a result of the recent threat. For example, surveillance efforts are supported by funding from the Global Emerging Infections Surveillance and Response section of the Armed Forces Health Surveillance Branch in the Defense Health Agency's Public Health Division.[13] According to a March 2016 DoD memo, 190 DoD installations are located in areas where mosquitoes capable of carrying ZIKAV occur, and increased vector monitoring will be conducted in installations in 27 states, the District of Columbia, Guam, and Puerto Rico.[14] Four regional commands exist under the US Army Medical Command, all of which have entomological sciences divisions that conduct mosquito surveillance. Additionally, the US Air Force School of Aerospace Medicine, the US Navy and Marine Corps Public Health Center and regional Navy Environmental and Preventive Medicine Units, and the Navy Entomology Center of Excellence assist those undertaking vector surveillance or arbovirus testing.

For a military entomologist tasked with establishing and maintaining an *Aedes* spp./ZIKAV surveillance program in temperate areas that experience high mosquito seasonality, two important questions arise: (1) is ZIKAV transmission possible here?; (2) when should vector surveillance be conducted? In temperate zones like the continental United States, the answers to these questions may vary depending on the time of year. In this article, we describe an Excel-based tool that is designed to assist entomologists and other health professionals address these 2 questions throughout the year.

Habitat suitability models displaying potential distribution have been published for both *Ae. aegypti* and *Ae.*

albopictus,[15-19] as well as for ZIKAV.[20-23] While these models often display average yearly suitability, they do not necessarily provide information that could be used for decisions about the timing of surveillance activities, and are global in extent rather than focused on particular areas where a surveillance program might be established. Questions about timing of mosquito monitoring and allocation of resources requires a consideration of what conditions limit adult mosquito activity and ZIKAV dissemination in the field.

Relative humidity, rainfall, drought, and wind velocity affect survival and behavior of mosquitoes, and therefore transmission.[24] However, temperature is the most important ecological determinant of the development rate of *Ae. aegypti*,[25] and one of the principal determinants of *Aedes* survival.[26] Temperature also directly affects the replication rate of arboviruses, thus affecting the extrinsic incubation period.[27] What then do we know about how temperature limits *Aedes* and arboviruses such as ZIKAV?

In their review of reports published in the early 20th century, Bonne-Wepster and Brug[28] presented data about the effects of temperature on the activity and survival of *Ae. aegypti* mosquitoes, summarized below:

- Female mosquitoes were observed to feed most readily between 26°C and 35°C, between 19°C and 25°C they are slow to blood feed, and below 15°C to 19°C they do not feed (Marchoux et al,[29] Howard et al,[30] Connor[31]).
- Female mosquitoes in Montevideo can continue to bite at 14°C to 15°C (Cossio[32]).
- Adults were observed to die when exposed to temperatures above 38°C, but Davis[33] reported some adults surviving exposure to 40°C during 7 hours, and amongst another group exposed to 45°C for 2 hours.
- At 7°C to 9°C, adult females may live up to 80 days, and the male up to 14 days (Guiteras; Otto; Newman[34]).
- The female dies within 24 hours, if exposed to a temperature of 6°C (Flu[35]).
- Dinger et al[36] reported survival of adult female mosquitoes kept for 6 days at 5°C.

More recently, in Saudi Arabia, Khormi et al[37] found that the minimum temperature range of 18°C to 25°C is suitable for *Ae. aegypti* survival, and the survival rate increases up to 38°C. Conner[38] and Wayne and Graham[39] found that *Ae. aegypti* is most active at temperatures between 15°C and 30°C, while other field and laboratory observations found survival rates from about 18°C to 38°C or less, based on daily or monthly minimum and maximum temperatures.[40-42] In a study of *Ae. aegypti* distribution using the program CLIMEX, Khormi and Kumar[18] set the limiting low temperature at 18°C, the lower optimal temperature at 25°C, the upper optimal temperature at 32°C, and the limiting high temperature at 38°C. Brady et al[16] limited their predictions of temperature suitability to areas with a maximum monthly temperature exceeding 13°C for *Ae. albopictus* and 14°C for *Ae. aegypti.* These threshold temperatures were based on previous studies of the observed temperatures below which biting and movement behaviors were impaired.[42-45]

Studies suggest that an increase between 14°C to 18°C and 35°C to 40°C can lead to higher transmission of dengue.[46] Xiao et al[47] found that oral infections of DENV2 did not produce antigens in the salivary glands of *Ae. albopictus* kept at 18°C for up to 25 days but did produce antigens at 21°C. It is not known if *Ae. albopictus* held longer at the lower temperature would have disseminated infections, but Dohm et al[48] found that *Culex pipiens* required 25 days at 18°C to disseminate infections of West Nile virus (WNV). For comparison, WNV is capable of replication from 14°C to 45°C.[49,50] Tilston et al[51] analyzed monthly average temperature of cities that experience chikungunya outbreaks and found that start and finish occurred when average monthly temperatures were 20°C or higher. At the upper temperature limit, Kostyuchenko et al[52] found that ZIKAV is more thermally stable than DENV, and is also structurally stable even when incubated at 40°C, mimicking the body temperature of extremely feverish patients after virus infection. However, a study by Goo et al[53] indicates that the thermal stability of some ZIKAV strains, including those involved in recent outbreaks, falls between those of DENV and WNV.

Remotely sensed temperature data is freely available from multiple sources as both near-real time recordings and forecast predictions. Combining remotely-sensed temperature data with predicted distributions of the vectors and virus could provide insight into when areas of interest are suitable for transmission and should be actively monitored. Our aim was to produce a knowledge product and surveillance decision tool that uses publicly available information about potential distribution and thermal requirements of the vectors and virus at US military facilities.

Materials and Methods

Areas of Interest

The location and boundary of US military facilities was obtained from the US Census Bureau's TIGER/Line

2015 shapefile product.[54] This shapefile lists facilities in the continental United States, Alaska, Hawaii, Puerto Rico, and Guam. As some facility names comprised multipart polygons, these were reduced from 804 to 733 to match the number of unique facility names, using the Dissolve tool in ArcMap 10.4 (ESRI, Redmond, CA). The centroid of each facility was selected to produce a shapefile of points using the Feature to Point tool (inside polygon option checked) of ArcMap. The georeference of each centroid was obtained by the Add XY Coordinates tool and joined to the points shapefile. Extraction of all facility centroid raster values was first obtained by the Extract Values to Points tool, then for polygons using the Zonal Statistics as Table tool, and the results merged. This approach was required because smaller polygons would not produce results using the Zonal Statistics as Table tool, which necessitated use of the raster data associated with the points for these facilities.

Temperature Data

To monitor temperature in near real-time, daily time averaged maps of air temperature at the surface (daytime/ascending) were downloaded from the Giovanni 4.19 (Released Date: 2016-04-12. Data provided by the National Air and Space Administration (NASA) Goddard Earth Sciences Data and Information Services Center) data portal at 1° spatial resolution.[55] Daily gridded temperature analyses were also collected from the National Oceanic and Atmospheric Administration (NOAA) US National Weather Service Climate Prediction Center (CPC).[56] Forecast temperature data was also provided by the CPC and the NOAA National Digital Forecast Database at 5 km spatial resolution.[57] For predictions based on monthly averages, monthly gridded climate data with a spatial resolution of 1 km were downloaded from WorldClim.[58]

Habitat Suitability Models

We chose the models of *Ae. aegypti* and *Ae. albopictus* by Kraemer et al,[59] which were based on an extensively documented set of presence observations for each vector. We also used the habitat suitability model for ZIKAV transmission by Messina et al.[21] This model used Zika case reports and data on temperature, precipitation, humidity, enhanced vegetation index, and urban versus rural. Both vector and virus maps are at a 5 km x 5 km spatial resolution. The 0.5 model suitability score was arbitrarily used as a presence/absence threshold.

Thresholds

The temperature suitable for activity of *Ae. aegypti* and *Ae. albopictus* combined was estimated as 13°C to 38°C, and 18°C to 42°C for ZIKAV. We chose to be conservative, using temperatures at the extremes of the reported suitable temperature range, and maximum rather than mean air temperatures.

Human Population Data

In order to more fully understand the potential impact of ZIKAV risk to military and nonmilitary personnel and their families in and around each facility, we explored risk in terms of human population data, with the following considerations. The flight range of *Ae. aegypti* and *Ae. albopictus* is in the order of hundreds of meters only,[60,61] and each facility would differ in the average distance that human carriers of ZIKAV would routinely travel to and from each facility. Additionally, some facilities were remote, while others were adjacent to or enclosed within urban and suburban areas. To address these complications, we created a buffer of 5 km around all facility polygons to capture the human population density according to LandScan 2011.[62] This was accomplished using the LandScan raster and the Buffer and Sum output in the Zonal Statistics as Table tools in ArcMap. A buffer of 5 km is a conservative estimate and is meant to give a uniform measure for each facility of the potential host density affected in an outbreak or vector control situation.

Excel-based Zika Risk Tool

A goal of this project was to display disparate data sources visually and in a simple and intuitive way in order to more effectively communicate the level of risk at each military facility. The risk estimation and alert system had to be in a format that was readily understandable and easily accessible by military users, who often have information system security restrictions or bandwidth caps. We chose MS Excel (Microsoft Corp, Seattle, WA), as a universal platform for performing calculations and reporting results. This software had the added advantage that the scatterplot function can be used to map each military facility,[63] with icons displaying various categories of risk, and using a geocorrected map background[64] for each state. Other notable features that were used in the Excel risk estimation tool were the formula functions, conditional formatting to represent categories of numbers as different types of symbols, dependent dropdown lists and hyperlinks to allow users to navigate more quickly to the results of individual facilities, and textualized results that users can read as statements describing the situation and as guidance for vector surveillance.

Calculations Within the Excel Risk Estimation Tool

Given that the maximum temperature is available for a site (ie, "Temp"), the following lists an example

sequence of tasks and their calculations, with explanations and the Excel formula (in square brackets), culminating in a risk rating:

1. Column A. "Was temperature suitable during period for the vector?", ie, if the maximum was 13°C to 38°C, it is 1, otherwise 0 [=IF((Temp>=13)-(Temp>38),1,0)],
2. Column B. "Was temperature suitable during period for virus replication in mosquito?", ie, if the maximum was 18°C to 42°C, it is 1, otherwise 0 [=IF((Temp>=18)-(Temp>42),1,0)]
3. Column C. "What is the sum of the thermal suitability values for vector (Column A) and virus (Column B)?", ie, possible choices are: 0, 1, or 2.
4. Column D. "If temperature for the vectors (Column A) was within the required range, what is the model suitability for vector?", ie, this was the maximum modeled suitability (0-1.00) for either *Ae. aegypti* or *Ae. albopictus.*
5. Column E. Score vector model suitability as 3 if ≥0.5, otherwise 2 [=IF(Column D<0.5,2,IF(Column D>=0.5,3))]. The 0.5 model suitability score was arbitrarily used as the cutoff for presence/absence.
6. Column F. "If temperature for the virus (Column B) was within the required range, what is the model suitability for the virus?" (0-1.00)
7. Column G. Score virus model suitability as 7 if ≥0.5, otherwise 5 [=IF(Column F<0.5,5,IF(Column F>=0.5,7))]. The 0.5 model suitability score was arbitrarily used as the cut-off for presence/absence.
8. Column H. "What is the 'Combined Score' for the interaction of temperature suitability of vector and virus, vector model suitability, and virus model suitability score (ie,=C×E×G)?" The use of 0, 1, and prime numbers for the component scores produces a unique semi-prime number for the product, ie, 0, 10 (=1×2×5), 14 (=1×2×7), 15 (=1×3×5), 21 (=1×3×7), 20 (=2×2×5), 28 (=2×2×7), 30 (=2×3×5), or 42 (=2×3×7). A zero indicated that the temperature at the site was unsuitable for both vector and pathogen, so suitability scores were irrelevant, and combination scores were all scored zero.
9. The 9 possible Combined Scores were divided into 6 categories based on whether preconditions do not exist for transmission, are unsuitable for transmission, are somewhat suitable for transmission, or are suitable for transmission (Figure 1).
10. Column I. An Overall Zika Risk Code was established based on the Combined Score (Figure 1), and rates conditions as low (Blue: Code 1) to high risk (Red: Code 4). The 9 possible Combined Scores are initially divided according to whether temperature conditions are not suitable (Code 1) or suitable for the vectors and virus (Codes 2-4). Codes 2-4 are then characterized according to increasing habitat suitability, with Code 4 being where models predict suitable habitat for vectors and virus.
11. Action statements were constructed based on the temperature and habitat model suitability scores (Figure 2). For example, if conditions are too cold for the development of the vectors and models predict that the location is unsuitable for the vectors, the Action statement would be: "Too cold or hot for vectors–surveillance unnecessary. When Temp suitable, model suggests vectors unlikely or low numbers." Alternatively, if conditions are warm enough for the development of the vectors and models predict that the location is highly suitable for the vectors, the Action statement would be: "Temp suitable for vectors–surveillance may be needed. When Temp suitable, model suggests vectors likely–may need control, education, and policies minimizing exposure."

Results

The Excel files comprise 12 monthly files based on average monthly maximum temperatures (suitable for longer term planning), and near real-time and forecast file, updated weekly. These files are freely available via the VectorMap website.[65] The tool provides risk maps of facilities as a continental overview (Figure 3), and on a state by state basis (Figure 4). Results for individual facilities are navigable via dropdown menus and hyperlinks (Figure 5). Statewide summary data of risk profile and humans potentially affected is shown in Figure 6. The temporal changes in average risk based on the 12 monthly files is given in Figure 7 in terms of the number of facilities affected (of 733) and the number of people within 5 km of the facilities. April to October was the period of greatest risk with suitable conditions for Zika transmission (ie, code 4) potentially affecting a maximum of 114 facilities in 12 states and territories,

Zika risk code	Detailed explanation	Preconditions for transmission	Combination score
1	Temperature not OK for Vector and Virus	Preconditions do not exist for Transmission	0
1	Temperature OK for Vector or Virus but not both. Model says habitat not very suitable for Vector	Preconditions do not exist for Transmission	10,14
1	Temperature OK for Vector or Virus but not both. Model says habitat suitable for Vector	Preconditions do not exist for Transmission	15,21
2	Temperature OK for Vector and Virus. Model says habitat not very suitable for Vector and Virus	Preconditions unsuitable for Transmission	20
3	Temperature OK for Vector and Virus. Model says habitat suitable for Vector or Virus but not both.	Preconditions somewhat suitable for Transmission	28,30
4	Temperature OK for Vector and Virus. Model says habitat suitable for Vector and Virus.	Preconditions suitable for Transmission	42

Figure 1. An overall Zika Risk Code based on the combined score, which rates conditions from low risk (Blue: Code 1) to high risk (Red: Code 4).

Variable	Outcome	Action Statement
Temperature threshold for Zika vectors	Temperature below threshold (< 13° C)	Too cold for vectors - surveillance unnecessary.
	Temperature above threshold (=> 13° C)	Warm enough for vectors - surveillance may be needed.
Vector Suitability	Low vector suitability (< 50%)	When warm enough, model suggests vectors unlikely or low numbers.
	High vector suitability (=> 50%)	When warm enough, model suggests vectors likely - may need control, education, and policies minimizing exposure.
Temperature threshold for Zika virus	Temperature below threshold (< 18° C)	Too cold for Zika - surveillance unnecessary.
	Temperature above threshold (=> 18° C)	Warm enough for Zika - surveillance may be needed.
Zika Suitability	Low Zika suitability (< 50%)	When warm enough, model suggests Zika unlikely.
	High Zika suitability (=> 50%)	When warm enough, model suggests Zika likely - may need control, education, and policies minimizing exposure.
	No data	No Model result for vector suitability.

Figure 2. Action statements constructed on the basis of the temperature and habitat model suitability scores.

and 4,546,505 people within the vicinity of these facilities, of a total of 32,811,618 within the vicinity of all 733 facilities. The maximum number of facilities recording code 4 in any one month (eg, August) were: Florida (36), Hawaii (16), Louisiana (12), Texas (11), and Virginia (11). Of these, the number of people within 5 km of these facilities were: Texas (1,215,230), Florida (1,125,032), Louisiana (462,586), Virginia (409,066), and Hawaii (247,918).

These data may assist public health planning, and can be seen as an indicator of potential disease burden, or of people potentially benefiting from a well-informed vector surveillance and control program conducted on military facilities. Results are provided in a variety of symbologies and as textualized statements of how the factors examined may affect ZIKAV transmission, and recommended actions for entomologists conducting routine vector surveillance. The action statement textualizes the data and is designed to assist a preparedness posture, particularly around vector surveillance and control. Changes in the action statement over the year, for example as a result of rising temperature, can be used as a guide to affect changes in vector surveillance and control activities at particular facilities.

COMMENT

The Excel tool is designed to provide insights into ZIKAV transmission potential at US military facilities, but could be applied to other arboviruses and situations, such as cities,[66] tire dumps, or parks. The spreadsheet is flexible in that vector and virus suitability model scores, temperature limits, and the wording of action statements can be replaced depending on the context, and as new information is compiled.

For US military situations, this tool could be used in conjunction with the Electronic Surveillance System for the Early Notification of Community-based Epidemics[67] or Medical Situational Awareness in Theater,[68] which

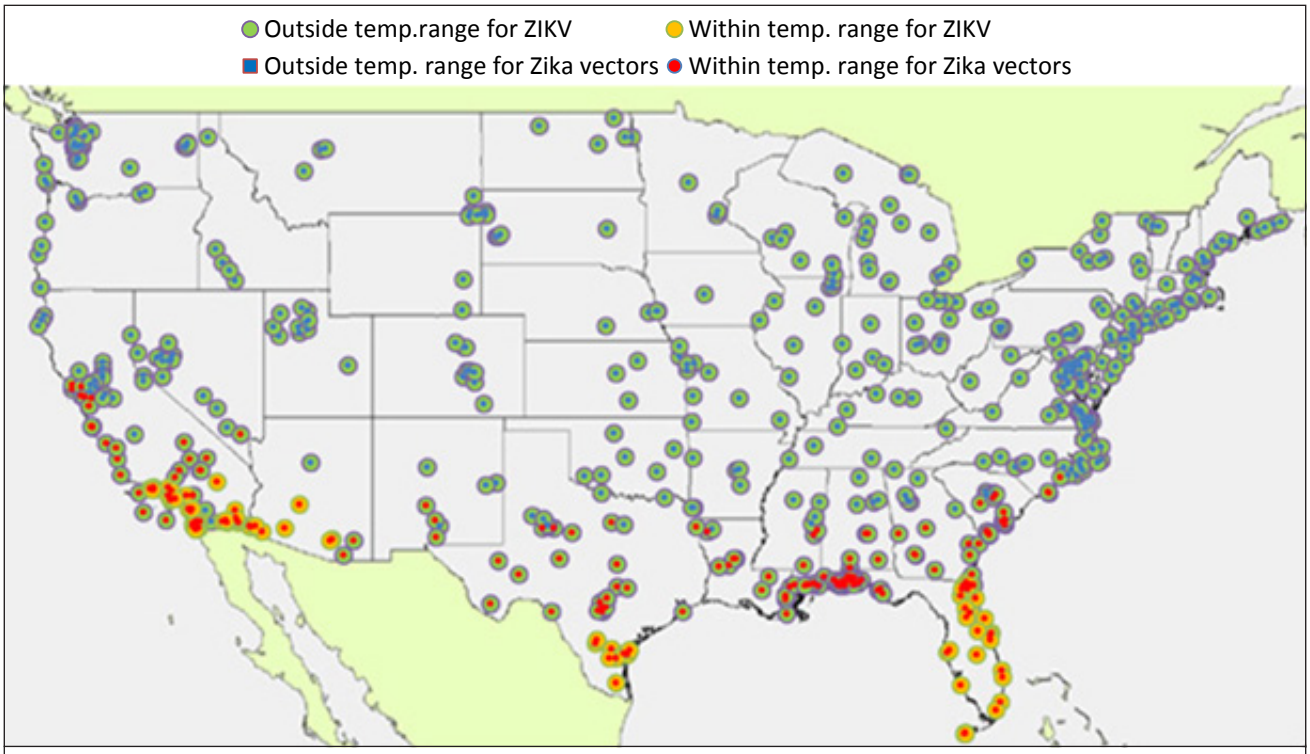

Figure 3. Average maximum temperature conditions for January for vectors and ZIKAV at military facilities within the contiguous 48 states.

reports on febrile illnesses and rash in the military population. Coordination of result reporting through the Armed Forces Pest Management Board[69] and VectorMap may also be desirable. The Navy and Marine Corps Public Health Center (NMCPHC) guide, *Aedes Surveillance and Control Plan for U.S. Navy and Marine Corps Installations*,[70] states that "each installation's medical personnel should conduct ongoing *Aedes* surveillance during the mosquito season appropriate to their region and take preventive and responsive action to reduce disease risk to active duty, government employees, and family member populations."[70(p4)] In addition, it points out that "OPNAVINST 6250.4C…requires all Navy and Marine Corps installations to have an Emergency Vector Control Plan (EVCP) for disease vector surveillance and control during disease outbreaks."[70(p4)] The spreadsheet described in this study should provide an additional resource for installations as they use the NMCPHC guide to "…complement installation pest management plans, including the EVCP, as a way to assess the risk of vector borne diseases, and implement strategies to reduce the risk to personnel assigned to installations."[70(p4)]

Knowing when conditions are suitable for vectors is crucial for monitoring the success or failure of any control program. Appendix C of the NMCPHC guide[70] is a chart to determine the risk of infection on an installation and when to apply vector control measures. This 4-level vector threat response plan relies on information about vector abundance and reports of disease transmission. We believe the Excel spreadsheet risk tool to be a valuable adjunct to the NMCPHC plan, as it would assist with defining the length of the mosquito season, and the judicious deployment and timing of entomological resources. Each military facility is unique and varies in size, function, human density, and suitable mosquito habitat, so not all of the 733 facilities addressed in this study will be at risk of mosquito-borne disease and suitable candidates for mosquito surveillance. However, in any case, all locations should be useful as points of reference for other nearby locations where mosquito surveillance is conducted.

Development of the Zika Risk Code in Figure 1 derived some inspiration from Figure 3 of Fischer et al,[71] who combined models of vector habitat suitability with temperature categories for CHIKV replication to produce a matrix of climate related risk classes.

It is important to note that each data source used in this analysis has the potential for errors which should be considered when determining risk. For example, habitat suitability models for each vector may not be accurate for all areas, and may only predict average yearly suitability. Traits like temperature vary continuously over the surface of the earth but are effected by elevation and use of

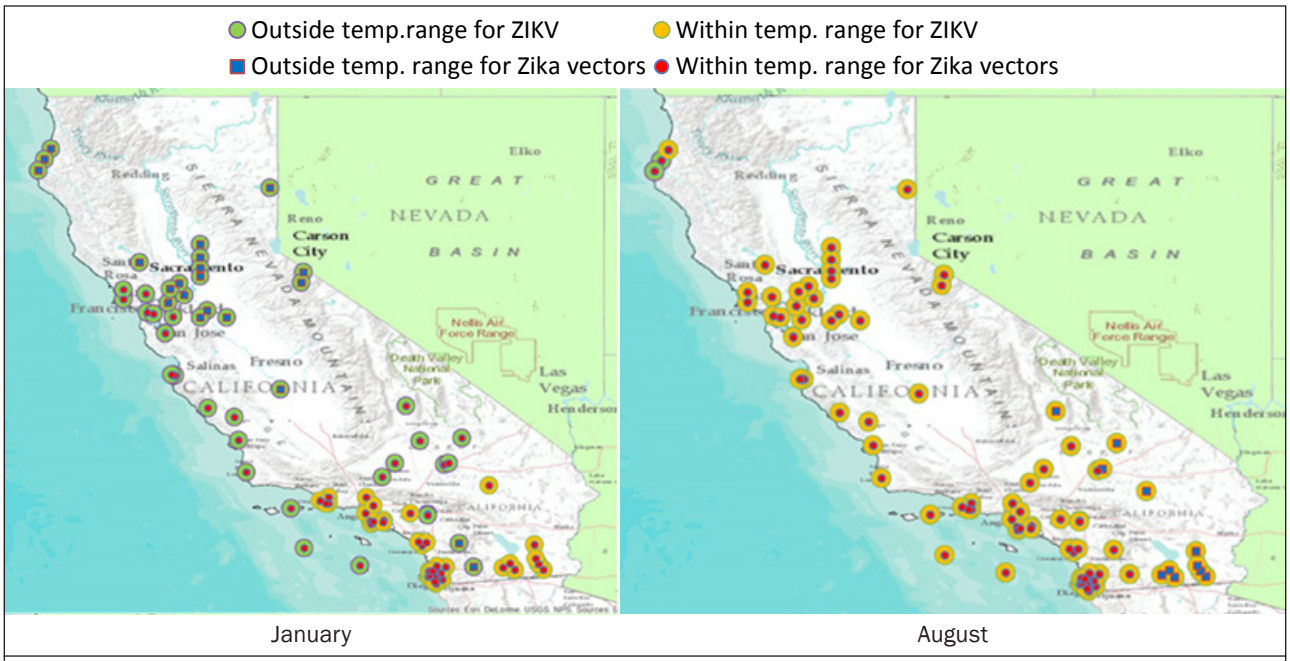

Figure 4. Thermal conditions for January and August for vectors and ZIKAV at military facilities in California. Note: unsuitable conditions in August in the south are due to temperatures too high for the vectors.

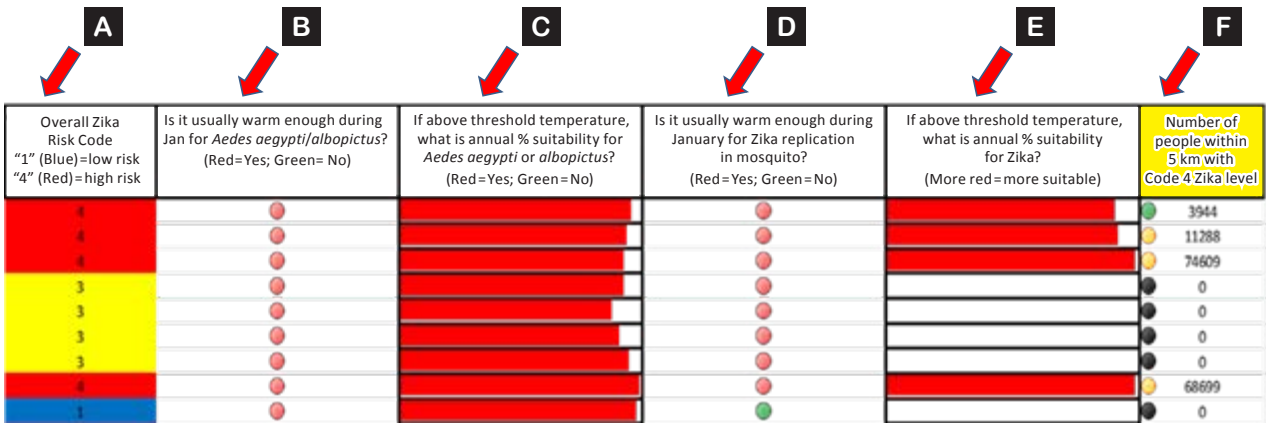

Figure 5. An overall Zika virus risk code for near real-time and forecast periods (A) is assigned based on a combination of: temperature suitability for adult activity of the vectors (*Ae. aegypti* and *Ae. albopictus*) (B), modeled habitat suitability of the vectors (C), temperature suitability for Zika virus replication within the vectors (D), and modeled habitat suitability of Zika virus transmission (E). If suitable conditions exist (Zika Risk Code 4), the number of people within 5 km is shown (F) as one indication of the number of potential hosts in the vicinity, or the number of people potentially benefiting from facility-wide vector surveillance and control programs.

multiple scale data, including weekly and monthly, and 1 km and 1° spatial resolution data used here will result in important spatial and temporal variations in accuracy. Temperature data refers to the maximum daytime air temperature near the surface (averaged over various spatial resolutions) from daily data for a recent date range, which NASA acknowledges has limitations. Vectors can also seek microclimates (eg, indoors, subterranean habitats) that may be warmer or cooler than the outside temperature that is estimated by remote sensing data.

Temperatures within the suitable range may not affect organisms uniformly. According to Westbrook et al,[72] adult female mosquitoes reared from immature stages at 18°C, were 6 times more likely to be infected with CHIKV than those reared at 32°C. Westbrook et al[72] noted that climate factors, such as temperature, experienced at the larval stage (which would not be detected by adult trapping programs) can influence the competence of adult female mosquitoes to vector arboviruses. We also do not account for temperature fluctuations; according to Lambrechts et al,[73] mosquitoes lived longer

Summary of Vector hazard during January for U.S. Military facilities by State for Zika virus

State	# Military facilities[1]	Zika Risk Code[2-5] (average)	# facilities where preconditions suitable for transmission (Zika Risk Code 4)[5]	# facilities with above threshold temperature for the vectors[6]	# facilities with above threshold temperature for the pathogen[7]	Total population within 5 km of facilities[8]	Total population within 5 km with suitable conditions for transmission
Alaska	20	1	0	0	0	357,044	0
Alabama	14	1	0	10	0	553,648	0
Arkansas	4	1	0	0	0	187,622	0
Arizona	11	2.19	0	10	0	556,120	0
California	100	1.71	0	79	0	6,655,724	0
Colorado	9	1	0	0	0	386,446	0
Connecticut	10	1	0	0	0	212,038	0
District of Columbia	8	1	0	0	0	842,772	0
Delaware	2	1	0	0	0	52,946	0
Florida	48	2.44	17	48	1	1,373,859	653,556
Georgia	16	1	0	9	0	1,021,113	0
Hawaii	36	3.2	16	36	1	980,370	247,918
Iowa	2	1	0	0	0	266,567	0

Figure 6. Summary risk data for each US state (top of list shown) to assist with public health and resource allocation planning.

and were more likely to become infected with DENV under moderate temperature fluctuations rather than under large temperature fluctuations. Thangamani et al[74] and Ferreira-de-Brito et al[6] found that ZIKAV can be vertically transmitted in *Ae. aegypti* but not *Ae. albopictus*. This capability suggests mechanisms for the virus to survive in eggs that can survive for months in a dried dormant state during adverse conditions, for example, a harsh winter that would normally kill adult mosquitoes.

The approach to estimating risk levels in this project deliberately uses simplified assumptions about temperature and mosquito physiology and relies on published models to consider other drivers, which could include: precipitation; interspecific competition; and anthropogenic factors such as imported cases, built-up areas, vegetation indices, human behavior, and economic indices that can modify risk in complex and less understood ways. Other models could be used in place of the ones included in this study. For example, Samy et al[23] used proxies for poverty and accessibility that may further increase the biological reality of estimates of transmission risk. As a risk factor for ZIKAV infections is congenital brain abnormalities including microcephaly,[75] risk estimates would be greatly enhanced by quantifying the proximity of pregnancies to areas of interest[76,77] (ie, military facilities). Among US military women, those of reproductive age represent the majority,[78] and from 2001 to 2010, an average of 15,600 children were born to active component women each year.[79] Data from the Defense Medical Surveillance System do not include records of nonreimbursed care received at medical facilities outside of the military health system, adding to the difficulty in estimating numbers of potentially at-risk mothers, or those in the vicinity who could benefit from vector control programs conducted within military facilities.

The Zika virus can be imported and spread by nonvector transmission routes (eg, sexual transmission[80]), so it is recommended that a level of caution be taken when interpreting the data provided by this system. It is wise to monitor activity in surrounding facilities and any reputable information from other sources before acting on any recommendations given here. It is further recommended that the near-real time and forecast analysis should be viewed in conjunction with the monthly average Excel vector hazard files which uses average monthly maximum temperature, to gain further longer-term insights into where thermal conditions will support vector activity.

While this tool could be improved with higher resolution data and more nuanced models, our aim was to create an accessible and adaptable, yet useful platform for entomological decision-making that uses readily available data and models. New models and higher resolution climate data can be easily incorporated into this tool as they become available. Validation of the output from this tool using mosquito surveillance results, and obtaining user feedback, would be useful goals for future research.

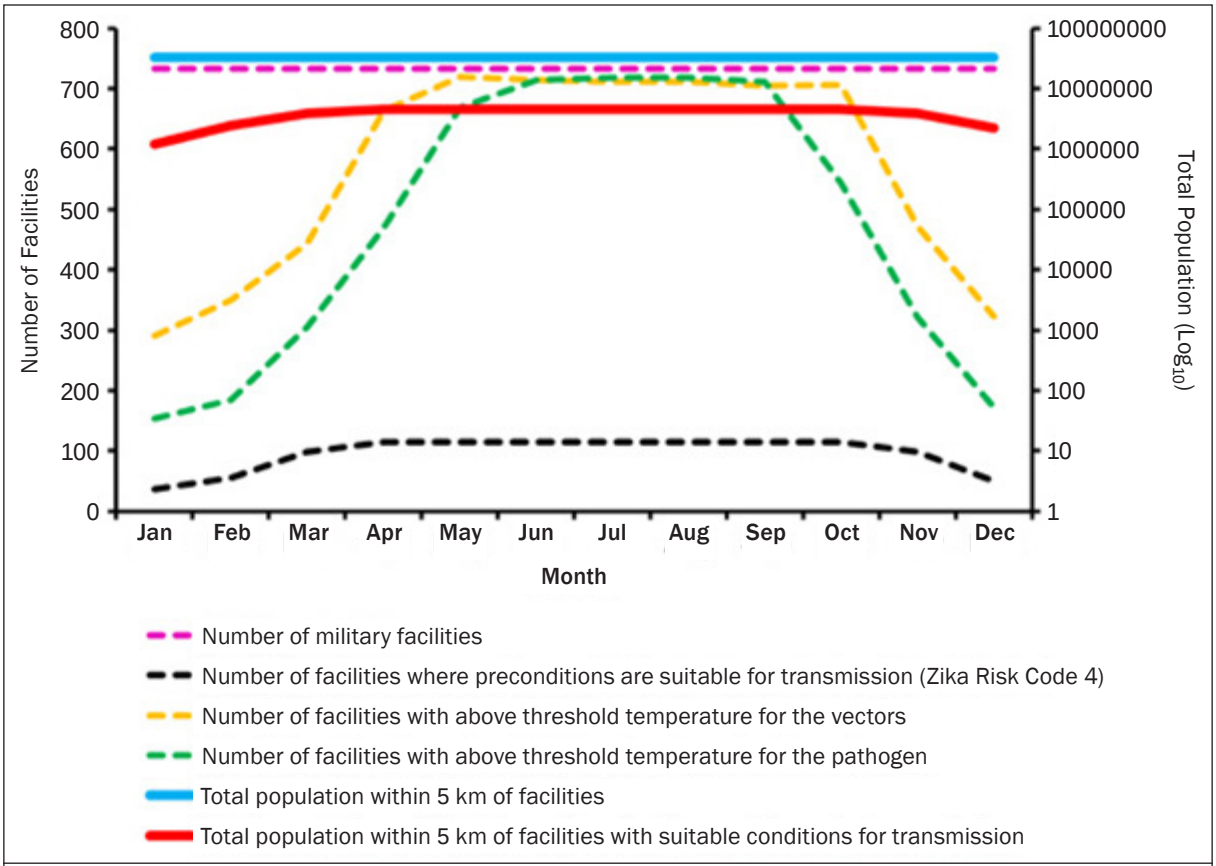

Figure 7. Summary risk data for 733 US military facilities over 12 months.

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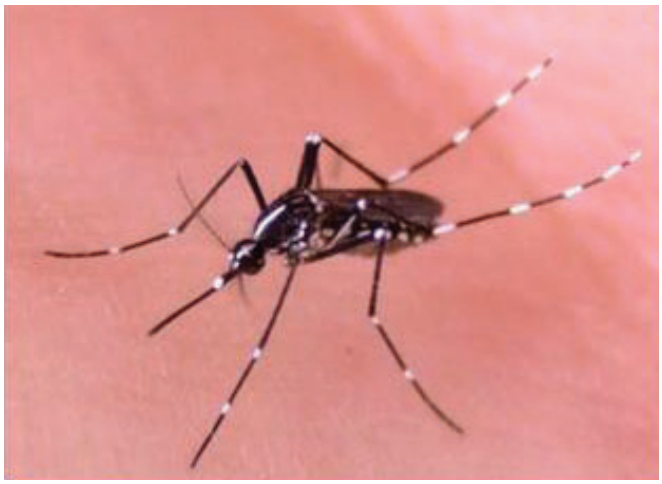
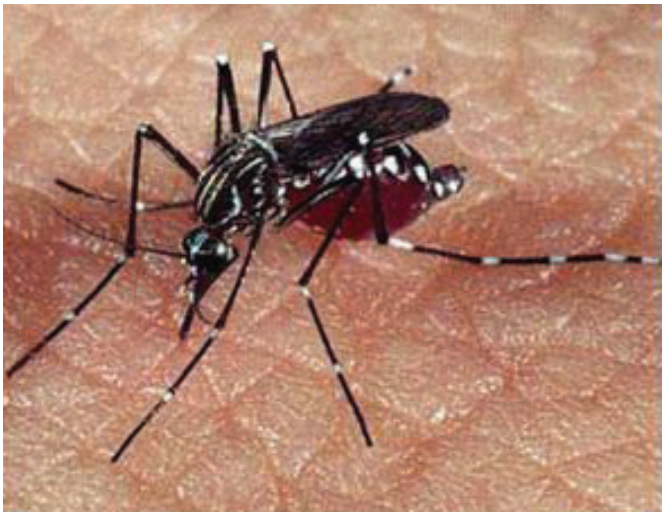


Aedes albopictus (courtesy of the CDC)



Aedes aegypti (courtesy of the CDC)

Biosurveillance and Morphological Variations of Larvae and Pupae of Common Malaria Vectors, *Anopheles* (*Anopheles*) Hyrcanus Group Species in the Republic of Korea

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ABSTRACT

A total of 4,576 *Anopheles* (*Anopheles*) Hyrcanus Group larvae belonging to 6 species (*An. belenrae*, *An. kleini*, *An. sinensis*, *An. pullus*, *An. lesteri*, and *An. sineroides*) were collected from 7 different habitat types in 3 provinces of the Republic of Korea. The occurrence and relative abundance of 6 *Anopheles* species were noted. The descriptions in the article of the waxy body ornamentations or patterns of *An.* (*Ano.*) Hyrcanus Group larvae and pupae may be useful for rapid field species identification when conducting larval mosquito surveillance.

Malaria is a major health threat to military and civilian personnel as well as family members deployed or residing in the Republic of Korea (ROK). Members of the *Anopheles* (*Anopheles*) are placed in 3 groups, namely the Hyrcanus Group (*An. belenrae* Rueda, *An. kleini* Rueda, *An. sinensis* Wiedemann, *An. pullus* Yamada, *An. lesteri* Baisas and Hu, *An. sineroides* Yamada), the Barbirostris Group (*An. koreicus* Yamada and Watanabe), and the Lindesayi Group (*An. lindesayi japonicus* Yamada).[1-3] *Anopheles kleini* and *An. lesteri* are the primary vectors of *Plasmodium vivax* malaria near the demilitarized zone (DMZ), while *An. sinensis* is a very poor vector and the status of the remaining species is undetermined.[4,5] Laboratory studies have shown the potential for *An. lesteri* as an efficient vector[6,7] and is considered a primary vector (as *An. anthropophagus* Xu and Feng) in parts of China.[8] In the ROK, the populations of *An. lesteri* are very low,[9] except on the northwest coastal islands.[10] Females of *An. belenrae* and *An. sineroides* have been found positive for *P. vivax* by enzyme-linked immunosorbent assay and polymerase chain reaction (PCR).[9,11] Species in Barbirostris and Lindesayi Groups have not been implicated as malaria vectors in the ROK.[9]

Knowledge of the species composition of common *Anopheles* (*Anopheles*) in various habitats in different provinces is needed for defining malaria vector distributions, biosurveillance and vector control. Data are limited on the collections and habitats for the occurrence and relative abundance of indigenous *Anopheles* mosquitoes in the ROK.

With several human malaria cases reported annually from the ROK,[10,12,13] there is an urgent need to accurately identify the potential *Anopheles* mosquito vectors. Misidentifications of species often lead to poor vector surveillance, misunderstanding of the epidemiology of disease transmission, and the development and implementation of ineffective strategies for control measures.[1] Both morphological and molecular analyses are essential in the taxonomy and identifications of members of the *Anopheles* Hyrcanus Group species, particularly those where their geographic distribution and vector potential are better understood. During the mosquito surveillance, field identifications of live larvae and pupae may also be convenient and necessary, when possible.

Waxy body ornamentations have been previously reported on various insects, particularly scale insects,[14] aphids,[15] and heliconian caterpillar,[16] and they have been shown to be useful for initial identifications during field surveillance efforts. Morphological variations in the body ornamentations for different stages of development, eg, leg paddles observed in the sabethine mosquitoes, *Sabethes cyaneus* (Fabricius)[17] are important. Knowledge on mosquito body ornamentation is very limited, and little is known about the waxy body covering of larvae and pupae of *Anopheles* species or its importance in identifications.

Immature stages (both larvae and pupae) and adults of members of the *An.* (*Ano.*) Hyrcanus Group in the ROK cannot be identified with certainty using morphological techniques. Although there are some slight differences in the branching patterns of the setae in the larval head (setae 3, 7, 9-C), thorax (14-M) and abdomen (5, 9-II; 4, 9-III; 4, 6-IV; 6-V),[3] they are still not very useful in performing accurate identifications of the species, thereby

requiring the need for the PCR techniques. Furthermore, specimens must be mounted on glass slides for microscopic morphological examinations of the branching patterns of setae of the head and other body parts, requiring excessive manpower and delays in identification. Some waxy ornamentations or patterns on the dorsal parts of the larval bodies were observed during the conduct of extensive field collections and surveillance of *Anopheles* mosquitoes from various parts of the ROK.

The main objectives of our study were: (1) to conduct a comparative survey of mosquito larvae from selected habitats using standardized sampling methods to obtain pertinent data on the occurrence and relative abundance of indigenous *Anopheles* species, and (2) to identify the waxy body ornamentations or patterns of *Anopheles* (*Anopheles*) Hyrcanus Group larvae and pupae which may be useful for rapid field identifications during mosquito vector surveillance and control.

Materials and Methods

Immature mosquito collections that included both anophelines and culicines were conducted from 3 provinces (Gyeonggi, Gyeongsangbuk, and Chungcheongbuk) of the ROK (Figure 1). Selected habitats were sampled including the following: rice paddies (uncultivated and cultivated), irrigation ditches (concrete and non-concrete ditches), drainage ditches (concrete and non-concrete ditches, culvert and dammed ditches), ponds (including pond reservoir), stream margins (including stream ponds, inlets, algal mats), and other habitats (including ground pools, ground depressions, flood pools, rock pools, uncultivated fields, artificial containers).

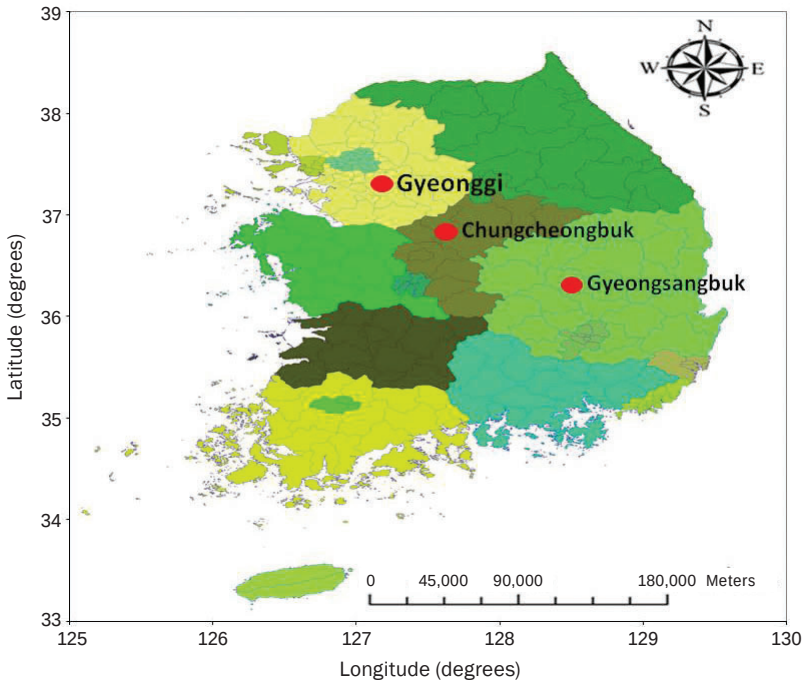

Figure 1. Geographic locations of the 3 provinces in the Republic of Korea within which mosquitoe collections were conducted.

As part of the mosquito biosurveillance, data were collected monthly from May 2007 to October 2007 and were combined over the sampling periods for each of the selected habitats in 3 provinces (Gyeonggi, Gyeongsangbuk, and Chungcheongbuk). The relative abundance of each *Anopheles* species in each habitat was calculated as the percentage of the total number of larvae recovered either per month or over the entire 6-month period.

As part of the morphological study of waxy body ornamentations, *Anopheles* larvae were collected from 9 locations in Gyeongsangbuk Province, including Hayang-eup, Gyeongsansi (Collection nos. KSK-509, 511, 513, 514, 520, 522, 523) and Cheongsong-eup, Jinbo-myeon (Collection nos. KSK-529, 533, 543, 545).

Depending on the habitats, larvae and pupae were collected using a standard larval dipper (350 ml, 13 cm diameter) or a white plastic larval tray (25 cm x 20 cm x 4 cm) (BioQuip, Rancho Dominguez, CA). Collected live larvae and pupae, with intact waxy ornamentations or body patterns were placed in plastic Whirl-Pak bags (118 ml, 8 cm x 18 cm) (BioQuip, Rancho Dominguez, CA) filled approximately ½ full with water from the collection site. The Whirl-Pak was tightly closed to retain air, placed in a cooler, and brought to the laboratory where selected specimens were photographed on the same day, and 3rd and 4th instar larvae then placed and directly preserved in 100% ethanol (EtOH) for molecular identification. The remaining larvae were individually link-reared to adult stage as morphological voucher specimens for this work. Emergent mosquito adults were pinned on paper points, each given a unique collection number, and identified using diagnostic morphological characters. For molecular species identifications, DNA was isolated from individual larval mosquitoes and adults (1 or 2 legs per adult) by extraction procedure and direct sequencing as described in Wilkerson et al.[12] Sequencing of *An. sinensis*, *An. pullus*, *An. lesteri*, *An. belenrae*, and *An. kleini* are those of Wilkerson et al[12] and Li et al[18] using the primers therein. GenBank accession numbers for the above species are in Wilkerson et al[12] and Li et al.[18] Voucher specimens and collection records were

deposited in the US National Museum of Natural History, Smithsonian Institution, Suitland, Maryland.

Results

Occurrence and Relative Abundance of Mosquito Larvae from Selected Habitats

A total of 4,576 specimens belonging to 6 species of *An.* (*Ano.*) Hyrcanus Group species (*An. belenrae*, *An. kleini*, *An. sinensis*, *An. pullus*, *An. lesteri*, and *An. sineroides*) were collected monthly from May 2007 to October 2007 from 7 general habitats by standard larval sampling method. As shown in the Table, all 6 species were collected from irrigation ditches, while only 5 species were collected from rice paddies, ponds, swamps, and other habitats, and only 4 species were collected from drainage ditches, and stream margins. In the irrigation ditches, *An. sinensis* (71.82%) was the most abundant species, followed by *An. kleini* (12.84%) and *An. sineroides* (9.68%). *Anopheles* larval population increased from 19.92% in June to 24.50% in August. In the rice paddies, *An. sinensis* (82.09%) had the greatest relative abundance followed by *An. pullus* (10.38%) and *An. kleini* (5.04%). Larval population increased from 16.03% in May to 23.81% in June. Except for *An. lesteri*, all species were collected from the rice paddies. In the pond reservoir, *An. sinensis* (54.63%) was the dominant species, followed by *An. kleini* (22.82%) and *An.sineroides* (12.55%). Larval population increased from 15.75% in July to a peak of 24.28% in August. In the stream margins and swamps, respectively, *An. sinensis* (80.07%, 36.86%) ranked first in abundance followed by *An. kleini* (13.07%, 27.78%) and *An. pullus* (5.85%, 30.38%). Larval population increased from 23.04% and 12.08% in July to 26.37% and 29.31% in August. In the drainage ditches, *An. sinensis* (51.98%) was the dominant species, followed by *An. pullus* (20.63%) and *An. kleini* (9.33%). Larval population increased from 15.38% in June to a peak of 35.90% in August. In all sampled habitats, *An. sinensis* had the highest relative abundance in all habitats, and its larvae were found in all 7 habitats similar to *An. kleini*, *An. pullus*, and *An. sineroides*. *Anopheles lesteri* larvae were only collected from the irrigation ditches and swamps, while *An. belenrae* larvae were only found in rice paddies, irrigation ditches, and ponds. Rueda et al[2] reported the associations of *An.* (*Ano.*) with other species of *Aedes* and *Culex* from different habitats in the ROK.

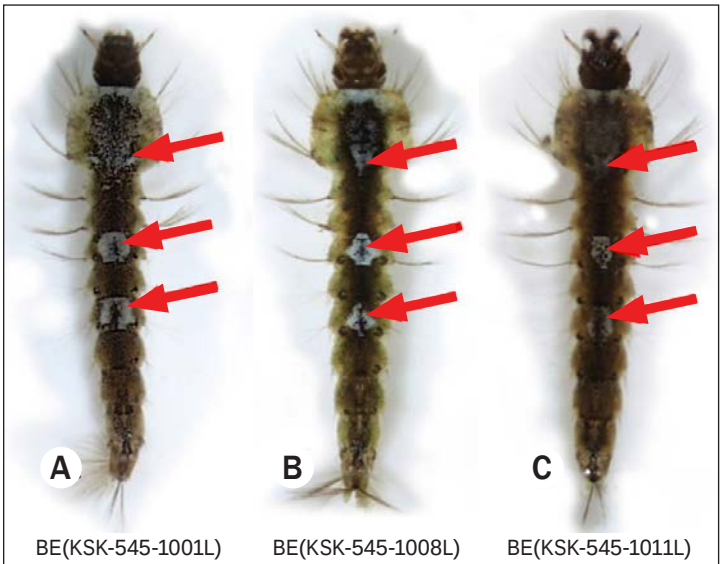

Figure 2. Mosquito larvae of *Anopheles belenrae*, dorsal view, showing waxy ornamentations of the thorax and abdomen (arrows).

Morphological Descriptions of 5 Common *Anopheles* (*Anopheles*) Species, with Emphasis on Waxy Ornamentations or Body Patterns, Based on Korean Specimens

1. *An. belenrae*, Larva: Body coloration is usually green or light reddish brown. Thorax has dorsal parts almost covered with white waxy materials, primarily along the anterior and lateral edges. It usually has a slightly pointed waxy ornamentation extending up to the anterior tip of the dorsum of abdominal segment I (Figure 2; collection nos. KSK-545-1001L, KSK-545-1008L, KSK-545-1011L). Abdomen has 2 distinct white waxy ornamentations on the dorsal parts of segments III and V. Abdominal segment III has a dorsal part with white waxy ornamentations of various shapes, including barrel shape (Figure 2A), diamond shape (Figure 2B), and chalice-shape (Figure 2C). Abdominal segment V has a dorsal part with a white waxy ornamentation that is almost similar to that one on abdominal segment I (Figure 2). Pupa: No live specimen was available to examine.

2. *An. kleini*, Larva: Body coloration is usually reddish brown to dark brown. Thorax has dorsal parts almost covered with white waxy materials, primarily along the anterior half and narrowly extending towards the anterior edge of abdominal segment I. The tip of this extension is usually blunt (Figure 3B, 3C) or rarely pointed (Figure 3A). Abdomen has one distinct white waxy ornamentation, barrel-shaped, on the dorsal part of segment III (Figure 3; collection nos. KSK-511-1004L, KSK-523-1004L, KSK-545-1005L), and nothing on abdominal segment V. Pupa: Abdominal segments III and IV have dorsal parts with white waxy ornamentations of various shapes, including Aladdin lamp shape (Figure 4A, collection no. KSK-511-1006P), blown tubular glass shape (Figure 4B, collection no. KSK-513-1004P) and cork crown shape (Figure 4C, collection no. KSK-514-1008P; Figure 5A, collection no. KSK-522-1004P).

Relative abundance of *Anopheles* (*Anopheles*) larvae on various habitats in 3 provinces (Geonggi, Gyeongsangbuk, Chungcheongbuk) of the Republic of Korea (May 2007 - October 2007) [part 1 of 2].

Habitat and Species	Relative Abundance (%)[a]						6-month Mean	Total Collected (n)
	May	June	July	August	Sepember	October		
Rice paddies (n=111)[b]								
An. belenrae	1.34	0.00	0.00	0.57	0.00	0.62	0.42	0.38 (7)
An. kleini	3.01	12.61	10.00	1.98	2.62	0.00	5.04	6.06 (113)
An. lesteri	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0)
An. pullus	54.18	4.50	0.88	1.98	0.75	0.00	10.38	10.4 (194)
An. sinensis	33.44	82.88	88.82	95.47	96.25	95.68	82.09	81.45 (1519)
An. sineroides	8.03	0.00	0.29	0.00	0.37	3.70	2.07	1.72 (32)
Total collected (n)	299	444	340	353	267	162		(1865)
Irrigation ditches (n=84)[c]								
An. belenrae	11.11	0.00	0.55	0.78	1.27	0.00	2.29	1.24 (13)
An. kleini	29.17	2.87	18.78	13.62	11.46	1.16	12.84	11.06 (116)
An. lesteri	0.00	0.96	0.00	0.00	0.00	0.58	0.26	0.29 (3)
An. pullus	4.17	6.70	2.21	1.95	2.55	1.16	3.12	3.05 (32)
An. sinensis	51.39	61.72	78.45	81.71	60.51	97.11	71.82	74.45 (781)
An. sineroides	4.17	27.75	0.00	1.95	24.20	0.00	9.68	9.91 (104)
Total collected (n)	72	209	181	257	157	173		(1049)
Drainage ditches (n=18)[d]								
An. belenrae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0)
An. kleini	0.00	0.00	14.29	25.00	16.67	0.00	9.33	12.82 (5)
An. lesteri	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0)
An. pullus	0.00	16.67	7.14	0.00	0.00	100.00	20.63	12.82 (5)
An. sinensis	0.00	83.33	78.57	75.00	75.00	0.00	51.98	71.79 (28)
An. sineroides	0.00	0.00	0.00	0.00	8.33	0.00	1.39	2.56 (1)
Total collected (n)	0	6	14	4	12	3		(39)
Ponds (n=61)[e]								
An. belenrae	0.16	0.00	0.00	0.99	1.10	0.00	3.86	1.44 (12)
An. kleini	50.00	16.16	41.22	16.83	12.15	0.55	22.82	17.55 (146)
An. lesteri	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0)
An. pullus	13.16	8.08	1.53	6.93	3.31	3.87	6.15	5.05 (42)
An. sinensis	13.16	8.08	57.25	75.25	78.45	95.58	54.63	66.71 (555)
An. sineroides	2.63	67.68	0.00	0.00	4.97	0.00	12.55	9.25 (77)
Total collected (n)	38	99	131	202	181	181		(832)
Stream margins (n=32)[f]								
An. belenrae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0)
An. kleini	66.67	4.76	2.06	0.90	4.05	0.00	13.07	2.14 (9)
An. lesteri	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0)
An. pullus	33.33	0.00	0.00	0.00	0.00	1.74	5.85	0.71 (3)
An. sinensis	0.00	95.24	97.94	99.10	95.95	92.17	80.07	95.49 (402)
An. sineroides	0.00	0.00	0.00	0.00	0.00	6.09	1.01	1.66 (7)
Total collected (n)	3	21	97	111	74	115		(421)

Notes:

a. Relative abundance means for 6 months are based on the total number of *Anopheles* larvae collected during the period.
b. Rice paddies, vacant or with rice plants
c. Irrigation ditches include concrete and nonconcrete ditches
d. Drainage ditches include concrete and nonconcrete ditches, culverts, dammed ditches
e. Ponds include pond reservoirs
f. Stream margins include stream ponds, stream inlets, algal mats

Relative abundance of *Anopheles* (*Anopheles*) larvae on various habitats in 3 provinces (Geonggi, Gyeongsangbuk, Chungcheongbuk) of the Republic of Korea (May 2007 - October 2007) [part 2 of 2].

Habitat and species	Relative Abundance (%)[a]							
	May	June	July	August	Sepember	October	6-month Mean	Total Collected (n)
Swamps (n=13)								
An. belenrae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0)
An. kleini	83.33	4.65	35.00	41.24	2.44	0.00	27.78	20.24 (67)
An. lesteri	0.00	0.00	5.00	1.03	7.32	0.00	2.22	1.81 (6)
An. pullus	16.67	90.70	10.00	2.06	2.44	1.02	20.48	14.80 (49)
An. sinensis	0.00	0.00	50.00	48.45	39.02	83.67	36.86	49.85 (165)
An. sineroides	0.00	4.65	0.00	7.22	48.78	15.31	12.66	13.29 (44)
Total collected (n)	12	43	40	97	41	98		(331)
Other habitats (n=22)[b]								
An. belenrae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0)
An. kleini	0.00	0.00	0.00	6.25	0.00	0.00	1.04	2.56 (1)
An. lesteri	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0)
An. pullus	0.00	0.00	8.33	0.00	0.00	0.00	1.39	2.56 (1)
An. sinensis	0.00	0.00	83.33	81.25	100.00	50.00	52.43	76.92 (30)
An. sineroides	100.00	100.00	8.33	12.50	0.00	50.00	45.14	17.95 (7)
Total collected (n)	2	1	12	16	6	2		(39)

Notes:
a. Relative abundance means for 6 months are based on the total number of *Anopheles* larvae collected during the period.
b. Other habitats including ground pools, ground depressions, flood pools, rock pools, uncultivated fields, artificial containers

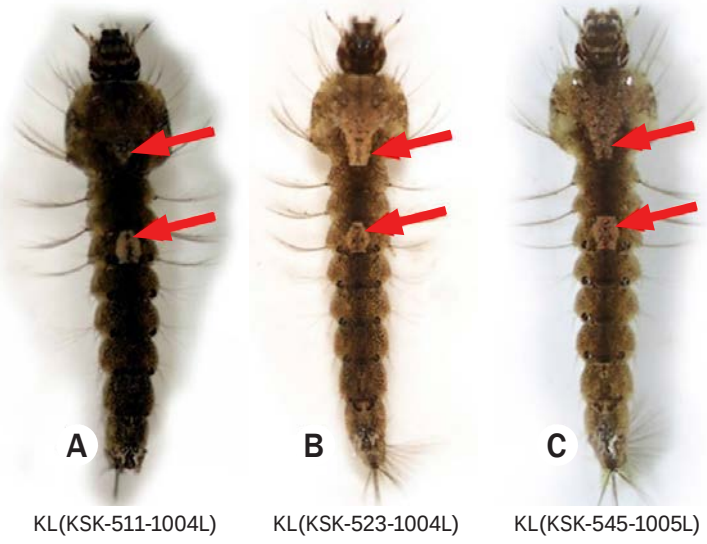

Figure 3. Mosquito larvae of *Anopheles kleini*, dorsal view, showing waxy ornamentations of the thorax and abdomen (arrows).

3. *An. pullus,* Larva: Body coloration is usually green or brown. Thorax has dorsal parts almost covered with white waxy materials, primarily along the middle part, and narrowly extending towards the anterior edge of abdominal segment I. The tips of this waxy extension are usually blunt (Figure 6A, 6C; collection nos. KSK-543-1005L, KSK-543-1013L) or seldom pointed (Figure 6B, collection no. KSK-543-1006L). Abdomen has one distinct white waxy ornamentation, barrel-shaped, on dorsal part of segment III (Figure 6), and nothing on the abdominal segment V. Pupa: Abdominal segments III and IV have dorsal parts with white waxy ornamentations of various shapes, including Aladdin lamp shape (Figure 7A, 7B; collection nos. KSK-514-1001P, KSK-523-1003P), and pear shape (Figure 7C, collection no. KSK-520-1001P).

4. *An. sinensis,* Larva: Body coloration is usually green, reddish brown, or dark brown. Thorax has dorsal parts almost completely covered with white or creamish colored waxy materials, primarily along the anterior half, and narrowly extending towards the anterior edge of abdominal segment I. The tip of this extension is usually blunt (Figure 8A, 8B; collection nos. KSK-543-1003L, KSK-545-1007L). Abdomen has one distinct white or creamish waxy ornamentation, barrel-shaped or pear-shaped, on dorsal part of segment III (Figure 8A, 8B), and usually lacking ornamentation on abdominal segment V. It is very rare that dorsal parts of the abdomen have 2 white waxy ornamentations. Greenish larvae usually have no or very light waxy ornamentations on the body. Pupa: Cephalothorax has a cup-shaped white waxy ornamentation on dorsal part. Abdominal segments I-V have median longitudinal white waxy ornamentations on dorsal parts (Figure 5B, 5C; collection nos. KSK-522-1001P, KSK-523-1002P).

5. *An. lesteri,* Larva: Body coloration is usually green or dark brown. Thorax has dorsal parts almost covered with white waxy materials, and approximately

cone-shaped along the median parts. It usually has a slightly pointed waxy ornamentation extending up to the anterior tip of the dorsum of abdominal segment I (Figure 8C, collection no. KSK-545-1009L). Abdomen has 2 distinct white waxy ornamentations on dorsal parts of segments III and V. Both ornamentations are barrel-shaped.

Pupa. No live specimen was available to examine.

COMMENT

The seasonal occurrence and relative abundance of *Anopheles* larvae varied from one type of habitat to another. These variations may be due to different environmental conditions found in most habitats. Although *Anopheles* (*Anopheles*) larvae and pupae have been previously described based on slide-mounted larval and pupal specimens,[2,3,19] little is known about the waxy ornamentation or body covering patterns of these immature mosquitoes in nature. In spite of detailed morphological descriptions of the larvae and pupae in various publications,[2,3,19] identifications keys are often difficult to construct due to much variation of supposed diagnostic characters, including position, types, forms, and number of branches of setae and other body parts. During intensive field surveillance, the use of waxy body ornamentations of the larvae may be useful characters for initial identifications of some *Anopheles* species, but there is still a need for larval rearing to obtain emerged adults. Available adult identification keys as well as PCR and DNA sequencing for larvae, pupae, and adults may be used to ascertain species identifications. The shapes or patterns of waxy body ornamentations are also not very distinct in first-instar and second-instar larvae, unlike third-instar and fourth-instar larvae of *Anopheles* mosquitoes. More specimens from the field, as well as from laboratory reared materials are required to further study the morphological variations or body patterns based on body ornamentations as diagnostic characters, in the mosquito larvae and pupae.

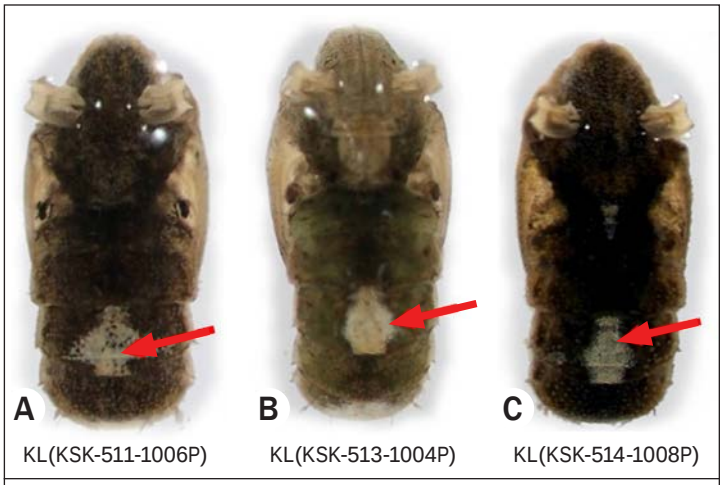

Figure 4. Mosquito pupae of *Anopheles kleini*, dorsal view, showing waxy ornamentations of the abdomen (arrow).

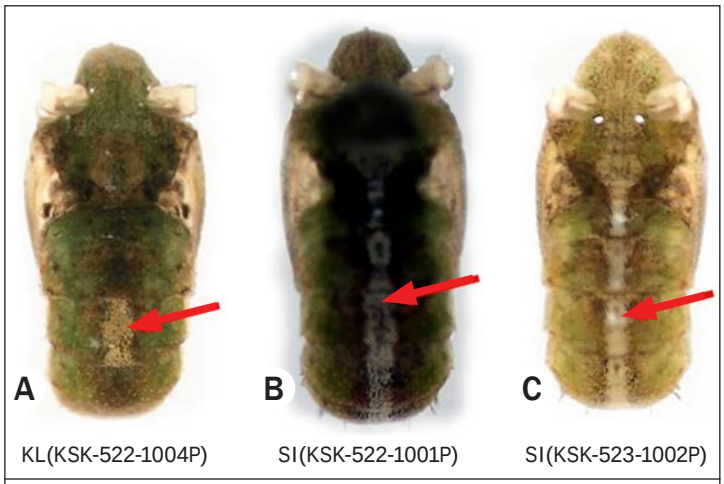

Figure 5. Mosquito pupae of *Anopheles kleini* (A), and *An. sinensis* (B,C), dorsal view, showing waxy ornamentations of the abdomen (arrow).

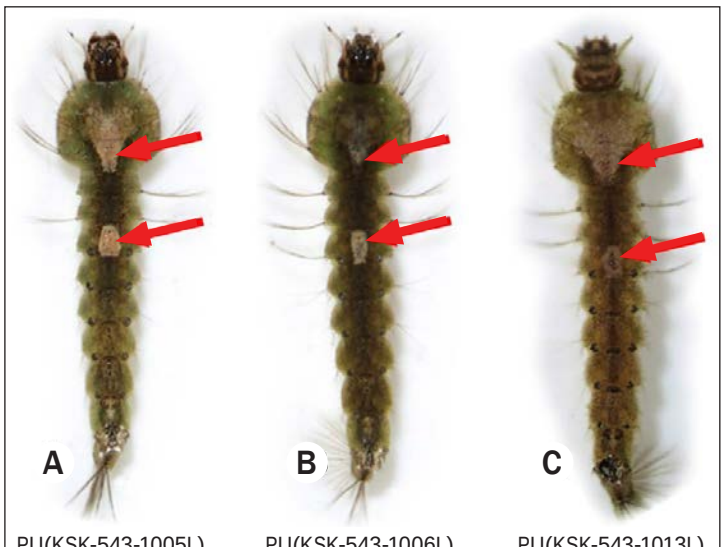

Figure 6. Mosquito larvae of *Anopheles pullus*, dorsal view, showing waxy ornamentations of the thorax and abdomen (arrows).

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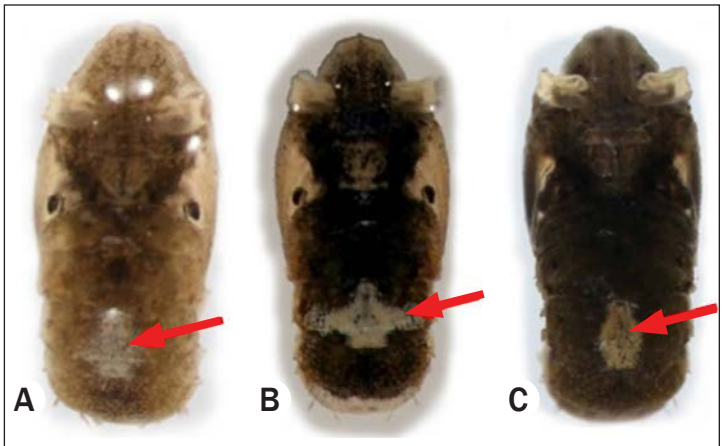

Figure 7. Mosquito pupae of *Anopheles pullus*, dorsal view, showing waxy ornamentations of the abdomen (arrows).

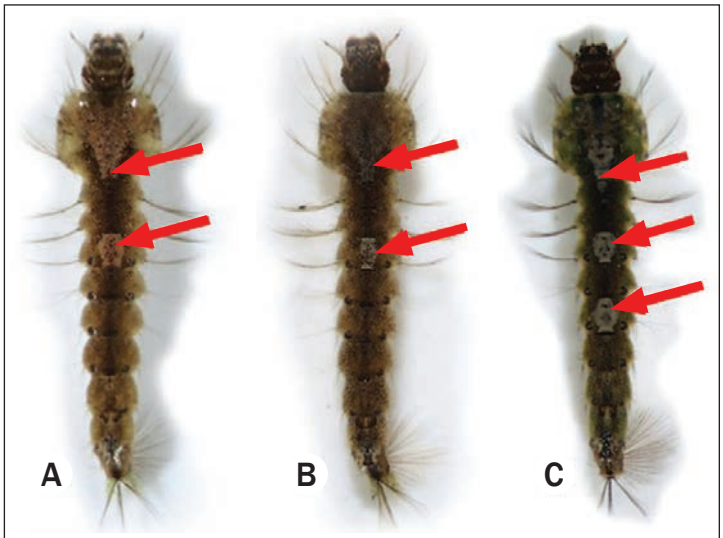

Figure 8. Mosquito larvae of *Anopheles sinensis (A,B)*, and *An. lesteri* (C), dorsal view, showing waxy ornamentations of the thorax and abdomen (arrows).

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A Case of Chagas Cardiomyopathy Following Infection in South Central Texas

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Abstract

Between 5 and 8 million people globally are infected with Trypanosoma cruzi, the causative parasitic agent of Chagas disease. The vast majority of incident infections originate in pockets of Latin America where domestic vector-borne transmission cycles are more common. Since 1955, when the first locally-acquired case was reported, fewer than 30 autochthonous cases have been documented in the United States. We describe the case of an 18-year-old US Air Force trainee, a native Texan with no travel history beyond the continental United States, who screened positive for T cruzi infection on blood donation and was subsequently found to have chronic Chagasic cardiomyopathy. This is the first documented case of Chagas disease in a US military trainee and one of the first known autochthonous cases of Chagasic cardiomyopathy in a Texas resident. Diagnostic, therapeutic, and military implications are discussed.

Human Chagas disease is caused by the protozoan parasite Trypanosoma cruzi, acquired primarily through contact with infected excreta of triatomine insects (known as "kissing bugs"). Although vector-borne transmission predominates, humans can also become infected congenitally, orally through contaminated food or beverages, or hematogenously through blood transfusion or organ transplantation.[1] During the first 4-8 weeks of infection, considered the acute phase, symptoms are usually mild, nonspecific, or unappreciable; potentially fatal myocarditis or meningoencephalitis occur rarely. Approximately 70% to 80% of infected persons enter a chronic indeterminate phase, characterized by lifelong infection without symptoms, electrocardiographic changes, and radiographic evidence of disease. The remaining 20% to 30% develop chronic disease, often presenting years or decades after infection as clinical cardiac and/or digestive disease.[2]

With approximately 5 to 8 million people infected worldwide,[1,3] Chagas is classified by the World Health Organization as one of the most important neglected tropical diseases.[1] There are an estimated 240,000 prevalent cases in the United States among immigrants from endemic areas of Latin America.[4] Fewer than 30 locally-acquired infections have been reported in the United States since 1955,[5] when a resident of Corpus Christi, Texas, became the first documented autochthonous case in the country.[6] In 2013, the Texas Department of State Health Services added Chagas disease to the state's Notifiable Conditions list, which requires the reporting of confirmed and suspected human cases to local or regional health departments. Twelve autochthonous human infections were confirmed in Texas during the first 2 years of mandatory reporting,[7] one of which was associated with left ventricular dysfunction.[8]

Case Report

In October 2016, an 18-year-old US Air Force trainee screened positive for *T cruzi* infection when he donated blood at Joint Base San Antonio (JBSA), Texas. Blood from all first-time donors at the JBSA-Lackland donation center is screened for *T cruzi* with an enzyme-linked immunosorbent assay from Ortho-Clinical Diagnostics. Per standard protocol, he was referred to the installation's trainee health clinic, where he was found to have normal vital signs and an unremarkable physical exam. He reported being in excellent health and had not experienced any recent chest pain, shortness of breath, dizziness, or gastrointestinal symptoms.

A chemiluminescence immunoassay and enzyme strip assay (Abbott Laboratories) were ordered and found to be positive for anti-*T cruzi* IgG antibodies. A whole blood sample was sent to the Reference Diagnostic Laboratory at the Centers for Disease Control and Prevention for further testing. An enzyme immunoassay was reactive and an immunoblot of trypomastigote excreted-secreted antigens was positive, confirming the diagnosis. The patient was notified of these results and referred to the Department of Infectious Disease at San Antonio Military Medical Center (SAMMC) for further evaluation and treatment.

After notifying the patient of his laboratory results, Infectious Disease conducted a 12-lead electrocardiogram

(ECG), which demonstrated normal sinus rhythm, first-degree atrioventricular block, and left anterior hemiblock with right bundle branch block. This prompted referral to the Division of Cardiology at SAMMC. Cardiovascular physical exam was benign with normal heart sounds, normal jugular venous pressure, normal apical impulse, and no evidence of congestive heart failure. A battery of noninvasive tests was performed in order to assess for common cardiac manifestations of Chagas disease, including, but not limited to, left ventricular dilatation and dysfunction, wall motion abnormalities, aneurysms, diastolic dysfunction, pathologic bradyarrhythmias and tachyarrhythmias, and ischemic heart disease.[9]

The majority of tests were within normal limits. Chest x-ray showed no evidence of cardiomegaly. Holter monitoring was negative for any pathologic dysrhythmias. Transthoracic echocardiogram demonstrated normal diastolic, valvular, and global systolic function. Exercise testing with Bruce protocol established no exercise-induced arrhythmias, ischemic electrical changes, or anginal symptoms. Cardiopulmonary exercise testing found an appropriate VO_2max, early anaerobic threshold, and normal VE/VCO_2 slope, consistent with a subclinical reduction in exercise capacity with preserved ventilatory efficiency. Cardiac magnetic resonance imaging confirmed the diagnosis of early heart disease, demonstrating left ventricular cavity dilation with preserved global systolic function (ejection fraction of 76%); the imaging was otherwise normal with no wall motion abnormalities, late gadolinium enhancement, abnormal T1 relaxation, or myocardial edema on T2 weighted images.

Given his exposure history, serologic findings, abnormal ECG, and left ventricular cavity dilation, the patient was determined to have chronic Chagasic cardiomyopathy. Per the Brazilian Consensus Classification and American College of Cardiology/American Heart Association classification schemes, he was classified as Stage B1 and Stage B, respectively,[10,11] and at low risk for cardiac death according to 2 validated risk calculators.[11,12] Since cardiomyopathy is a disqualifiable condition for accession into the US military,[13] the patient was processed for medical discharge from training. Infectious Disease advised the patient to complete a 60-day regimen of oral benznidazole,[14] but he declined. He was strongly encouraged to seek follow-up in the civilian health care sector and to notify household contacts that they should be screened for Chagas disease.[15]

Public health personnel interviewed the patient to facilitate case reporting to the Texas Department of State Health Services. The patient was raised on a ranch in south central Texas and had never traveled outside the continental United States. He camped occasionally near his home but never hunted or skinned animals. When shown a display case with triatomine insects of various species and at different stages of development, the patient immediately recognized them, saying they "were all over the place" on the ranch, including within the residence. He did not recall ever receiving a bite. A number of reservoir animals were also present on the property, including cats, dogs, raccoons, and armadillos. The patient was not aware of any relatives having Chagas disease, although he was adopted at a young age and had no knowledge of his biological mother. He had never received a blood transfusion. A week before his blood donation, he had spent 5 days and 4 nights on the JBSA Medina Training Annex for a field training exercise, during which he slept in a permethrin-treated bed net and reported no known insect bites.

Comment

Although neither congenital acquisition nor vector-borne acquisition during military training can be definitively ruled out, this patient was likely infected with *T cruzi* while growing up on a ranch in south central Texas. Ecologic modeling has predicted that this region of the United States is at increased risk for autochthonous Chagas disease.[16] Situated at the interface of tropical and temperate biomes, south central Texas has a number of environmental and cultural factors that may facilitate human exposure to *T cruzi*: a diverse array of wildlife reservoirs and indigenous triatomine species; the popularity of high-risk outdoor activities, especially hunting and camping; and the presence of scattered colonias (impoverished, primarily Hispanic communities). As compared to modern urban and suburban houses, poorly constructed ranches, cabins, and colonias are more susceptible to colonization by triatomine insects and wildlife reservoirs, thus increasing the likelihood of human exposure to infected vectors.[17]

The southern United States is inhabited by 11 recognized species of triatomine insects, listed in the Table, most of which are competent *T cruzi* vectors and likely to be involved in enzootic transmission cycles among indigenous wildlife reservoirs.[18] All species may exist as nest parasites and feed on a variety of vertebrate hosts. Competence for transmitting the parasite to humans is affected by environmental distribution, dispersal capacity, feeding and defecation behaviors, and ability to invade human domiciles, attributes of which vary according to species.[18-20]

In south central Texas, *Triatoma gerstaeckeri* insects have been found to enter human dwellings and to feed upon humans and domestic animals.[21,22] This

Triatoma in the United States.

Genus and species (subspecies)	Discoverer	Photograph
Paratriatoma hirsuta	Barber	Figure 1
Triatoma gerstaeckeri	Stål	
Triatoma incrassata	Usinger	Figure 2
Triatoma indictiva	Neiva	
Triatoma lecticularia	Stål	Figure 3
Triatoma neotomae	Neiva	
Triatoma protracta protracta	Uhler	Figure 4
Triatoma protracta woodi	Usinger	
Triatoma recurva	Stål	Figure 5
Triatoma rubida	Uhler	
Triatoma rubrofasciata	DeGeer	Figure 6
Triatoma sanguisuga	Leconte	

Paratriatoma hirsuta
Adult 12-14.5 mm
Photo by Christiane Weirauch.

Triatoma gerstaeckeri
Adult 23-28.5 mm
Photo by Edward Wozniak

Figure 1. *Triatoma* in the United States (continued).

medium-to-large triatomine species inhabits much of the Edwards Plateau and South Texas Brush Country between the 96th and 103rd parallels, the southeastern corner of New Mexico, and northeastern Mexico.[23] The *T cruzi* infection rate of this species may exceed 60% in south central Texas,[21,22] and adult insects often have detectable human blood in their midgut.[24]

Although the case patient was likely infected prior to arrival at JBSA, this report highlights the risk for autochthonous Chagas disease in the southern United States and underscores the importance of preventing Chagas and other vector-borne diseases while training in endemic areas. In order to decrease vector habitats, engineering controls should focus on reducing vegetation around military field sites to the maximum extent possible without disrupting the training mission. Administrative controls emphasizing site cleanliness should help minimize the population of woodrats, an important reservoir animal.[25] Finally, the 4 components of optimal personal protective measures should be meticulously used: a properly-worn field uniform (sleeves rolled down, wrist openings secured, undershirt tucked into the pants, and pant legs tucked into the boots); permethrin treatment of the uniform blouse and pants; the application of either DEET*-based (20% to 40% concentration) or picaridin†-based (20% concentration) insect repellent to exposed skin; and sleeping in a permethrin-treated bed net.[26] Finally, diligent public health surveillance and health care provider education for Chagas disease are warranted.

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*N,N-diethyl-meta-toluamide
†Hydroxyethyl isobutyl piperidine carboxylate

Triatoma incrassata
Adult 17.5-19.8 mm
No photo available..

Triatoma indictiva
Adult 18-22 mm
Photo by Christina Wozniak.

Figure 2. *Triatoma* in the United States (continued).

Triatoma lecticularia
Adult 19.5-25 mm
Photo by Edward Wozniak.

Triatoma neotomae
Adult 16.5-19.5 mm
Photo by Mike Quinn
TexasEnto.net.

Figure 3. *Triatoma* in the United States (continued).

Triatoma protracta protracta
Adult 16-23 mm
Photo by Justin Schmidt and
Steven Klotz.

Triatoma protracta woodi
Adult 14-16 mm
Photo by Edward Wozniak.

Figure 4. *Triatoma* in the United States (continued).

Triatoma recurva
Adult 25.5-33 mm
Photo by Justin Schmidt and
Steven Klotz.

Triatoma rubida
Adult 15.5-23 mm
Photo by Edward Wozniak.

Figure 5. *Triatoma* in the United States (continued).

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Triatoma rubrofasciata
Adult 19.5-25 mm
Photo by Gabe Hamer.

Triatoma sanguisuga
Adult 15.5-23 mm
Photo by Edward Wozniak.

Figure 6. *Triatoma* in the United States (end).

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Direct Detection of *Leishmania* from Clinical Samples

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ABSTRACT

The ability to rapidly and accurately diagnose leishmaniasis is a military priority. Testing was conducted to evaluate diagnostic sensitivity and specificity of field-expedient *Leishmania* genus and visceral *Leishmania* specific dual-fluorogenic, hydrolysis probe (TaqMan), polymerase chain reaction assays previously established for use in vector surveillance. Blood samples of patients with confirmed visceral leishmaniasis and controls without the disease from Baringo District, Kenya, were tested. *Leishmania* genus assay sensitivity was 100% (14/14) and specificity was 84% (16/19). Visceral *Leishmania* assay sensitivity was 93% (13/14) and specificity 80% (4/5). Cutaneous leishmaniasis (CL) skin scrapes of patients from Honduras were also evaluated. *Leishmania* genus assay sensitivity was 100% (10/10). Visceral *Leishmania* assay specificity was 100% (10/10) from cutaneous leishmaniasis samples; no fluorescence above background was reported. These results show promise in a rapid, sensitive, and specific method for *Leishmania* direct detection from clinical samples.

Leishmaniasis is recognized as a significant threat to deployed military forces and is a public health priority.[1] It is an emerging zoonotic disease caused by obligate intracellular parasites of the genus *Leishmania* which are transmitted by *Phlebotomine* sand flies. Currently, over 350 million people are at risk and 12 million people in 90 countries are infected.[2] The number of new cases of the non-fatal form of the disease, cutaneous leishmaniasis (CL), and the potentially fatal visceral (VL) form of the disease is 1.5 and 0.5 million annually. The most prevalent regions at risk are the Middle East, Africa (primarily east and north), India, Asia, Central and South America (primarily Honduras and Brazil). Leishmaniasis is recognized as an emerging disease in the United States. Visceral leishmaniasis is caused by *Leishmania donovani* complex *L donovani* and *L infantum* in the Old World and *L infantum*/*L chagasi* in the New World. Cutaneous leishmaniasis is caused primarily by *L tropica* and *L major* complex in the Old World and *L mexicana* complex (*L mexicana, L amazonensis, and L venezuelensis*); and subgenus *Viannia* complex *L brazilliensis* and *L guyanensis* in the New World. A licensed vaccine or drug prophylaxis does not exist.

Visceral leishmaniasis is fatal if untreated.[3,4] Cutaneous leishmaniasis can cause severe scarring with permanent disfigurement and physical impairment.[5,6] Both forms of the disease often require lengthy and painful treatment and may present complex issues associated with drug efficacy, adverse effects, and cost.[7] Early and accurate diagnosis is critical to efficacious treatment. Early treatment decreases VL mortality, CL physical impairment and disfigurement, and the length and expense of care.

Visceral and cutaneous leishmaniasis clinical samples are obtained from venous blood, spleen and bone marrow aspirate and skin lesion biopsy, respectively. Laboratory methods used for diagnoses are primarily microscopy and immunoassay.[7,8] Isoenzyme analysis is typically used to identify *Leishmania* species. However, these methods can take days to weeks to produce results. Commercially available immunochromatographic (ICT) strip assays are available that detect *L donovani* antibody in human serum, providing an aid in presumptive diagnosis of VL. Moreover, ICT strip assays are easily transported and require minimal training, 2 key traits to providing an efficacious field-expedient methodology.[9] Detection by ICT augmented by molecular-based methods achieves optimal sensitivity and specificity.[10,11]

Polymerase chain reaction (PCR) detection tests are finding a place as an aid in leishmaniasis diagnosis.[12-17] However, detection by PCR is challenged by the number of infecting parasites from potentially low load CL patient samples of 2 to 11e6 parasites/skin biopsy and VL sample loads of 30 to 2e5 parasites/mL blood with a median of ≈1e3 parasites/mL.[18,19] The load of CL parasites in blood is presumably at single log or less concentration based on PCR and isoenzyme test results.[20,21]

We describe here sensitive and specific *Leishmania* genus (LEIS) and visceral *Leishmania* (LVL) TaqMan PCR assays (ThermoFisher Scientific, Waltham, MA) which clearly show promise as an aid in diagnosis.

Methods

Clinical Samples

Test panels were prepared consisting of well characterized nucleic acid extracts of VL confirmed patient and healthy control blood samples and CL confirmed patient skin scrapes.

Blood samples of patients with VL and controls without the disease were obtained in endemic areas of Baringo District, Kenya, during routine surveillance for VL.[22] These samples were used to construct a test panel of 14 VL patients and 19 control samples. Testing was conducted at the Basic Science Department, USAMRU-K in February 2012. Clearance was obtained from the institutional ethical committee of the Kenya Medical Research Institute (KEMRI ERC #1282) and the Institutional Review Board (IRB), Walter Reed Army Institute of Research (WRAIR), Silver Spring, Maryland (WRAIR IRB #1402).

Prior to evaluations conducted in Kenya reported here, LEIS and LVL assays were tested under a separate study using total nucleic acid extracts from isolates of 10 CL patient skin scrape samples obtained in Honduras. Samples were provided by the Honduras Ministry of Public Health. Serology was used to identify the causative agent as *L mexicana*. Extracts were prepared and transported to the United States Army Center for Health Promotion and Preventive Medicine-West (CHPPM-W), Fort Lewis, WA. Testing was conducted at the Department of Entomology, CHPPM-W. Test results were used for PCR assay evaluation purposes only. Aliquots of specimens routinely ordered for conventional diagnostic purposes were used in this study and all patient identifiers were removed prior to the receipt of specimens in compliance with the human use protocol, "Surveillance of Emerging Infectious Disease Agents in Human Populations of Central America," USAMRIID, Fort Detrick, Maryland, approved 2001 (FY01-08).

Nucleic Acid Preparation

Extracts were prepared using commercially available, off the shelf, preformatted, guanidinium thiocyanate based total nucleic acid purification kits following the manufacturer's instructions (QIAGEN, Valencia, California).

PCR Assay Design

Previously established *Leishmania* genus primer and probe sequences were obtained from the literature for LEIS freeze-dried assay development.[23] Design of LVL primer and probe oligonucleotides and LVL and LEIS PCR reaction conditions were previously described.[24] *Leishmania* genus and LVL assay optimization, limit of detection (LoD), analytic sensitivity, and specificity test results are previously described.[24] Primer and probe synthesis, proprietary master mix preparation and freeze-drying process, and quality control were conducted commercially (BioFire Diagnostics Inc., Salt Lake City, Utah).

Analyses

The analytic platform was a Department of Defense approved, field-expedient, real-time PCR thermocycler, the Ruggedized Advanced Pathogen Identification Device (RAPID) (Idaho Technology, Inc, Salt Lake City, Utah) as shown in the Figure. The analytic system and PCR cycling conditions were previously described.[24] The comparator test for this study was a well-established *L donovani* PCR assay targeting a 360 base-pair region of the 6-phosphate isomerase gene.[25] Comparator test analysis was conducted using a SmartCycler (Cepheid, Inc, Sunnyvale, California). Blind testing was conducted.

Data Management and Statistical Analyses

Sample identification and experiment information were entered electronically in the RAPID operating system run protocol. A single data point at the end of each annealing extension cycle was collected and reported as TaqMan probe fluorescence released by 5'-nuclease activity during primer extension. The criterion for a positive result was a significant increase in fluorescence over background levels, that is, cycle threshold (Ct), defined by an algorithm provided in the RAPID analytical software (Roche Molecular Biochemicals, Basel, CH). The Ct is defined as the first PCR cycle with significant fluorescence when normalized against background fluorescence. Samples with a mean Ct more than 40 were considered negative, whereas samples with a mean Ct of 40 or less were considered positive by RAPID analyses. Sample results and statistical analyses were automatically archived.

Results

Test panels consisting of well characterized nucleic acid extracts of blood samples from 14 VL positive patients and 19 controls without VL, and 10 CL positive patient skin scrape samples were used to evaluate LEIS and LVL assay diagnostic sensitivity and specificity. The results are presented in the Table.

Visceral Leishmaniasis Diagnostic Sensitivity from Blood Samples

Visceral leishmaniasis test results were LEIS sensitivity 100% (14/14) and specificity 84% (16/19) and LVL sensitivity 93% (13/14) and specificity 80% (4/5). Mean LEIS Ct and standard deviation were 26.96 and 4.32, respectively, where n=14, SE=1.16 (95% CI, 24.69-29.23). Specificity of the LEIS assay was 84% (16/19). False positive results occurred for 3 samples. The Ct values were 30.63, 30.9, and 31.48. The sensitivity of the LVL assay was 93% (13/14). Mean LVL Ct and standard deviation were 31.80 and 4.36, respectively, where n=13, SE=1.21 (95% CI, 29.43-34.17). Specificity of the LVL assay was 80% (4/5). One false positive occurred with a Ct value of 34.49.

Cutaneous Leishmaniasis Diagnostic Sensitivity from Skin Lesion Samples

Test panel results of CL skin scrapes of patients from Honduras were LEIS sensitivity 100% (10/10). Honduras test panel LEIS results were mean Ct and standard deviation of 26.51 and 2.87, respectively, where n=10, SE=0.91 (95% CI, 24.73-28.29). The specificity of the LVL assay was 100% (10/10); no detectable fluorescence above background was observed.

Controls

Throughout laboratory testing and field evaluations, positive template control reactions reported fluorescence at the expected LEIS assay Ct value (≈18) and LVL assay Ct value (≈28).[24] Negative template control reactions did not report fluorescence above background. No fluorescence above background was observed in testing of LEIS and LVL assay specificity using human genomic DNA (1ng/reaction).

COMMENT

This preliminary evaluation of LEIS and LVL TaqMan PCR assay diagnostic sensitivity and specificity shows promise in a rapid, sensitive, and specific method for *Leishmania* direct detection from VL patient blood and CL skin scrape samples. However, enhancement of our method and additional testing are needed.

The LEIS assay serves as a valuable screening tool for subsequent *Leishmania* species identification by molecular and/or isoenzyme analyses. The diagnostic sensitivity of the LVL assay was 93% (n=14) which is approximately in line with previous reports describing direct detection from VL patient blood samples by real-time PCR; 79% (n=15), 80% (n=45), and 94% (n=100).[13-15] However, it is unlikely that we can reliably reproduce greater than 90% sensitivity results without enhancing our methods. False negative results are inevitable because VL patient blood samples potentially harbor parasites at concentrations as low as a single log. While PCR limit of detection is achieved at single copy and regularly at single log concentrations, there is no expectation of reliable and reproducible results from low load samples using conventional methods. The issue is that only a fraction of the sample is extracted when using commercial preformatted kits (100 µL-200 µL) or robotic systems (100 µL-400 µL).[26-28] Thus, for samples with a low parasite load, the resulting extract concentration of target template is likely to be diluted below detection limits. Reports of ≈80% diagnostic sensitivity are likely to better represent consistently achievable direct detection given the limitations of conventional sample preparation methodologies.

In this study, the specificity of LEIS (84%, 16/19) and LVL (80%, 4/5) assays from VL patient blood samples were probably underestimated. Both LEIS and LVL assays reported robust fluorescence from the same "healthy control," indicating incorrect reporting by the *L donovani* comparator test result. Better than reported LEIS assay specificity was also evidenced by subsequent use of our early development phase "Human *Leishmania* (LHL)" assay which is designed to detect VL and CL causative agents. Both, LEIS and LHL results were positive (Ct 31.48 and 35.82, respectively) and negative by LVL for the same healthy control, indicating that the sample harbored a CL causative agent.

The field-expediency and clinical implications associated with VL diagnosis from noninvasively obtained body fluids versus invasively obtained samples are profound. Direct detection of VL parasites from noninvasively obtained body fluids is challenged by characteristically single log loads. Various degrees of success have been achieved from urine (88%, n=17) and oral fluid (100%, n=37 and 83%, n=148).[29-31] Detection from canine oral

Results of LEIS and LVL RT-PCR assay sensitivity and specificity testing using clinical samples.

Disease	Source	Origin	Sensitivity	LEIS PCR Ct Mean/SD	Specificity	Sensitivity	LVL PCR Ct Mean/SD	Specificity
VL	Blood	Kenya	100% (14/14)	26.96/4.32	84% (16/19)	93% (13/14)	31.80/4.36	80% (4/5)
CL	Lesion	Honduras	100% (10/10)	26.51/2.87				100% (10/10)

VL indicates visceral leishmaniasis; CL, cutaneous leishmaniasis; Ct, critical threshold; LEIS, *Leishmania* genus; LVL, visceral *Leismania*; and PCR, polymerase chain reaction [detection test].

and conjunctival fluid show promise as well.[32] In these studies, conventional sample preparation methods were augmented with centrifugation to concentrate parasites prior to extraction.

We are currently developing an alternative approach. Microfiltration methodology versus centrifugation mitigates variables affecting relative yields of pathogens obtained from patient body fluids. The specific gravity (SG) of body fluids varies widely across patients and for samples obtained from the same patient over time. It has been shown that centrifugation of high SG urine results in significantly higher yields of organism compared to low SG urine samples.[33] To help ensure consistently high relative yields, we are developing a filtration approach to eliminate SG variables. Preliminary results of our leptospirosis diagnostic system show promise in increasing analytical limit of detection by 1-2 log concentration compared to centrifugation. This will be described in a future article. The low cost, simple, and portable characteristics of filtration technology also influenced our decision to take a microfiltration/capture approach.

The results of LEIS and LVL detection assay testing show promise in a rapid, sensitive, and specific method for *Leishmania* direct detection from clinical samples. It is our intent to augment analytical capabilities with standardized, field-expedient, detection enhancing, pre-analytical technologies.

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New Records, Distribution, and Updated Checklists of Old World Phlebotomine Sand Flies, With Emphasis on Africa, Southwest Asia, and Central Asia

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ABSTRACT

This article includes new records, distribution, and updated checklist of Phlebotomine sand flies (Psychodidae, Diptera) in the Old World (Africa including West Indian Ocean Islands, Southwest Asia, and Central Asia) based on specimen collections housed in different repositories worldwide. About 124 species have primary types housed in 5 repositories including holotypes (45 species, 4 subspecies), syntypes (28 species, 3 subspecies), "types" (14 species), allotypes (10 species), paratypes (36 species, 3 subspecies), lectotypes (13 species), and cotype (5 species), mounted on 671 slides. New abbreviations were proposed for 2 subgenera in the genus *Phlebotomus* and 6 subgenera in the genus *Sergentomyia*. New country records were noted in *Phlebotomus* (4 species in 4 subgenera in 7 countries) and *Sergentomyia* (10 species in 4 subgenera in 8 countries). For species diversity in the Old World, *Phlebotomus* includes 92 species and 7 subspecies in 9 subgenera, while *Sergentomyia* includes 166 species and 16 subspecies in 12 subgenera. A total of 95 species and 7 subspecies of 2 genera (*Phlebotomus* and *Sergentomyia*) were recorded in Africa while about 26 species and 16 subspecies in Southwest Asia and Central Asia.

Phlebotomine sand flies (Subfamily Phlebotominae, Family Psychodidae, Order Diptera) are vicious biting insects that cause extreme nuisance and transmit diseases to humans. In Africa and Asia (Figure 1), the peridomestic species bite insatiably and also are of major public health concern in many parts of the world, particularly in the Old World, where they are capable of transmitting pathogens, including protozoans (*Leishmania*) and viruses (Phleboviruses, sand fly fever).[1] *Phlebotomus* females suck blood, including humans, while *Sergentomyia* females primarily feed on reptiles, and rarely bite humans.[2] Subgenera (*Adlerius*, *Euphlebotomus*, *Larroussius*, *Phlebotomus*, *Paraphlebotomus*, and *Synphlebotomus*) in the genus *Phlebotomus* contain species that are vectors or suspected vectors in the Old World.[2] Of approximately 266 sand fly species in the Old World (Southwest Asia, Central Asia, and Africa including Western Indian Ocean Islands), only about 27 species are capable of transmitting protozoan *Leishmania* parasites that cause visceral leishmaniasis (kala-azar) and various forms of cutaneous leishmaniasis (oriental sore, espundia, etc) in humans.[2-4] A few sand fly species have been associated with *Phlebovirus* and other viruses.[3-6] Numerous reports are available on the biology, and medical importance (as disease vectors) of sand flies and leishmaniasis control.[2,7-10] In the Old World, the human biting *Phlebotomus* sand flies and major leishmaniasis transmissions are confined in the subtropics (particularly in dry, semiarid areas), with some human biting species in Africa south of the Sahara.[2]

Among diseases transmitted by Phlebotomine sand flies, leishmaniasis has the most significant effect on military operations, particularly those of the United States.[11] In Afghanistan (Operation Enduring Freedom, OEF) and Iraq (Operation Iraqi Freedom, OIF), numerous US Soldiers were exposed to significant leishmaniasis risk and about 1,287 incident diagnoses/reports of leishmaniasis, both cutaneous (1,283 cases) and visceral (4 cases) forms, were reported from 2001-2006 among OEF/OIF deployed troops.[12] During OIF, US military entomologists conducted the sand fly surveillance at Tallil Air Base, Iraq, from April 2003 to November 2004, and they noted the strong effect of sand fly vectors on military operations, including the leishmanial threat to deployed troops in Iraq.[13-15]

In this article, we examine the types and related specimens of Old World Phlebotomine sand flies deposited in the following: US National Museum of Natural History

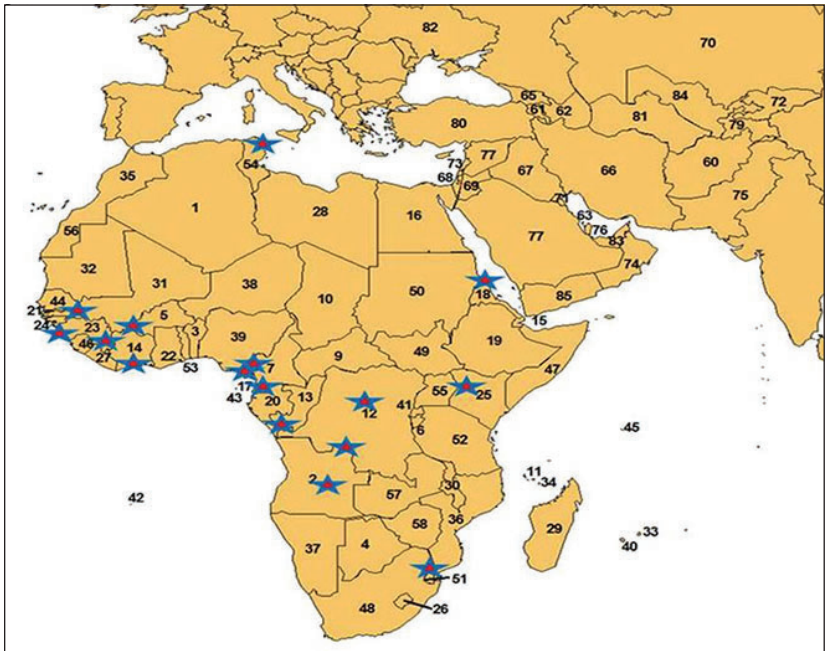

Figure 1. Map showing different countries in the Old World, particularly Africa, Southwest Asia, and Central Asia, and respective country codes (see Table 4 for code–country definitions). Note: a star indicates the location of various primary types.

(USNMNH), Smithsonian Institution, Suitland, MD; The Museum of Natural History (MNH), London, UK; Institut de Recherche pour le Développement (IRD), Montpellier, France; Musée National d'Histoire Naturelle Entomologie (MNHE), Paris, France; Institut Pasteur (IP), Paris, France; Musée Royal de L'Afrique Centrale (MRAC), Dept Entomologie, Tervuren, Belgium. Additional specimens were borrowed from the following institutions: Reimer University (RU), Reimer, France; US Army/Kenya Medical Research Institute (KEMRI), Nairobi, Kenya; Florida State Collection of Arthropods (FSCA), Gainesville, Florida; and US Navy laboratories (UNL). In addition, 8 interactive computerized identification keys (LUCID software[16]) of selected African and Asian sand fly species (potential or known vectors), based on specimens from different depositories are presently posted on the Walter Reed Biosystematics Unit (WRBU) website (wrbu.si.edu).[17]

We record the collection data of sand flies, including their geographical distribution, past and present taxonomic arrangement, and related information. The species occurrence and diversity of these sand flies, according to the number of collections for each country over certain periods, were analyzed and reported. Other collections or occurrence data of sand fly specimens (including non-types, from the Old World) from the 6 repositories (USNMNH, MNH, IRD, MNHE, IP, MRC) were also examined and recorded, and will be posted to the WRBU/VectorMap website (vectormap.si.edu).[18] These data may be helpful in developing world sand fly taxonomic catalogs, sand fly vector risk maps, and predictive distribution models. In addition to increasing the knowledge of sand fly distribution, the collection holdings in these repositories, particularly the primary types, will assist future phlebotomine researchers in their taxonomic and related studies.

Materials and Methods

Old World Phlebotomine sand fly specimens used in this study were deposited in 6 repositories (USNMNH, MNH, IRD, MNHE, IP, MRC), or borrowed from various agencies (RU, KEMRI, FSCA,UNL). The slide mounted specimens were examined and their collection data were recorded. All collection data from selected repositories were recorded verbatim and entered into a standard specimen label data capture spreadsheet. The data were georeferenced using the point-radius method of georeferencing locality descriptions that do not have associated geographic coordinates. The georeferenced data were processed and used for analyses. The primary types (holotypes, allotypes, paratypes, syntypes) of sand flies were examined for collection records and related information. Photographs of primary types of selected sand fly species were taken, and will be processed for another report.

Results

Species Types and Abbreviations

The proposed abbreviations for genera and subgenera of Phlebotominae (Psychodidae, Diptera) of the Old World is shown in Table 1, based primarily on Marcondes,[20] with additions of new abbreviations for the 2 subgenera in genus *Phlebotomus*, ie, *Legeromyia*, *Leg.*; *Madaphlebotomus*, *Mad.*; and the 6 subgenera in genus *Sergentomyia*, ie, *Capensomyia*, *Cap.*; *Parvidens*, *Pav.*; *Rondanomyia*, *Ron.*; *Spelaeomyia*, *Spe.*; *Trouilletomyia*, *Tro.*, and *Vattieromyia*, *Vat.*

The list of Old World Phlebotomine species with type specimens deposited in the various repositories is shown in Table 2 (see pages 71-73), using the available taxonomic arrangements.[19] The number of slides for each type, species, sex, and the country of type origin are also included in Table 2. About 124 species have primary types deposited in 5 repositories (MNH, IRD, MNHE, IP, MRC), including holotypes (45 species, 4 subspecies), syntypes (28 species, 3 subspecies), "types"

(14 species), allotypes (10 species), paratypes (36 species, 3 subspecies), lectotypes (13 species), and cotype (5 species), mounted on 671 slides. Those specimens on slides labeled "Type" could be considered as holotypes, but proper designations should be done later.

New Country Records and Updated Checklist of Phlebotomine Species

An updated checklist of Phlebotomine sand fly species in different countries of Africa (including Western Indian Ocean Islands), Southwest Asia, and Central Asia is presented in Table 3 (see pages 74-82). It also includes distribution of these species in Africa (58 countries), Southwest and Central Asia (26 countries) and other countries (35), as well as their selected references.[3,9,32-49] Several islands of Western Indian Ocean, ie, Comoros, Madagascar, Mauritius, Mayotte (France), Réunion (France), and Seychelles are included as Africa for the purposes of this article.[50]

The genus *Phlebotomus* includes 92 species and 7 subspecies in 9 subgenera (*Adlerius*, 17 species; *Anaphlebotomus*, 6 species; *Euphlebotomus*, 5 species; *Larroussius*, 27 species, 5 subspecies); *Legeromyia*, 1 species; *Madaphlebotomus*, 4 species; *Paraphlebotomus*, 12 species, 2 subspecies; *Phlebotomus*, 6 species; *Synphlebotomus*, 10 species; *Transphlebotomus*, 2 species). The genus *Sergentomyia* includes 166 species and 16 subspecies in 12 subgenera (*Capensomyia*, 10 species; *Demeillonius*, 1 species; *Grassomyia*, 6 species, 2 subspecies; *Neophlebotomus*, 22 species; *Parrotomyia*, 16 species, 4 subspecies; *Rondanomyia*, 1 species, 2 subspecies; *Sergentomyia*, 45 species, 6 subspecies; *Sintonius*, 27 species; *Spelaeomyia*, 6 species; *Trouilletomyia*, 2 species; *Vattieromyia*, 4 species), and one ungrouped taxon (24 species). Further, we recorded a total of 95 species and 7 subspecies of 2 genera (*Phlebotomus* and *Sergentomyia*) in Africa, while about 26 species and 16 subspecies in Southwest Asia and Central Asia. About 22 species of both genera had wide distributions and were found in 35 countries other than Africa, Southwest Asia, and Central Asia.

Table 1. Proposed abbreviations for genera and subgenera of Phlebotominae (Psychodidae, Diptera) of the Old World.

Genus	Subgenus	Abbreviation*
Phlebotomus		*Ph.*
	Adlerius	*Adl.*
	Anaphlebotomus	*Ana.*
	Euphlebotomus	*Euo.*
	Larroussius	*Lar.*
	Legeromyia	*Leg.***
	Madaphlebotomus	*Mad.***
	Paraphlebotomus	*Par.*
	Phlebotomus	*Phl.*
	Synphlebotomus	*Syn.*
	Transphlebotomus	*Tra.*
Sergentomyia		*Se.*
	Capensomyia	*Cap.***
	Grassomyia	*Gra.*
	Neophelebotomus	*Neo.*
	Parrotomyia	*Par.*
	Parvidens	*Pav.***
	Rondanomyia	*Ron.***
	Sergentomyia	*Ser.*
	Sintonius	*Sin.*
	Spelaeomyia	*Spe.***
	Trouilletomyia	*Tro.***
	Vattieromyia	*Vat.***
Chinius		*Ch.*

*Primarily based on Marcondes.[29]
**Proposed new abbreviation

The new records of species in different countries are shown in Table 3 (with country codes (CN) as listed in Table 4, see page 83). In the genus *Phlebotomus*, 4 species in 4 subgenera were recorded for the first time in 7 countries. These species included the following: *Phlebotomus* (*Adlerius*) *chinensis* Newstead, Iran (CN 66); *Ph*. (*Anaphlebotomus*) *rodhaini* Parrot, Côte d'Ivoire (CN 14), Ghana (CN 22), Mozambique (CN 36); *Ph*. (*Paraphlebotomus*) *jacusieli* Theodor, Lebanon (CN 73); and *Ph*. (*Phl*.) *papatasi* (Scopoli), Kenya (CN 25). In the genus *Sergentomyia*, 10 species in 4 subgenera were recorded for the first time in 8 countries. These species included the following: *Sergentomyia* (*Grassomyia*) *ghesquierei* (Parrot), Ghana (CN 22) Somalia (CN 47); *Se*. (*Neophlebotomus*) *decipiens* (Theodor), Tanzania (CN 52); *Se*. (*Neo*.) *dureni* Parrot, Ethiopia (CN 19); *Se*. (*Par*.) *freetownensis freetownensis* (Sinton), Democratic Republic of Congo (CN 12); *Se*. (*Par*.) *magna* (Sinton) Ghana (CN 22); *Se*. (*Sergentomyia*) *antennata* (Newstead), Tanzania (CN 52); *Se*. (*Ser*.) *buxtoni* (Theodor), Côte d'Ivoire CN (14); *Se*. (*Ser*.) *magnidentata* Davidson, Kenya (CN 25); *Se*. (*Ser*.) *minuta minuta* Rondani, Kenya (CN 25); and *Se*. (*Ser*.) *schwetzi* (Adler, Theodor & Parrot), Tanzania (CN 52).

Three taxa[46] were not included in the updated list shown in Table 3 pending clarification of their taxonomic identities, namely: *Se*. (*Ser*.) *bereiri*, *Se*. (*Ser*.) *distinctus* (as *distincta*), and *Se*. (*Ser*.) *fermatus* (as *firmata*). They cannot be treated as subspecies of *Se*. (*Ser*.) *bedfordi* because of their overlapping distributions.[3] These 3 taxa were treated by Davidson[51] as good species based on a unique and maybe aberrant holotype female of *Se. bedfordi*. Seccombe et al[3] did not support it due to the absence of statistical and/or biological analysis of sympatric populations. They noted that the morphological, ecological, and geographical data of Davidson[51] may help in future taxonomic studies. Niang et al[46] provided diagnostic morphological features and distribution records of these 3 taxa, in addition to *Se. bedfordi*. Although it was suggested[46,51] that the distribution of *Se. bedfordi* is confined to South Africa,

other reports[3,19,47] and recent examinations of specimens from different depositories or museums showed its wider distribution (Table 3). Recent analysis[49] of the molecular taxonomy of Phlebotomine sand flies based on 3 mitochondrial gene fragments (mtDNA) confirmed the presence of *Se. congolensis* (Bequart & Walravens), *Se. salisburiensis* (Abonnenc) and *Se. bedfordi* "Maun" form from Botswana. Kruger[49] proposed this form for *Se. caliginosa* Davidson and unassigned specimens of *Se. bedfordi* group found in Botswana. Since no formal name is available for this form, we temporarily include it with *Se. bedfordi* in Table 3. Unless the taxonomy of this group is well clarified, routine identification of these taxa will be very difficult and their distribution records will not be accurate.

Comment

The Phlebotomine sand fly collections, including primary types and voucher specimens, are very important for vector identifications, surveillance, and control efforts. Different repositories (USNMNH, MNH, IRD, MNHE, IP, MRC) have voucher specimens of the major species incriminated and/or suspected as vectors[7] of various *Leishmania* species involved in the transmission of human leishmaniasis in the Old World. Nine of 15 suspected vector species and 6 of 10 incriminated vector species[9] of *Leishmania* in the Old World have primary types deposited in the 2 repositories (MNH, IP). For example, MNH has the holotypes and/or "syntypes" of incriminated vectors, ie, *Phlebotomus* (*Larroussius*) *perniciosus* Newstead, *Ph.* (*Lar.*) *orientalis* Parrot, *Ph.* (*Phlebotomus*) *duboscqi* Neveu-Lemaire, and suspected vectors, ie, *Ph.* (*Synphlebotomus*) *celiae* Minter, *Ph.* (*Lar.*) *perfiliewi perfiliewi* Parrot, *Ph.* (*Lar.*) *smirnovi* Perfil'ev, *Ph.* (*Lar.*) *tobbi* Adler & Theodor). Considering the importance of the distribution records of sand flies, particularly vector species, there is an exigent need to conduct additional collections of sand flies from the Old World in order to obtain fresh voucher specimens for both molecular and morphological studies, and for safe deposits in the USNMNH and other pertinent repositories.

Concerning specimen collections from various repositories, they were used in the development of LUCID interactive keys for the Old World Phlebotomine sand flies,[24] particularly Africa, Southwest Asia, and Central Asia (US Africa Command (AFRICOM), Central Command (CENTCOM), Europe Command (EUCOM)). Eight morphological keys for males and females of the Old World were created by author L. M. Rueda, with assistance from WRBU staff (particularly J. Stoffer for Automontage images), and are posted at the WRBU website[17] as:

- Key to identification of male and female adult Phlebotomine sand fly genera of the Old World (PACOM [Pacific Command], CENTCOM, EUCOM, AFRICOM).
- Key to identification of male and female adult *Phlebotomus* subgenera of Southwest and Central Asia (PACOM, CENTCOM, EUCOM, AFRICOM).
- Key to identification of male adult species of subgenus *Larroussius* of *Phlebotomus*, Africa (AFRICOM), with emphasis on medically important species.
- Key to identification of male adult species of subgenus *Paraphlebotomus* of *Phlebotomus*, Africa (AFRICOM), with emphasis on medically important species.
- Key to identification of male adult species of subgenus *Phlebotomus* of *Phlebotomus*, Africa (AFRICOM), with emphasis on medically important species.
- Key to identification of male adult species of subgenus *Larroussius* of *Phlebotomus*, Southwest and Central Asia (CENTCOM), with emphasis on medically important species.
- Key to identification of male adult species of subgenus *Paraphlebotomus* of *Phlebotomus*, Southwest and Central Asia (CENTCOM), with emphasis on medically important species.
- Key to identification of male adult species of subgenus *Phlebotomus* of *Phlebotomus*, Southwest and Central Asia (CENTCOM), with emphasis on medically important species.

An example of a screen shot of LUCID interactive key for species of *Phlebotomus* (*Larroussius*) male adults from Africa is shown in Figure 2, and posted at the WRBU website.[17] Male genitalia are important morphological characters to identify different species of sand flies. Examples are shown in Figures 3 and 4 for primary types of Phlebotomine sand flies, ie, *Ph.* (*Paraphlebotomus*) *alexandri* Sinton, paratype; *Ph.* (*Adlerius*) *angustus* Artemiev, paratype; *Ph.* (*Larroussius*) *aculeatus* Lewis, Minter & Ashford, paratype; *Ph.* (*Lar.*) *longipes* Parrot & Martin, paratype; *Ph.* (*Phlebotomus*) *minteri* Lewis, holotype; *Ph.* (*Synphlebotomus*) *celiae* Minter, holotype; *Ph.* (*Syn.*) *vansomerenae* Heisch, Guggisberg & Teesdale, paratype; and *Se.* (*Sergentomyia*) *ashfordi* Davidson, paratype.

About 12,000 slides of Phlebotomine sand flies were donated by COL (Ret) Philip Lawyer to the USNMNH for safe keeping. These slides were temporarily mounted

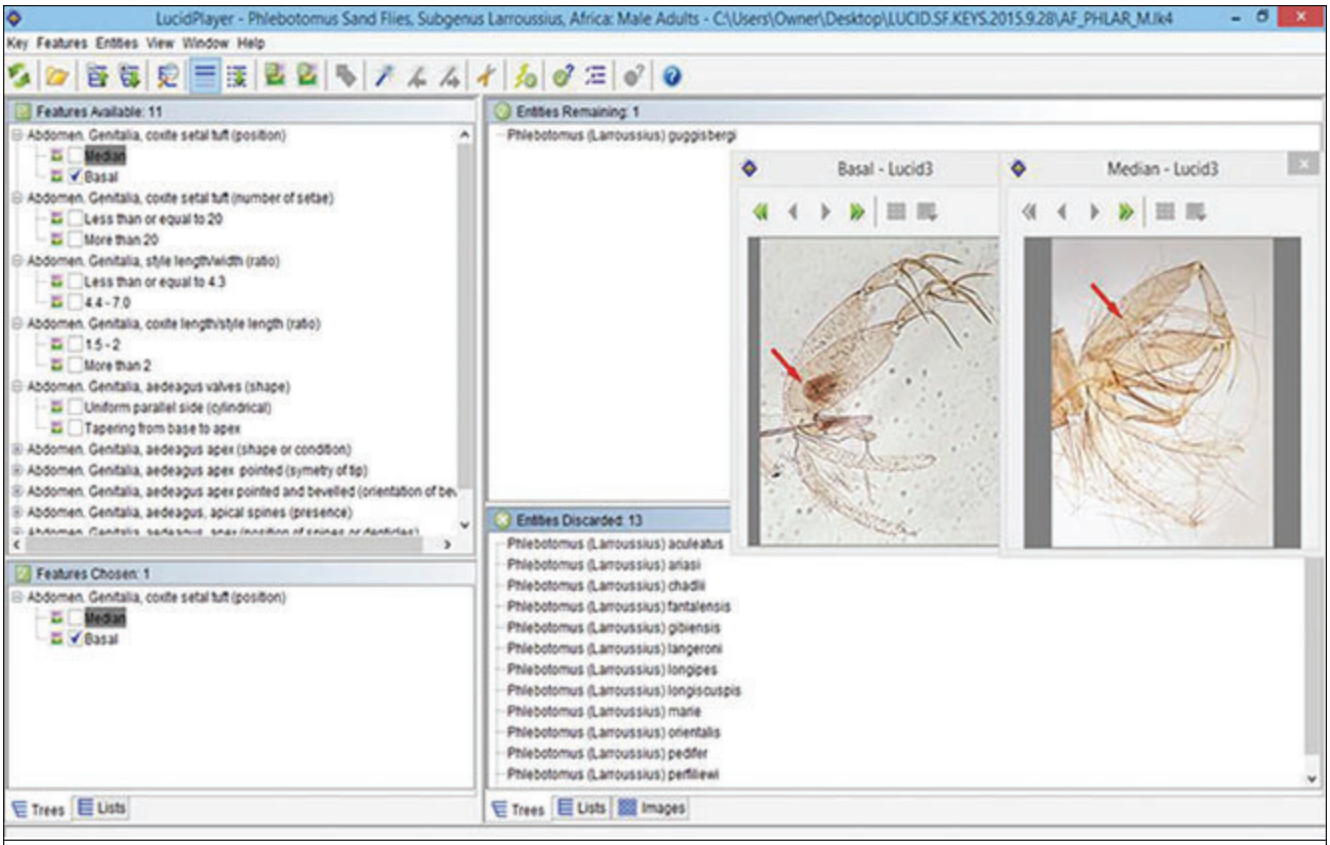

Figure 2. Example of screen shot of LUCID[16] interactive key for species of *Phlebotomus (Larroussius)* male adults, Africa (source: US Africa Command).

using Hoyer's medium before, and they will be remounted permanently and identified correctly. Their locality data from slide labels and collection sheets will be retrieved, recorded, and processed. Additional collection data from Old World countries generated by ongoing US military entomological surveillance efforts and newly published scientific literature will be entered into VectorMap regularly, to enable further analysis of species diversity and to create sand fly vector distribution models that will be useful for leishmaniasis risk assessments.

Sand fly slides in various repositories or museums labeled as "types" should be carefully examined, and if necessary, must be designated as holotype or appropriate type to avoid future confusions in taxonomic clarification and related systematic studies. For instance, the taxa/species without designated "holotype," but which have slides labeled "syntypes" or "types" should have proper designations.

ACKNOWLEDGMENTS

We express our sincere appreciation and gratitude to the following individuals for the loans of sand fly types and other specimens, and for their hospitality during the visits of Dr Rueda to examine their collections: D. Fontenille, F. Simard and N. Rahola, Maladies Infectieuses et Vecteurs: Ecologie, Génétique, Evolution et Contrôle, Institut de Recherche pour le Développement, Montpellier, France; D. Sivell, T. M. Howard and Z. Adams, Entomological Collections, Molecular Collections and Plants, Department of Life Sciences, The Natural History Museum, London, UK; E. Coninck and H. Stephane, Musée Royal de L'Afrique Centrale, Dept. Entomologie, Tervuren, Belgium; C. Daugeron, Musée National d'Histoire Naturelle Entomologie, Paris, France; P. Reiter, A.-B. Failloux, J. C. Ganthier, Institut Pasteur, Paris, France. We also thank K.A. Barbara, J.W. Diclaro, II, and P.J. Obenauer, US Navy Laboratories; N. Leger, Reimer University, Reimer, France; and J. Bast and R. Sang, US Army Kenya Medical Research Institute, Nairobi, Kenya, for sand fly slides loaned and deposited at the US National Museum of Natural History, Washington, DC.

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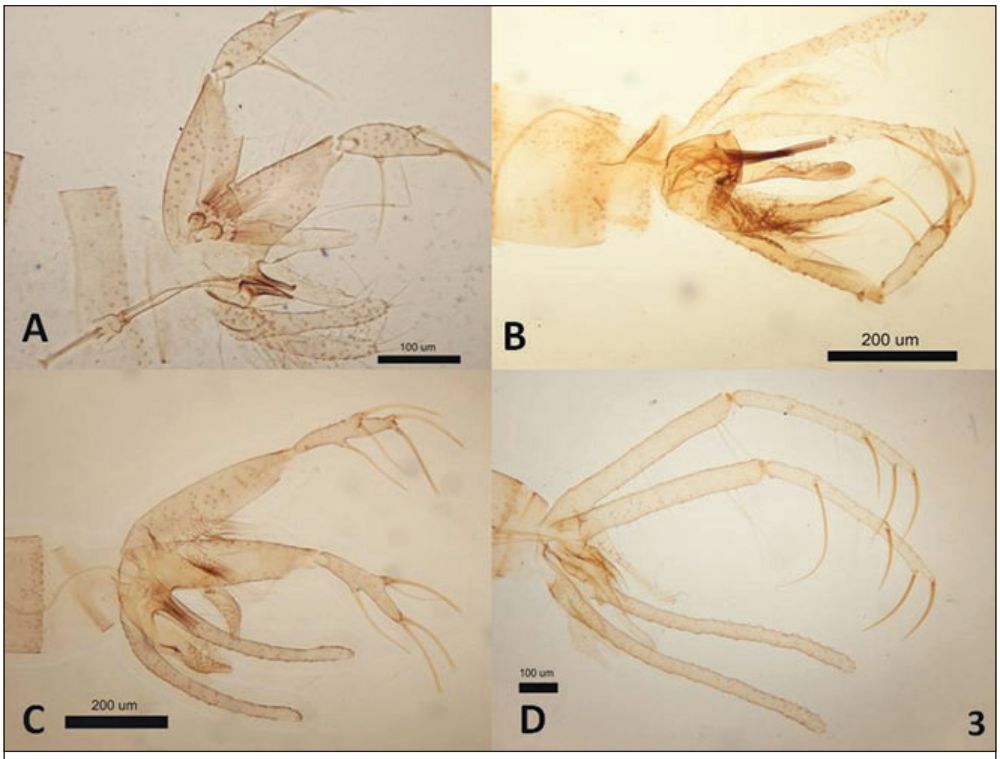

Figure 3. Male genitalia of *Phlebotomus* sand flies. (A). *Phlebotomus (Paraphlebotomus) alexandri* Sinton, paratype; (B). *Phlebotomus (Adlerius) angustus* Artemiev, paratype; (C). *Phlebotomus (Larroussius) aculeatus* Lewis, Minter & Ashford, paratype; (D). *Phlebotomus (Phlebotomus) minteri* Lewis, holotype.

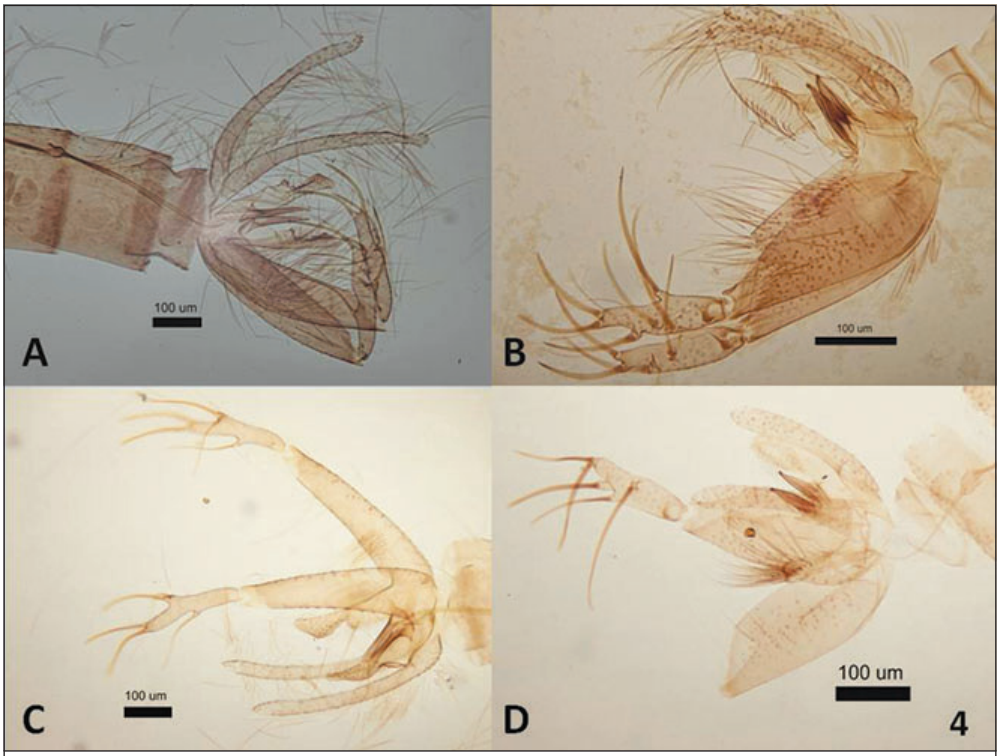

Figure 4. Male genitalia of *Phlebotomus* and *Sergentomyia* sand flies. (A). *Phlebotomus (Larroussius) longipes* Parrot & Martin, paratype; (B). *Phlebotomus (Synphlebotomus) celiae* Minter, holotype; (C). *Sergentomyia (Sergentomyia) ashfordi* Davidson, paratype; (D). *Phlebotomus (Synphlebotomus) vansomerenae* Heisch, Guggisberg & Teesdale, paratype.

Table 2 (part 1 of 3). Types of sand flies (Phlebotominae, Psychodidae) from Africa and Southwest and Central Asia, examined from different repositories, including country of type origin.

Species	[a]Depository [b]Type (number of slides by [c]sex)	Country of Type Origin
Phlebotomus aculeatus	NH: P (1 F, 1 M)	Kenya
Phlebotomus alexandri	NH: P (3 F)	Iraq
Phlebotomus angustus	NH: P (1 F, 5 M, 4 U)	Afghanistan
Phlebotomus arabicus	NH: L (M); PL (5 F)	Yemen
Phlebotomus ariasi	NH: P (14 M)	Ethiopia
Phlebotomus ashfordi	NH: H (F); P (1 F, 1 M)	Ethiopia
Phlebotomus bergeroti	NH: S (2 F, 4 M)	Algeria
Phlebotomus caudatus	NH: H (M)	Afghanistan
Phlebotomus celiae	NH: H (M)	Kenya
Phlebotomus chabaudi	IP: H (M); IR: P (2 U)	Tunisia
Phlebotomus chadlii	NH: P (3 F, 4 M)	Kenya
Phlebotomus comatus	NH: P (2 M)	Afghanistan
Phlebotomus duboscqi	NH: H (M)	Mauritania
Phlebotomus elgonensis	ME: P (1 F, 1 M)	Kenya
Phlebotomus fantalensis	NH: H (M), P (6 M)	Ethiopia
Phlebotomus gibiensis	NH: H (M); P (6 F, 20 M)	Ethiopia (H; P: 6 F, 16 M); Kenya (4 M)
Phlebotomus gigas	BE: L (F); T (1 F, 1M)	Dem. Rep. Congo
Phlebotomus guggisbergi	NH: L (M); PL (3 F, 1 M)	Kenya
Phlebotomus halepensis	NH: S (5 M)	Iran
Phlebotomus jacusieli	NH: H (M)	Israel
Phlebotomus kabulensis	NH: P (2 M)	Afghanistan
Phlebotomus kandelakii burneyi	NH: H (M); P (11 F, 23 M)	Pakistan
Phlebotomus katangensis	BE: T (1 M)	Dem. Rep. Congo
Phlebotomus kyreniae	NH: S (11 F, 9 M)	Cyprus
Phlebotomus longipes	NH: S (3 F, 6 M)	Ethiopia
Phlebotomus marismortui	NH: S (4 F, 1 M)	Israel
Phlebotomus mesghalii	NH: H (M); P (15 F, 12 M)	Kenya (H; P: 13 F, 8 M); Iran (P: 2 F, 4 M)
Phlebotomus minteri	NH: H (M); P (1 M)	Tanzania
Phlebotomus mongolensis	NH: S (1 F, 1 M)	Afghanistan
Phlebotomus naqbenius	NH: H (M), P (10 F, 10 M)	Saudi Arabia
Phlebotomus nuri	NH: H (M); P (17 M)	Pakistan
Phlebotomus orientalis	NH: S (1 F, 2 M)	Ethiopia
Phlebotomus pedifer	NH: H (M); P (4 F, 4 M)	Kenya
Phlebotomus perfiliewi galilaeus	NH: S (10 F, 13 M)	Cyprus (S: 1 F, 5 M); Israel (S: 9 F, 8 M)
Phlebotomus perfiliewi perfiliewi	NH: S (2 M)	Ukraine
Phlebotomus perniciosus	NH: S (6 M)	Malta
Phlebotomus rodhaini	BE: T (1 M); A (F)	Mozambique
Phlebotomus rupester	NH: P (1 F, 4 M)	Afghanistan
Phlebotomus saevus	NH: S (1 F)	Ethiopia
Phlebotomus salangensis	NH: P (1 F, 10 M)	Afghanistan
Phlebotomus smirnovi	NH: S (1 M)	Belarus
Phlebotomus syriacus	NH: S (5 F, 2 M)	Israel
Phlebotomus tobbi	NH: S (10 F, 16 M, 11 U)	Iran (S: 5 F, 5 M); Israel (S: 5 F, 11 M, 9 U)
Phlebotomus vansomerenae	NH: P (1 M)	Kenya
Phlebotomus wenyoni	NH:: S (3 M)	Iran

[a]**Depository Codes:**
BE - Royal Museum of Central Africa, Tervuren, Belgium
IP - Institut Pasteur, Paris, France
IR - Inst. Res. Dev., Montpellier, France
ME - Museum of Entomology, Florida State Collection of Arthropods
NH - Museum of Natural History, London, UK

[b]**Type Codes:**
H - holotype (1 specimen)
L - lectotype (1 specimen)
P - paratype
PL - paralectotype
S - syntype
T - type

[c]**Sex Codes:**
F - female
M - male
U - undetermined

Table 2 (continued; part 2 of 3). Types of sand flies (Phlebotominae, Psychodidae) from Africa and Southwest and Central Asia, examined from different repositories, including country of type origin.

Species	[a]Depository [b]Type (number of slides by [c]sex)	Country of Type Origin
Sergentomyia adleri	NH: S (1 M)	Ghana
Sergentomyia africana africana	NH: L (1 F); P (1 F)	Nigeria
Sergentomyia africana asiatica	NH: S (4 F, 7 M)	Palestine
Sergentomyia ansarii	IR: T (1 M, 1F)	Iran
Sergentomyia antennata	NH: H (F); P (1 M)	Ghana
Sergentomyia azizi	NH: S (1 F)	Cyprus
Sergentomyia baghdadis	NH: S (12 F, 10 M)	Iraq
Sergentomyia balmicola	NH: P (1 M)	Cameroon
Sergentomyia bedfordi	NH: H (F); BE: P (7 F, 18 M)	South Africa (H: F); D. R. Congo (P: 7 F, 18 M)
Sergentomyia blossi	NH: S (2 F)	Kenya
Sergentomyia buxtoni	NH: L (F); P (2 M); P (2 M)	Ghana (L: f), P: 2 M; Mali (P: 2 M)
Sergentomyia christophersi	NH: L (F); P (1 F)	Pakistan
Sergentomyia clastrieri	NH: P (1 F, 1 M)	Guinea
Sergentomyia clydei	NH: L (F); P (1 M)	Pakistan
Sergentomyia collarti	BE: C (1 F, 1 M); NH: S (6 F, 5 M)	Dem. Rep. Congo
Sergentomyia corneti	IR: H (F); P (1 F)	Senegal
Sergentomyia crosarai	BE: C (7 F)	Dem. Rep. Congo
Sergentomyia darlingi	IP: P (1 F, 1 M); IR: P (4 U)	Sudan
Sergentomyia decipiens	BE:T (11 F, 13 M)	Dem. Rep. Congo
Sergentomyia dentata dentata	NH: H (M); P (2 F)	Pakistan
Sergentomyia diapagai	IR: P (1 M); NH: P (1 M)	Burkina Faso
Sergentomyia dolichopa	IP: H (M); A (F); P (1 F, 1 M)	Djibouti
Sergentomyia dubia	NH: S (3 F)	Ghana (S: 2 F); Nigeria (1 F)
Sergentomyia dureni	BE: T (1 M); NH: S (1 F)	Dem. Rep. Congo
Sergentomyia dyemkoumai	IR: T (M)	Côte d'Ivoire
Sergentomyia edentulus	IR: H (F); P (2 F)	Senegal
Sergentomyia emilii	IR: H (M); P (4 M); IP: P (1 M)	Rep. Congo (H: M, P: 4 M); Dem. Rep. Congo (P: 1 M)
Sergentomyia fallax cypriotica	NH: S (1 F, 1 M)	Cyprus
Sergentomyia formica	NH: P (1 F)	Namibia
Sergentomyia freetownensis freetownensis	NH: H (F)	Sierra Leone
Sergentomyia freetownensis furanus	NH: H (F)	Sudan
Sergentomyia garnhami	NH: P (2 M)	Kenya
Sergentomyia gracilis	NH: L (1 F); P (1 M)	Kenya
Sergentomyia graingeri	NH: H (F)	Kenya
Sergentomyia grilloti	IR: H (F)	Rep. Congo
Sergentomyia grjebinei	IR: H (F); A (M)	Rep. Congo
Sergentomyia hamoni	IP: H (M); A (F)	Senegal
Sergentomyia harveyi	NH; H (F); P (6 F, 2 M)	Kenya
Sergentomyia heischi	NH: H (F)	Kenya
Sergentomyia herollandi	NH: P (F)	Mali
Sergentomyia hodgsoni	NH: L (F); P (1 M)	Pakistan
Sergentomyia inermis	IR: A (M); NH: S (11 F)	Guinea (M); Nigeria (11 F)

[a]**Depository Codes:**

BE - Royal Museum of Central Africa, Tervuren, Belgium
IP - Institut Pasteur, Paris, France
IR - Inst. Res. Dev., Montpellier, France
KE - Kenya National Museum, Nairobi
ME - Museum of Entomology, Florida State Collection of Arthropods
NH - Museum of Natural History, London, UK
NM - National Museum of Natural History, Paris
UM - US National Museum of Natural History, Smithsonian Institution Suitland, Maryland

[b]**Type Codes:**

A - allotype (1 specimen)
C - cotype
H - holotype (1 specimen)
L - lectotype (1 specimen)
P - paratype
PL - paralectotype
S - syntype
T - type

[c]**Sex Codes:**

F - female
M - male
U - undetermined

Table 2 (continued; part 3 of 3). Types of sand flies (Phlebotominae, Psychodidae) from Africa and Southwest and Central Asia, examined from different repositories, including country of type origin.

Species	[a]Depository [b]Type (number of slides by [c]sex)	Country of Type Origin
Sergentomyia ingrami	NH: H (M); P (1 M)	Ghana
Sergentomyia iranica	NH: H (F); P (2 M)	Iran
Sergentomyia kirki	NH: S (2 F)	South Sudan
Sergentomyia kitonyii	NH: H (F); P (8 F)	Kenya
Sergentomyia lesleyae	NH: H (F); P (1 M)	Sudan
Sergentomyia lewisiana	IR: H (F); A (M); P (5 F, 8 M)	Senegal
Sergentomyia logonensis	IR: T (F)	Cameroon
Sergentomyia machadoi	IR: P (1 M); NH: P (5 F, 2 M)	Angola
Sergentomyia macinthosi	IR: H (F)	South Africa
Sergentomyia madagascariensis	IR: H (M); A (F); P (1 F); NH: P (2 F)	Madagascar
Sergentomyia magnidentata	NH: H (F); P (1 F)	Kenya
Sergentomyia meilloni	NH: H (M)	South Africa
Sergentomyia minuta	NH: P (2 F, 5 M)	Ghana (1 M, 1 F); Zimbabwe (1 F, 4 M)
Sergentomyia mirabilis	BE: T (1 F, 1 M)	Dem. Rep. Congo
Sergentomyia montana	NH: H (F); P (1 F)	Ethiopia
Sergentomyia moucheti	IR: H (F)	Cameroon
Sergentomyia ovazzai	IR: H (F)	Guinea
Sergentomyia palestinensis	NH: H (F)	Palestine
Sergentomyia punjabensis	NH: S (1 M)	Pakistan
Sergentomyia renauxi	BE: P (1 F)	Dem. Rep. Congo
Sergentomyia richardi	BE: H (1 F)	Dem. Rep. Congo
Sergentomyia rimi	NH: P (2 F, 1 M)	Namibia (1 F, 1M); South Africa (1 F)
Sergentomyia roberti	IR: P (F)	Dem. Rep. Congo
Sergentomyia rogeri	IR: H (F); A (M); P (3 F)	Senegal
Sergentomyia rosannae	NH: H (F); P (3 F, 4 M)	Kenya
Sergentomyia schoutedeni	BE: L (M); P (3 M); C (2 F)	Dem. Rep. Congo
Sergentomyia schwetzi	BE: C (5 F, 1 M); NH: S (1 M)	Dem. Rep. Congo
Sergentomyia sidioliensis	IR: H (F); A (M); P (4 M)	Senegal
Sergentomyia simillima	NM: P (2 M)	Ivory Coast
Sergentomyia sonyae	NH: H (F); P (2 F, 5 M)	Oman
Sergentomyia squamipleuris	NH: L (1 F)	Sudan
Sergentomyia taizi	NH: H (F); P (2 F)	Yemen
Sergentomyia tauffliebi	IP: H (M); A (F); P (1 M)	Senegal
Sergentomyia teesdalei	NM: T (1 M)	Kenya
Sergentomyia thomsoni	NH: P (1 F)	Malawi
Sergentomyia tiberiadis	NH: L (F)	Israel
Sergentomyia trouilleti	IR: T (F)	Rep. Congo
Sergentomyia wansoni	BE: T (1 F, 1 M); C (1 F, 1 M)	Dem. Rep. Congo
Sergentomyia yusafi	NH: L (M); P (1 F)	Kenya
Sergentomyia yvonnae	BE: T (F)	Dem. Rep. Congo

[a]**Depository Codes:**

BE - Royal Museum of Central Africa, Tervuren, Belgium
IP - Institut Pasteur, Paris, France
IR - Inst. Res. Dev., Montpellier, France
KE - Kenya National Museum, Nairobi
ME - Museum of Entomology, Florida State Collection of Arthropods
NH - Museum of Natural History, London, UK
NM - National Museum of Natural History, Paris
UM - US National Museum of Natural History, Smithsonian Institution Suitland, Maryland

[b]**Type Codes:**

A - allotype (1 specimen)
C - cotype
H - holotype (1 specimen)
L - lectotype (1 specimen)
P - paratype
PL - paralectotype
S - syntype
T - type

[c]**Sex Codes:**

F - female
M - male
U - undetermined

Table 3 (part 1 of 9). Checklist of Phlebotomine sand flies of Africa, Southwest and Central Asia, including distribution. Note: countries are represented by numerical codes which are defined in Table 4.

Species	Africa*	Southwest Asia and Central Asia*	Other Countries	Selected References
Phlebotomus (Adlerius) angustus Artemiev, 1978		60, 77, 81, 84		3, 19
Phlebotomus (Adlerius) arabicus Theodor, 1953	10, 19, 27	64,77, 85		3, 19, 46, 47, X
Phlebotomus (Adlerius) balcanicus Theodor, 1958		62, 65, 66, 80	96, 102, 109, 111, 118	3, 19, 43, 47
Phlebotomus (Adlerius) brevis Theodor & Mesghali, 1964		62, 65, 66, 80	96, 104	3, 19, 43, 45
Phlebotomus (Adlerius) chinensis Newstead, 1916		66*		X
Phlebotomus (Adlerius) comatus Artemiev, 1978		60, 75	107	3, 19
Phlebotomus (Adlerius) halepensis Theodor, 1958		62, 65, 68, 66, 78, 80	107	3, 19, 43
Phlebotomus (Adlerius) hindustanicus Theodor, 1958		65, 75	98, 107	3, 19
Phlebotomus (Adlerius) kabulensis Artemiev, 1978		60, 65, 66		3, 19, 3
Phlebotomus (Adlerius) kyreniae Theodor, 1958		80	94	3, 19
Phlebotomus (Adlerius) longiductus Parrot, 1928		60, 66, 75, 84	92, 98, 109	3, 19, 43
Phlebotomus (Adlerius) naqbenius Lewis & Buttiker, 1986		77		3, 19
Phlebotomus (Adlerius) rupester Artemiev, 1978		60		3, 19
Phlebotomus (Adlerius) salangensis Artemiev, 1978		60, 66		3, 19
Phlebotomus (Adlerius) simici Nitzulescu, 1931		66, 68, 78, 80	96, 102	3, 19
Phlebotomus (Adlerius) turanicus Artemiev, 1974		60, 79, 81, 84		3, 19
Phlebotomus (Adlerius) zulfagarensis Artemiev, 1978		66, 81		3, 19
Phlebotomus (Anaphlebotomus) colabaensis Young & Chalam, 1927		75	98	3, 19
Phlebotomus (Anaphlebotomus) huberti Depaquit, Leger & Robert, 2002	29			36
Phlebotomus (Anaphlebotomus) rodhaini Parrot, 1930	2, 3, 4, 7, 9, 12, 13, 14*, 19, 21, 22*, 23, 25, 27, 36*, 39, 44, 48, 50, 53, 55			3, 19, 44, 47, 49, X
Phlebotomus (Anaphlebotomus) rousettus Davidson, 1981	48			3, 19
Phlebotomus (Anaphlebotomus) vincenti Randrianambinintsoa & Depaquit, 2013	29			36
Phlebotomus (Euphlebotomus) argentipes Annandale and Brunetti, 1908		75	87, 98, 99, 101, 103, 106, 107, 113, 117, 120	3, 19
Phlebotomus (Euphlebotomus) autumnalis Artemiev, 1980		60		3, 19
Phlebotomus (Euphlebotomus) caudatus Artemiev, 1978		60		3, 19
Phlebotomus (Euphlebotomus) mesghalii Rashti & Nadim, 1970		60, 66		3, 19, 43
Phlebotomus (Euphlebotomus) nadimi Javadian, Jalali-Galousang & Seyedi-Rashti, 1997		66		3, 19, 43
Phlebotomus (Larroussius) aculeatus Lewis, Minter & Ashford, 1974	19, 25			3, 19, X
Phlebotomus (Larroussius) ariasi Tonnoir, 1921	1, 35, 54		95, 100, 108, 112	3, 19, X
Phlebotomus (Larroussius) ashfordi Gebre-Michael and Lane, 1996	19			3, 19, 28, X
Phlebotomus (Larroussius) chadlii Rioux, Juminer and Gibily, 1966	1, 35, 54			3, 19
Phlebotomus (Larroussius) elgonensis Ngoka, Madel & Mutinga, 1975	19, 25, 29			3, 19, 46, X
Phlebotomus (Larroussius) fantalensis Lewis, Minter & Ashford, 1974	19			3, 19
Phlebotomus (Larroussius) gibiensis Lewis, Minter & Ashford, 1974	19			3, 19
Phlebotomus (Larroussius) guggisbergi Kirk & Lewis, 1952	25, 52, 55			3, 19
Phlebotomus (Larroussius) ilami Javadian, Jalali-Galousang & Seyedi-Rashti, 1997		66		43, 45

*New country species record. Notes: Africa includes several Western Indian Ocean islands (Comoros, Madagascar, Mauritius, Mayotte, Réunion, and Seychelles); X indicates observed.

Table 3 (continued; part 2 of 9). Checklist of Phlebotomine sand flies of Africa, Southwest and Central Asia, including distribution. Note: countries are represented by numerical codes which are defined in Table 4.

Species	Africa*	Southwest Asia and Central Asia*	Other Countries	Selected References
Phlebotomus (Larroussius) kandelakii kandelakii Shchurenkova, 1929		60, 61, 62, 65, 66, 73, 80		3, 19, 43
Phlebotomus (Larroussius) kandelakii burneyi Lewis, 1967		66, 75		3, 19
Phlebotomus (Larroussius) keshishiani Shchurenkova, 1936		60, 66, 75, 79		3, 19, 43
Phlebotomus (Larroussius) langeroni Nitzulescu, 1930	1, 35, 54	64, 66		3, 19
Phlebotomus (Larroussius) longicuspis Nitzulescu, 1930	1, 5, 28, 35, 54		112	3, 19, 25
Phlebotomus (Larroussius) longipes Parrot & Martin, 1939	19, 25, 50			3, 19, X
Phlebotomus (Larroussius) major Annandale, 1910		66, 75	107, 117	3, 19
Phlebotomus (Larroussius) mariae Rioux, Croset, Leger & Bailly-Choumara, 1974	35			3, 19
Phlebotomus (Larroussius) neglectus Tonnoir, 1921		66, 80, 82	86, 88, 96, 100, 110	3, 19, 43
Phlebotomus (Larroussius) notus Artemiev & Neronov, 1984		60		3, 19
Phlebotomus (Larroussius) orientalis Parrot, 1936	10, 15, 16, 19, 25, 38, 41, 50, 55	64, 77, 85		3, 19, 46, X
Phlebotomus (Larroussius) pedifer Lewis, Mutinga & Ashford, 1972	19, 25, 50			3, 19, X
Phlebotomus (Larroussius) perfiliewi perfiliewi Parrot, 1930	1,35, 54	62, 66, 80, 82	96, 97, 100, 104, 109	3, 19, 43
Phlebotomus (Larroussius) perfiliewi galilaeus Theodor, 1958		68, 80	94	3, 19
Phlebotomus (Larroussius) perfiliewi transcaucasicus Perfil'ev, 1937		62, 65, 66. 67, 80		3, 19
Phlebotomus (Larroussius) perniciosus Newstead, 1911	1, 28, 35	60, 65, 78, 80,	94, 95, 100, 104, 108, 111, 112	3, 19
Phlebotomus (Larroussius) smirnovi Perfil'ev, 1941		66, 70, 81, 84	92	3, 19
Phlebotomus (Larroussius) somaliensis Abonnenc, Adam & Bailly-Choumara, 1959	19, 47			3, 19, X
Phlebotomus (Larroussius) syriacus Adler & Theodor, 1931		61, 62, 65, 68, 69, 78, 80, 82		3, 19
Phlebotomus (Larroussius) tobbi Adler & Theodor, 1930		62, 65, 66, 67, 68, 73, 78, 80	86, 94, 96, 100	3, 19, 43
Phlebotomus (Larroussius) wenyoni Adler & Theodor, 1930		66, 67, 80, 81		3, 19, 43
Phlebotomus (Legeromyia) multihamatus Rahola, Depaquit, Makanga & Paupy, 2013	20			35
Phlebotomus (Madaphlebotomus) berentiensis (Leger & Rodhain, 1978)	29			3, 19, 26, 33
Phlebotomus (Madaphlebotomus) fertei Depaquit, Leger & Robert, 2002	29			21, 26, 36
Phlebotomus (Madaphlebotomus) fontenillei Depaquit, Leger & Robert, 2004	29			23, 26
Phlebotomus (Madaphlebotomus) vaomalalae Randrianambinintsoa, Leger & Depaquit, 2013	29			26, 37
Phlebotomus (Madaphlebotomus) vincenti Randrianambinintsoa & Depaquit, 2013	29			37
Phlebotomus (Paraphlebotomus) alexandri Sinton, 1928	1, 15, 16, 19, 38, 50, 54	60, 62, 66, 67, 68, 69, 70, 71, 74, 75, 77, 80, 81, 83, 85	92, 94, 96, 98, 105, 109, 112	3, 19, 43, X
Phlebotomus (Paraphlebotomus) andrejevi Shakirzyanova, 1953		60, 66, 70, 84	92, 105	3, 19, 43
Phlebotomus (Paraphlebotomus) caucasicus Marzinowsky, 1917		60, 61, 62, 65, 66, 70, 84	92	3, 19, 43
Phlebotomus (Paraphlebotomus) chabaudi Croset, Abonnenc & Rioux, 1970	1, 28, 35, 54		112	3, 19

*New country species record. Notes: Africa includes several Western Indian Ocean islands (Comoros, Madagascar, Mauritius, Mayotte, Réunion, and Seychelles); X indicates observed.

Table 3 (continued; part 3 of 9). Checklist of Phlebotomine sand flies of Africa, Southwest and Central Asia, including distribution. Note: countries are represented by numerical codes which are defined in Table 4.

Species	Africa*	Southwest Asia and Central Asia*	Other Countries	Selected References
Phlebotomus (Paraphlebotomus) gemetchi Gebre-Michael and Balkew, 2003	19			29
Phlebotomus (Paraphlebotomus) jacusieli Theodor, 1947		73*, 82, 66, 68, 80		3, 19, 43, X
Phlebotomus (Paraphlebotomus) kazeruni Theodor & Mesghali, 1964	1, 16, 27, 35	60, 66, 69, 77, 80		3, 19, 43, 47, X
Phlebotomus (Paraphlebotomus) marismortui Theodor, 1947		68		3, 19
Phlebotomus (Paraphlebotomus) mireillae Kellick-Kendrick, Tang, Johnson, Ngumbi & Robert, 1997	19, 25			46, X
Phlebotomus (Paraphlebotomus) mongolensis Sinton, 1928		60, 62, 66, 70, 84	92	3, 19, 43
Phlebotomus (Paraphlebotomus) nuri Lewis, 1967		60, 66, 75		3, 19
Phlebotomus (Paraphlebotomus) saevus Parrot & Martin, 1939	7, 19, 25, 50	77, 85		3, 19
Phlebotomus (Paraphlebotomus) sergenti sergenti Parrot, 1917	1, 15, 16, 18, 19, 25, 27, 28, 31, 35, 37, 38, 47, 54	60, 61 64, 65, 66, 67, 68, 69, 70, 73, 75, 77, 78, 80, 85	94, 95, 96, 98, 100, 104, 107, 108, 109, 111, 112	3, 19, 43, 47, X
Phlebotomus (Paraphlebotomus) sergenti similis Perfil'ev, 1963		63, 66, 82	118, 119	3, 19, 43
Phlebotomus (Phlebotomus) bergeroti Parrot, 1934	1, 5, 10, 15, 16, 18, 19, 32, 35, 38, 44, 47, 50	66, 68, 69, 75, 77, 83, 85		3, 19, 43, 46, X
Phlebotomus (Phlebotomus) duboscqi Neveu-Lemaire, 1906	5, 7, 9, 10, 19, 21, 22, 25, 31, 32, 33, 38, 39, 44, 46, 50, 53, 59	77, 85		3, 19, 40, 46, X
Phlebotomus (Phlebotomus) gigas Parrot & Schwetz, 1937	2, 7, 9, 12, 13, 20, 23, 31			3, 19, 46, X
Phlebotomus (Phlebotomus) minteri Lewis, 1982	52			3, 19
Phlebotomus (Phlebotomus) papatasi (Scopoli, 1786)	1, 16, 18, 19, 25*, 28, 35, 38, 47, 50, 54	60, 61, 62, 65, 66. 67. 68, 69, 70, 71, 73, 74, 75, 77, 78, 80, 81, 84, 85	87, 89, 94, 96, 96, 97, 98, 100, 104, 107, 109, 112, 113	3, 19, 42, 43, X
Phlebotomus (Phlebotomus) salehi Mesghali, 1965		66	98	3, 19, 43, X
Phlebotomus (Synphlebotomus) ansarii Lewis, 1957		66		3, 19, 43
Phlebotomus (Synphlebotomus) celiae Minter, 1962	19, 25			46, X
Phlebotomus (Synphlebotomus) eleanorae Sinton, 1931		66	98	3, 19, 43
Phlebotomus (Synphlebotomus) grovei Downes, 1971	37			3, 19
Phlebotomus (Synphlebotomus) katangensis Bequaert & Walravens, 1930	12, 58			3, 19, 46, X
Phlebotomus (Synphlebotomus) martini Parrot, 1936	19, 25, 47, 50, 55			3, 19, X
Phlebotomus (Synphlebotomus) rossi de Meillon & Lavoipierre, 1944	37, 48, 58			3, 19
Phlebotomus (Synphlebotomus) saltiae Leger, Haddad and Chaker, 1997		73		32
Phlebotomus (Synphlebotomus) taylori Davidson, 1982	58			3, 19
Phlebotomus (Synphlebotomus) vansomerenae Heisch, Guggisberg & Teesdale, 1956	19, 25			3, 19
Phlebotomus (Transphlebotomus) canaaniticus Adler & Theodor, 1931		68, 69, 78		3, 19
Phlebotomus (Transphlebotomus) mascittii Grassi, 1908	1	6	94, 95, 96, 100, 102, 112, 115	3, 19
Sergentomyia (Capensomyia) caffrarica (de Meillon & Lavoipierre, 1944)	48			3, 19
Sergentomyia (Capensomyia) capensis (de Meillon, 1955)	48			3, 19

*New country species record. Notes: Africa includes several Western Indian Ocean islands (Comoros, Madagascar, Mauritius, Mayotte, Réunion, and Seychelles); X indicates observed.

Table 3 (continued; part 4 of 9). Checklist of Phlebotomine sand flies of Africa, Southwest and Central Asia, including distribution. Note: countries are represented by numerical codes which are defined in Table 4.

Species	Africa*	Southwest Asia and Central Asia*	Other Countries	Selected References
Sergentomyia (Capensomyia) drakensbergi Davidson, 1979	48			3, 19
Sergentomyia (Capensomyia) haeselbarthi (Abonnenc, 1967)	48			3, 19
Sergentomyia (Capensomyia) kalaharia Davidson, 1979	37, 48			3, 19
Sergentomyia (Capensomyia) luteola Davidson, 1983	48			3, 19
Sergentomyia (Capensomyia) meeseri (de Meillon & Hard, 1953)	48, 55, 58			3, 19
Sergentomyia (Capensomyia) nama Davidson, 1953	37, 48			3, 19, X
Sergentomyia (Capensomyia) namibensis (de Meillon & Hardy, 1953)	37, 48			3, 19, X
Sergentomyia (Capensomyia) xera Davidson, 1979	37, 48			3, 19, X
Sergentomyia (Demeillonius) transvaalensis Sinton, 1933	48			3, 19
Sergentomyia (Grassomyia) dreyfussi dreyfussi (Parrot, 1933)	1, 15, 18, 19, 25, 35, 47, 54, 66	66, 69, 77, 85		3, 19, 43
Sergentomyia (Grassomyia) dreyfussi turkestanica Theodor & Mesghali, 1964		60, 68, 81		3, 19
Sergentomyia (Grassomyia) ghesquierei (Parrot, 1929)	3, 5, 12, 13, 14, 19, 21, 22*, 23, 44, 47*			3, 19, X
Sergentomyia (Grassomyia) indica (Theodor, 1931)		66, 75	90, 92, 93, 98, 99, 101, 103, 107, 116, 117	3, 19
Sergentomyia (Grassomyia) inermis (Theodor, 1938)	2, 3, 4, 5, 9, 10, 19, 21, 22, 23, 25, 30, 39, 44, 48, 50			3, 19, 49
Sergentomyia (Grassomyia) madagascariensis (Abonnenc, 1969)	29			3, 19
Sergentomyia (Grassomyia) squamipleuris (Newstead, 1912)	2, 3, 5, 9, 10, 12, 13, 14, 15, 16, 19, 21, 22, 23, 25, 29, 30, 31, 36, 39, 44, 46, 48, 50, 52, 53, 55, 57	66, 67, 68, 77		3, 19, 27, 43, X
Sergentomyia (Neophlebotomus) angolensis (Abonnenc, 1968)	2, 13			3, 19
Sergentomyia (Neophlebotomus) collarti (Adler, Theodor & Parrot, 1929)	2, 7, 9, 12, 13, 14, 21, 22, 23, 39, 42, 46, 50, 52, 55			3, 19, X
Sergentomyia (Neophlebotomus) corneti Pastre, 1975	44			3, 19, X
Sergentomyia (Neophlebotomus) decipiens (Theodor, 1931)	7, 9, 12, 13, 14, 23, 25, 39, 44, 50, 52*, 55			3, 19, X
Sergentomyia (Neophlebotomus) dolichopa (Abonnenc & Courtois, 1970)	15	66, 85		3, 19, X
Sergentomyia (Neophlebotomus) dureni Parrot, 1934	2, 5, 9, 10, 12, 13, 14, 19*, 21, 22*, 23, 25, 27, 39, 44, 50, 52			3, 19, X
Sergentomyia (Neophlebotomus) dyemkoumai (Abonnenc, 1964)	13, 14			3, 19, 46
*Sergentomyia (Neophlebotomus) grejbinei (*Vattier-Bernard, 1971)	12, 13			3, 19
Sergentomyia (Neophlebotomus) grilloti (Vattier-Bernard & Bimangou, 1975)	13			3, 19
Sergentomyia (Neophlebotomus) harveyi (Heisch, Guggisberg & Teesdale), 1956	25			3, 19
Sergentomyia (Neophlebotomus) hodgsoni (Sinton, 1933)		60, 66, 75	98, 117	3, 19

*New country species record. Notes: Africa includes several Western Indian Ocean islands (Comoros, Madagascar, Mauritius, Mayotte, Réunion, and Seychelles); X indicates observed.

Table 3 (continued; part 5 of 9). Checklist of Phlebotomine sand flies of Africa, Southwest and Central Asia, including distribution. Note: countries are represented by numerical codes which are defined in Table 4.

Species	Africa*	Southwest Asia and Central Asia*	Other Countries	Selected References
Sergentomyia (Neophlebotomus) ingrami (Newstead, 1914)	2, 3, 5, 7, 9, 12, 13, 14, 19*, 21, 22, 23, 25, 27, 39, 44, 50, 53, 55			19, 47, X
Sergentomyia (Neophlebotomus) kirki (Parrot, 1948)	25, 30, 50			3, 19
Sergentomyia (Neophlebotomus) kitonyii (Minter, 1963)	19, 25			3, 19, X
Sergentomyia (Neophlebotomus) machadoi (Abonnenc, 1968)	2			3, 19, X
Sergentomyia (Neophlebotomus) macintoshi (Abonnenc & Pastre, 1972)	48			3, 19
Sergentomyia (Neophlebotomus) notata (Parrot, 1938)	19			3, 19
Sergentomyia (Neophlebotomus) pawlowskyi Perfil'ev, 1933		60, 61, 66, 67, 80, 81, 84		3, 19
Sergentomyia (Neophlebotomus) serrata (Parrot & Malbrant, 1945)	12, 13, 19, 25, 50, 55			3, 19
Sergentomyia (Neophlebotomus) sonyae Lewis, 1982	74, 77			3, 19
Sergentomyia (Neophlebotomus) teesdalei Minter, 1963	25			3, 19, X
Sergentomyia (Neophlebotomus) trouilleti Vattier-Bernard, 1976	13			3, 19
Sergentomyia (Parrotomyia) africana africana (Newstead, 1912)	3, 5, 7, 9, 12, 13, 15, 14, 19, 21, 22,23, 25, 27, 31, 32, 38, 39, 44, 46, 48, 50, 53, 55, 57, 85	66		3, 27, 34, 43, 46, 47, X
Sergentomyia (Parrotomyia) africana asiatica (Theodor, 1933)	35	68, 69, 75, 77	98	3, 19
Sergentomyia (Parrotomyia) babu babu (Annandale, 1910)	33	60, 75	87, 98	3, 19
Sergentomyia (Parrotomyia) baghdadis Adler & Theodor, 1929		60, 66, 67, 75	98	3, 19, 43, X
Sergentomyia (Parrotomyia) bailyi (Sinton, 1931)		66, 75	90, 92, 98, 101, 117, 120	3, 19
Sergentomyia (Parrotomyia) crosarai (Parrot & Wanson, 1946)	2, 12, 13, 27			3, 19, 47, X
Sergentomyia (Parrotomyia) eremitis (Parrot & Bouquet de Joliniere, 1945)	1, 27, 50, 53			3, 19, 47, X
Sergentomyia (Parrotomyia) freetownensis freetownensis (Sinton, 1930)	2, 3, 9, 14, 18, 12*, 13, 19, 25, 23, 32, 44, 46, 48, 50, 58, 77			3, 19, X
Sergentomyia (Parrotomyia) freetownensis furanus (Lewis & Kirk, 1958)	50			3, 19
Sergentomyia (Parrotomyia) grekovi Khodukin, 1929		60, 66, 70, 75, 79, 81, 82, 84	98	3, 19, 43
Sergentomyia (Parrotomyia) magna (Sinton, 1932)	2, 5, 7, 9, 12, 14, 21, 19, 22*, 23, 31, 32, 39, 53, 44, 48, 50, 58	77		3, 19, X
Sergentomyia (Parrotomyia) palestinensis (Adler & Theodor, 1927)	1, 15, 16, 18, 19, 35, 50	62, 66, 67, 68, 69, 75, 77	98	3, 19, 43, X
Sergentomyia (Parrotomyia) rhodesiensis (de Meillon & Hardy, 1953)		58		3, 19
Sergentomyia (Parrotomyia) shorttii (Adler & Theodor, 1927)		75	87, 98, 106	3, 19
Sergentomyia (Parrotomyia) sogdiana (Parrot, 1928)		66, 79, 84		3, 19, 43
Sergentomyia (Parrotomyia) sumbarica Perfil'ev, 1933		60, 66, 67, 81, 84	92	3, 19, 43, X
Sergentomyia (Parrotomyia) yvonnae (Parrot & Schwetz, 1937)	12			3, 19, X

*New country species record. Notes: Africa includes several Western Indian Ocean islands (Comoros, Madagascar, Mauritius, Mayotte, Réunion, and Seychelles); X indicates observed.

Table 3 (continued; part 6 of 9). Checklist of Phlebotomine sand flies of Africa, Southwest and Central Asia, including distribution. Note: countries are represented by numerical codes which are defined in Table 4.

Species	Africa*	Southwest Asia and Central Asia*	Other Countries	Selected References
Sergentomyia (Parrotomyia) zielkei Seccombe, Ready and Huddleston, 1993	48			3, 19
Sergentomyia (Parvidens) arida Davidson, 1982	37, 58			3, 19, X
Sergentomyia (Parvidens) heischi Kirk & Lewis, 1950	19, 25, 36, 37, 50			3, 19, X
Sergentomyia (Parvidens) iranica (Lewis & Mesghali, 1961)		66		3, 19, 43
Sergentomyia (Parvidens) lesleyae (Lewis & Kirk, 1946)	19, 32, 50			3, 19
Sergentomyia (Rondanomyia) goodmani goodmani Depaquit & Robert, 2005	29			22
Sergentomyia (Rondanomyia) goodmani comorensis Depaquit, Randrianamnini & Leger, 2012	11			39
Sergentomyia (Sergentomyia) antennata (Newstead, 1912)	1, 3, 5, 7, 9, 10, 19, 12, 14, 15, 16, 19, 20, 21, 22, 23, 25, 27, 30, 31, 32, 35, 39, 44, 46, 47, 48, 50, 52*, 53, 54	66, 67, 68, 69, 71, 77, 83, 55		3, 19, 43, 46, 47, X
Sergentomyia (Sergentomyia) arpaklensis Perfil'ev, 1933		81, 84		3, 19
Sergentomyia (Sergentomyia) ashfordi Davidson, 1987	19			3, 19
Sergentomyia (Sergentomyia) bedfordi (Newstead, 1914)	2, 3, 4**, 7, 9, 10, 12, 14, 19, 21, 22, 23,25, 27,31, 36, 37, 39, 44, 48, 50, 52, 53, 55, 58			3, 19, 47, 49, X
Sergentomyia (Sergentomyia) bergerardi Trouillet & Vattier-Bernard, 1978	13			3, 19
Sergentomyia (Sergentomyia) bimangoui Davidson, 1990	13			3, 19
Sergentomyia (Sergentomyia) blossi (Kirk & Lewis, 1952)	25			3, 19, X
Sergentomyia (Sergentomyia) buxtoni (Theodor, 1933)	3, 5, 10, 14*, 21, 22, 31, 38, 39, 44			3, 19, X
Sergentomyia (Sergentomyia) caliginosa Davidson, 1987	30, 36, 48, 57, 58			3, 19
Sergentomyia (Sergentomyia) cincta (Parrot & Martin, 1944)	9, 15, 16, 19, 22, 25, 50, 55			3, 19, X
Sergentomyia (Sergentomyia) congolensis (Bequaert &Walravens, 1930)	4, 12			3, 49, X
Sergentomyia (Sergentomyia) davidsoni Seccombe, Ready and Huddleston, 1993	18, 19, 50			3, 19, X
Sergentomyia (Sergentomyia) dentata dentata (Sinton, 1933)		60, 62, 65, 66, 67, 75, 78, 80, 83, 85		3, 19, 43
Sergentomyia (Sergentomyia) dentata agdamica Artemiev, 1982		61, 62, 65, 66, 67		3, 19
Sergentomyia (Sergentomyia) dentata bruchoni Parrot, 1935		80	96	3, 19
Sergentomyia (Sergentomyia) dubia (Parrot, Mornet & Cadenat, 1934)	5, 7, 13, 14, 21, 22, 23, 31, 32, 39, 44			3, 19, X
Sergentomyia (Sergentomyia) fallax fallax Parrot, 1921	1, 5, 15, 16, 19, 35, 38, 54	60, 64, 77, 85		3, 19, X
Sergentomyia (Sergentomyia) fallax afghanica Artemiev, 1974		60		3, 19
Sergentomyia (Sergentomyia) fallax cypriotica (Adler, 1946)		67, 68, 73, 80	94	3, 19
Sergentomyia (Sergentomyia) formica Davidson, 1987	37, 48, 58			3, 19
Sergentomyia (Sergentomyia) gracilis (Kirk & Lewis, 1952)	25			3, 19

*New country species record; ***Sergentomyia* (*Sergentomyia*) *bedfordi* "Maun form". Notes: Africa includes several Western Indian Ocean islands (Comoros, Madagascar, Mauritius, Mayotte, Réunion, and Seychelles); X indicates observed.

Table 3 (continued; part 7 of 9). Checklist of Phlebotomine sand flies of Africa, Southwest and Central Asia, including distribution. Note: countries are represented by numerical codes which are defined in Table 4.

Species	Africa*	Southwest Asia and Central Asia*	Other Countries	Selected References
Sergentomyia (Sergentomyia) impudica Abonnenc, 1968	2, 13, 27, 55			3, 19, 44, X
Sergentomyia (Sergentomyia) logonensis (Rageau, 1951)	7, 9			3, 19, X
Sergentomyia (Sergentomyia) magnidentata Davidson, 1987	25, 47*			3, 19, X
Sergentomyia (Sergentomyia) mervynae Pringle, 1953	54	60, 66, 75		3, 19, 43
Sergentomyia (Sergentomyia) minuta minuta Rondani, 1843	25*	80, 82	95, 96, 100, 104, 108, 109, 112	3, 19, X
Sergentomyia (Sergentomyia) minuta parroti (Adler & Theodor, 1927)	1, 28, 35, 54	78	94, 96, 112	3, 19
Sergentomyia (Sergentomyia) murgabiensis murgabiensis Perfil'ev, 1939		60, 66, 70, 81		3, 19
Sergentomyia (Sergentomyia) murgabiensis pashtunica Artemiev, 1974		60, 66, 75	105	3, 19
Sergentomyia (Sergentomyia) murgabiensis sintoni Pringle, 1953	16	60, 66, 67, 71, 80, 81		3, 19
Sergentomyia (Sergentomyia) pastoriana (Parrot, Mornet & Cadenat, 1945)	14, 19, 22, 23, 39, 46, 50, 55			3, 19, X
Sergentomyia (Sergentomyia) punjabensis (Sinton, 1933)		75	113, 117	3, 19
Sergentomyia (Sergentomyia) renauxi (Parrot & Schwetz, 1937)	12, 13			3, 19, X
Sergentomyia (Sergentomyia) richardi (Parrot & Wanson, 1946)	7, 12, 27, 53, 55			3, 19, X
Sergentomyia (Sergentomyia) rima Davidson, 1987	30, 36, 37, 48, 57, 58			3, 19, X
Sergentomyia (Sergentomyia) ruttledgei (Lewis & Kirk, 1946)	50, 55			3, 19
Sergentomyia (Sergentomyia) salisburiensis (Abonnenc, 1967)	4, 37, 48, 49, 57, 58			3, 19, 49, X
Sergentomyia (Sergentomyia) schoutedeni schoutedeni (Adler, Theodor & Parrot, 1929)	3, 7, 12, 14, 23, 25, 26, 39, 50, 53, 55			3, 19, X
Sergentomyia (Sergentomyia) schoutedeni nocens (Parrot, 1951)	25, 50			3, 19
Sergentomyia (Sergentomyia) schwetzi (Adler, Theodor & Parrot, 1929)	1, 2, 3, 5, 7, 9, 10, 12, 13, 14, 15, 16, 19, 20, 21, 22, 23, 25, 27, 31, 32, 35, 35, 37, 39, 44, 46, 47, 48, 50, 52*, 53, 55	77, 85		24, 25, 27, X
Sergentomyia (Sergentomyia) serridentata Davidson, 1990	7, 55			3, 19, X
Sergentomyia (Sergentomyia) silva Trouillet, 1985	13			3, 19
Sergentomyia (Sergentomyia) taizi Lewis, 1974	15, 16	77, 85		3, 19, X
Sergentomyia (Sergentomyia) teteica Artemiev, 1985	36			3, 19
Sergentomyia (Sergentomyia) theodori (Parrot, 1919)	16	60, 66, 67, 68, 69, 73, 75, 77, 78, 80	94, 96, 98, 101	3, 19, 43
Sergentomyia (Sergentomyia) waqqasi Artemiev, 1982		67		3, 19
Sergentomyia (Sergentomyia) yusafi (Sinton, 1930)	25, 39, 52, 55, 57	85		3, 19, 46, X
Sergentomyia (Sergentomyia) zumpti (Abonnenc, 1967)	37, 48			3, 19
Sergentomyia (Sintonius) adami (Abonnenc, 1960)	9, 31			3, 19, X
Sergentomyia (Sintonius) adleri (Theodor, 1933)	3, 5, 7, 9, 10, 14, 15, 16, 19, 21, 22, 25, 38, 39, 44, 50, 53, 55	66, 69, 77, 83		3, 19, 27, 46, X

*New country species record. Notes: Africa includes several Western Indian Ocean islands (Comoros, Madagascar, Mauritius, Mayotte, Réunion, and Seychelles); X indicates observed.

Table 3 (continued; part 8 of 9). Checklist of Phlebotomine sand flies of Africa, Southwest and Central Asia, including distribution. Note: countries are represented by numerical codes which are defined in Table 4.

Species	Africa*	Southwest Asia and Central Asia*	Other Countries	Selected References
Sergentomyia (Sintonius) affinis affinis (Theodor, 1933)	9, 19, 23, 25, 50			3, 19, 46, X
Sergentomyia (Sintonius) affinis vorax (Parrot, 1948)	2, 3, 5, 9, 10, 19, 22, 23, 25, 31, 39, 44, 50			3, 19, X
Sergentomyia (Sintonius) balmicola Abonnenc, Adam & Bailly-Choumara, 1959	7			3, 19
Sergentomyia (Sintonius) calcarata (Parrot, 1948)	10, 15, 18, 19, 50	77		3, 19, X
Sergentomyia (Sintonius) choumarai (Abonnenc, 1960)	47			3, 19
Sergentomyia (Sintonius) christophersi (Sinton, 1927)	1, 5, 9, 10, 15, 16, 18, 19, 21, 23, 25, 29, 35, 38, 39, 44, 50, 54, 55	60, 66, 69, 74, 75, 77, 85	98	3, 19, 27, 43, 46, X
Sergentomyia (Sintonius) clastrieri (Abonnenc, 1964)	15, 23			3, 19
Sergentomyia (Sintonius) clydei (Sinton, 1928)	1, 5, 7, 9, 10, 15, 16, 18, 19, 21, 22, 25, 31, 35, 38, 39, 44, 45, 47, 50, 53, 54, 55	60, 66, 67, 68, 70, 71, 75, 77, 79, 81, 83, 85		3, 19, 44, X
Sergentomyia (Sintonius) diapagai (Abonnenc, 1962)	5, 31			3, 19
Sergentomyia (Sintonius) edentulus Pastre, 1982	44			3, 19, X
Sergentomyia (Sintonius) graingeri (Heisch, Guggisberg & Teesdale, 1956)	25			3, 19
Sergentomyia (Sintonius) herollandi (Abonnenc, 1960)	31, 44			3, 19, X
Sergentomyia (Sintonius) hospitii (Sinton, 1924)		75	98	3, 19
Sergentomyia (Sintonius) lewisiana Pastre, 1982	44			3, 19, X
Sergentomyia (Sintonius) mbandakai Abonnenc, 1970	12			3, 19
Sergentomyia (Sintonius) meilloni Sinton, 1932	25, 36, 37, 48, 51, 58			3, 19, X
Sergentomyia (Sintonius) rogeri Pastre, 1982	44			3, 19, X
Sergentomyia (Sintonius) sattii Qutubuddin, 1962	50			3, 19
Sergentomyia (Sintonius) sidioliensis Pastre, 1982	44			3, 19, X
Sergentomyia (Sintonius) suberecta (Sinton, 1932)	25, 50			3, 19, X
Sergentomyia (Sintonius) tauffliebi (Abonnenc & Cornet, 1971)	13, 14, 44			3, 19, X
Sergentomyia (Sintonius) thomsoni (Theodor, 1938)	10, 19, 25, 30			3, 19
Sergentomyia (Sintonius) tiberiadis tiberiadis (Adler, Theodor & Lourie, 1930)	15, 16, 18, 19, 50	60, 66, 68, 74, 77, 83, 85		3, 19, 43, X
Sergentomyia (Sintonius) tiberiadis pakistanica Artemiev & Saf'janova, 1974		60, 75, 81		3, 19
Sergentomyia (Sintonius) wansoni (Parrot, 1938)	2, 5, 9, 12, 13, 19, 31, 55	66		3, 19, X
Sergentomyia (Spelaeomyia) bailyi (Sinton, 1931)	7	75	92, 98, 117, 120	3, 19
Sergentomyia (Spelaeomyia) darlingi (Lewis & Kirk, 1954)	5, 9, 31,50			3, 19, X
Sergentomyia (Spelaeomyia) emilii (Vattier-Bernard, 1966)	5, 9, 13			3, 19, X
Sergentomyia (Spelaeomyia) hunti (Lewis & Kirk, 1946)	9, 50			3, 19
Sergentomyia (Spelaeomyia) mirabilis (Parrot & Wanson, 1939)	2, 9, 12, 13, 52, 55			3, 19, X
Sergentomyia (Spelaeomyia) moucheti (Vattier-Bernard & Abonnenc, 1967)	7, 9, 13			3, 19

*New country species record. Notes: Africa includes several Western Indian Ocean islands (Comoros, Madagascar, Mauritius, Mayotte, Réunion, and Seychelles); X indicates observed.

Table 3 (continued; part 9 of 9). Checklist of Phlebotomine sand flies of Africa, Southwest and Central Asia, including distribution. Note: countries are represented by numerical codes which are defined in Table 4.

Species	Africa*	Southwest Asia and Central Asia*	Other Countries	Selected References
Sergentomyia (Trouilletomyia) boironis Ranadrianambinintspa & Depaquit, 2014	29			38
Sergentomyia (Trouilletomyia) huberti Depaquit, Leger & Robert, 2002	29			24
Sergentomyia (Vattieromyia) anka Depaquit, Leger & Robert, 2008	29			24
Sergentomyia (Vattieromyia) namo Depaquit, Leger & Robert, 2008	29			24
Sergentomyia (Vattieromyia) pessoni Depaquit, Randrianamnini & Leger, 2012	11			39
Sergentomyia (Vattieromyia) schlerpsiphon Depaquit, Leger & Robert, 2008	29			24
*Sergentomyia (Ungrouped)** *berentiensis* Leger & Rodhain, 1978	29			3, 19
Sergentomyia (Ungrouped) bernardae Trouillet, 1982	13			3, 19
Sergentomyia (Ungrouped) cunicula Davidson, 1979	37			3, 19
Sergentomyia (Ungrouped) dissimillima (Abonnenc, 1972)	5, 13, 14, 23			3, 19
Sergentomyia (Ungrouped) garnhami (Heisch, Guggisberg & Teesdale, 1956)	25, 52			3, 19
Sergentomyia (Ungrouped) hamoni (Abonnenc, 1958)	2, 5, 7, 9, 12, 13, 14, 31, 44, 50			3, 19, X
Sergentomyia (Ungrouped) hirta (Parrot & Bouquet de Joliniere, 1945)	1			3, 19
Sergentomyia (Ungrouped) horridula Vattier-Bernard & Trouillet, 1982	13			3, 19
Sergentomyia (Ungrouped) leponti Vattier-Bernard, 1973	13			3, 19
Sergentomyia (Ungrouped) lumsdeni Kirk & Lewis, 1950	13, 55			3, 19
Sergentomyia (Ungrouped) majungaensis Depaquit, Leger and Robert, 2007	29			48
Sergentomyia (Ungrouped) metzi Davidson, 1979	37, 48			3, 19, X
Sergentomyia (Ungrouped) montana (Sinton, 1924)		75	98, 107	3, 19
Sergentomyia (Ungrouped) moreli (Abonnenc & Hamon, 1958)	13, 14, 27, 55			3, 43, 47, X
*Sergentomyia (Ungrouped) multid*ens (Heisch, Guggisberg & Teesdale, 1956)	15, 19, 25	85		3, 19
Sergentomyia (Ungrouped) ovazzai (Pastre, 1973)	23			3, 19
Sergentomyia (Ungrouped) roberti Vattier-Bernard & Trouillet, 1081	13			3, 19
Sergentomyia (Ungrouped) rosannae (Heisch, Guggisberg & Teesdale, 1956)	25			3, 19
Sergentomyia (Ungrouped) simillima (Newstead, 1914)	3, 5, 7, 9, 12, 13, 14, 19, 22, 23, 24, 27, 37, 39, 46, 50, 53, 55			3, 43, 47, X
Sergentomyia (Ungrouped) villosa Davidson, 1979	37, 48, 58			3, 19, X
Sergentomyia (Ungrouped) vulpes Davidson, 1979	37, 48			3, 19, X
Sergentomyia (Ungrouped) welwitschii Davidson, 1979	37, 48			3, 19, X
Sergentomyia (Ungrouped) wurtzi (Parrot, 1938)	19			3, 19
Sergentomyia (Ungrouped) wynnae (Watson, 1951)	25, 55			3, 19

*New country species record. Notes: Africa includes several Western Indian Ocean islands (Comoros, Madagascar, Mauritius, Mayotte, Réunion, and Seychelles); X indicates observed.
** Ungrouped indicates subgenus uncertain.

Table 4. List of countries and their numerical codes as shown in Figure 1 and Table 3.

Code No.	Country*	Code No.	Country*	Code No.	Country*	Code No.	Country*
1	Algeria	31	Mali	62	Azerbaijan	92	China (mainland)
2	Angola	32	Mauritania	63	Bahrain	93	China (Hong Kong)
3	Benin	33	Mauritius	64	Egypt	94	Cyprus
4	Botswana	34	Mayotte (France)	65	Georgia	95	France
5	Burkina Faso	35	Morocco (excluding Western Sahara)	66	Iran	96	Greece
6	Burundi	36	Mozambique	67	Iraq	97	Hungary
7	Cameroon	37	Namibia	68	Israel	98	India
8	Cape Verde	38	Niger	69	Jordan	99	Indonesia
9	Central African Republic	39	Nigeria	70	Kazakhstan	100	Italy
10	Chad	40	Réunion (France)	71	Kuwait	101	Laos
11	Comoros	41	Rwanda	72	Kyrgyzstan	102	Macedonia
12	Congo, Democratic Republic of	42	Saint Helena, Ascension and Tristan da Cunha (UK)	73	Lebanon	103	Malaysia
13	Congo, Republic of	43	São Tomé and Príncipe	74	Oman	104	Malta
14	Côte d'Ivoire	44	Senegal	75	Pakistan	105	Mongolia
15	Djibouti	45	Seychelles	76	Qatar	106	Myanmar
16	Egypt	46	Sierra Leone	77	Saudi Arabia	107	Nepal
17	Equatorial Guinea	47	Somalia	78	Syria	108	Portugal
18	Eritrea	48	South Africa	79	Tajikistan	109	Romania
19	Ethiopia	49	South Sudan	80	Turkey	110	Russia
20	Gabon	50	Sudan	81	Turkmenistan	111	Serbia & Montenegro (former Yugoslavia)
21	Gambia	51	Swaziland	82	Ukraine	112	Spain
22	Ghana	52	Tanzania	83	United Arab Emirates	113	Sri Lanka
23	Guinea	53	Togo	84	Uzbekistan (including Crimea)	114	Sri Lanka (Ubduam)
24	Guinea-Bissau	54	Tunisia	85	Yemen	115	Switzerland
25	Kenya	55	Uganda	86	Albania	116	Taiwan
26	Lesotho	56	Western Sahara	87	Bangladesh	117	Thailand
27	Liberia	57	Zambia	88	Bosnia & Herzegovina	118	Ukraine
28	Libya	58	Zimbabwe	89	Bulgaria	119	Ukraine (Crimea)
29	Madagascar	60	Afghanistan	90	Cambodia	120	Vietnam
30	Malawi	61	Armenia	91	Cameroon		

*Africa (code nos. 1-58, including West Indian Ocean Islands); Southwest Asia and Central Asia (code nos. 60-85, including Middle East/CENTCOM; part of EUCOM, former Russian Republics); other countries (Code nos. 86-121)

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Operational Mosquito and Vector-Borne Diseases Surveillance at Incirlik Air Base, Turkey

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Abstract

Arboviruses on Incirlik Air Base, Turkey, pose a threat to military personnel and civilians, but might also be relevant for understanding the threats in neighboring conflict zones such as Syria. We reviewed 6 years of mosquito and arbovirus surveillance at Incirlik Air Base. Over 6,000 mosquitoes were identified as *Aedes caspius*, *Anopheles claviger*, *Culex mimeticus*, *Cx. perexiguus*, *Cx. pipiens*, *Cx. sinaiticus*, and *Culiseta longiareolata*. Almost all of the mosquitoes (more than 90%) were *Cx. perexiguus* or *Cx. pipiens*. Both West Nile virus and Sindbis virus were detected in 6 mosquito pools among collections made in 2013, 2014, and 2015.

Vector-borne and zoonotic diseases pose a threat to active military personnel both overseas and in the United States. Deaths among service members from vector-borne diseases have dropped in recent decades, but morbidity from vector-borne diseases and injuries from stinging arthropods remain a real threat.[1-4] The US Air Force (USAF) maintains arthropod identification and pathogen surveillance capability at the USAF School of Aerospace Medicine at Wright-Patterson Air Force Base, OH, through the Epidemiology Consult Service. Entomology services include the capability to identify both wide range of arthropods from around the world and pathogen surveillance including the detection of several arboviruses, *Bartonella* spp., *Leishmania* spp., *Plasmodium* spp., and *Rickettsia* spp.[5-10] Many of these identifications and pathogen screening services are used for both threat assessments and as part of standard pest management operations.

Long-term data on both vectors and associated pathogens are maintained for later review and determination of epidemiologic patterns. With the ongoing conflicts in the Middle East, analysis of trends in pathogen surveillance continues to be relevant. Several mosquito-borne arboviruses including West Nile virus (WNV) and Sindbis virus (SINV) were historically reported from both military members and local mosquitoes in the region.[11,12] We reviewed the arbovirus surveillance data for Incirlik Air Base (AB) in Turkey from 2011-2016 with a discussion of 2 major viruses.

Incirlik AB is in a strategically relevant location. It is located in far southern Turkey relatively near Syria and other Middle Eastern countries, including Iraq, Lebanon, and Israel. Surveillance is relevant for ongoing force health protection. In addition, pathogens detected in Incirlik could be useful in understanding viruses circulating in neighboring countries. Several mosquito-borne viruses such as West Nile and Sindbis are endemic in Turkey.[13,14]

Methods

The US Air Force currently maintains several military bases in the Middle East, but Incirlik AB, which opened in February 1955, has one of the longest established records. Most bases conduct vector and pathogen surveillance programs. Mosquitoes on many of these bases breed in water retention ponds or subsurface catch basins below parking lots or streets. Mosquitoes were trapped on the air base using the solid-state Army miniature traps with dry ice. Traps were set out seasonally from April through October and run on a weekly basis. Mosquito fogging typically occurred 3 days a week. Two traps were used, both near the perimeter fence, one near the sewage treatment plant, and one near base quarters. Mosquitoes were killed by freezing and shipped dry to the USAF School of Aerospace Medicine for identification and pathogen detection. Mosquitoes were morphologically identified using regional keys.[15,16] Voucher specimens were deposited in the Ohio State University Museum of Biological Diversity, Columbus, OH, or the Walter Reed Biosystematics Unit, Silver Spring, MD.

After identification, mosquitoes were pooled by species and tested for arboviruses. Pools ranged from 1-25 mosquitoes depending on submission numbers. Most

mosquitoes were tested with a RAMP* WNV test and the VecTOR Test Systems (Thousand Oaks, California) *Alphavirus* panel that detects chikungunya (CHIK), equine encephalitis, Mayaro, SINV, Venezuelan equine encephalitis, and Western equine encephalitis viruses. These rapid assays which had been designed for field deployment and rapid screening of mosquitoes[17] were among the tools used from 2011-2016 by the USAF School of Aerospace Medicine. Both the VecTOR Test Systems and RAMP assays were performed in accordance with manufacturers' guidelines. We occasionally used commercially available inactivated West Nile and chikungunya virus antigens as a positive control to validate the assay sensitivity as suggested.[18] As a further validation, we verified 2 of the positive pools by reverse transcription polymerase chain reaction (RT-PCR). West Nile virus positive pools were verified using the protocols by Lanciotti et al.[19] The PCR products were sequenced and compared to sequences in GenBank using the BLAST† program.

There were 6 pools of *Culex perexiguus* Theobald or *Cx. pipiens* L. that tested positive for arboviruses as shown in the Table. There were 4 pools positive for WNV: one each on October 2 and 9, 2013; August 29, 2014; and September 16, 2015. We validated the October 9, 2013 results with both the RAMP and VecTOR Test Systems WNV assays. The RAMP scores ranged from 150 to the maximum limit of 740. Both pools tested by RT-PCR were positive with cycle threshold scores of 18.14 and 21.04 respectively which was less than the viral culture positive controls. The 919 base-pair sequence of WNV envelope protein was a 100% match to WNV isolate Spain/2010/H-1b (GenBank # JF719069).

In addition, 2 pools of combined *Cx. perexiguus* and *Cx. pipiens* from June 18 and 25, 2014, tested strongly positive for an *Alphavirus* on the VecTOR Test System assay. This assay is designed to detect specific viruses, and Sindbis is the detectable virus in the region.[13]

Comment

We detected West Nile and Sindbis viruses in pools of *Culex* spp. from Incirlik AB. The WNV was in the Western Mediterranean WNV subtype in the WNV lineage 1 based on sequence similarity. While often underappreciated, both viruses are a true threat to the military. Petersen et al[20] estimated that WNV caused hundreds of thousands of infections in the United States annually from 1999 to 2010. Further, WNV was the leading cause of vector-borne disease death in USAF-associated individuals since the 1970s.[2] Sindbis virus infection causes severe arthritis, fever, and, in rare cases, encephalitis.[21]

West Nile virus was detected in a total of 6 collections made in 2013, 2014, and 2015. This indicates that the virus circulates at some regularity and could pose a threat to both active duty, contractors, civilian personnel, and dependents. We separated mosquito collection data between sewage or waste water treatment plants and residential areas and detected infected mosquitoes in both areas. This virus requires avian hosts for the natural enzootic cycle but mosquitoes can be transovarially infected.[22] We could not determine the route of infection in mosquitoes from Incirlik AB.

Incirlik AB is close to Syria and other conflict zones in the Middle East. Detection of 2 potentially serious arbovirus threats on a military base in southern Turkey could raise concerns for similar disease threats in neighboring countries. This is especially true in conflict zones with little to no infrastructure. Infections by either West Nile or Sindbis virus often lead to mild disease but can lead to fatal or debilitating infections and might result in costly medical evacuations.

In summary, we reviewed 6 years (2011-2016) of WNV and SINV detection in mosquitoes from Incirlik AB. *Culex perexiguus* was the most commonly associated mosquito with WNV from the base. It is a member of the *Cx. univittatus* Theobald complex and several of these have previously been associated with both WNV and SINV with evidence of vertical transmission of WNV.[23] Our surveillance data suggests that there are repeated and predictable threats from WNV and possibly SINV on the base.

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*RAMP (rapid analyte measurement platform) is a registered trademark of Response Biomedical, Vancouver, Canada.

†Basic Local Alignment Search Tool; National Center for Biotechnology Information, Bethesda, MD.

Mosquito surveillance data from Incirlik Air Base, Turkey, from 2011-2016 with WNV virus and Sindbis virus screening data.

Locality name	Collection Dates	Species* and Number	Arbovirus Test Results
Housing	2 June-19 August 2011	*Culex perexiguus* (95) *Culex pipiens* (23) *Aedes caspius* (1) *Culiseta longiareolata* (7)	WNV/Alphavirus Negative
Housing	27 April-14 September 2012	*Culex perexiguus* (133) *Cules pipiens* (388) *Culex mimeticus* (7) *Aedes caspius* (7) *Culiseta longiareolata* (6)	WNV/Alphavirus Negative
Running Track	1 June 2012	*Culex perexiguus* (5) *Culex pipiens* (13)	WNV/Alphavirus Negative
Housing	18 April-20 November 2013	*Culex perexiguus* (73) *Culex pipiens* (339) *Culex sinaiticus* (148) *Aedes caspius* (17) *Culiseta longiareolata* (7)	One pool of 25 *Culex perexiguus* from 9 October 2013 was positive for WNV virus.
Sewage Treatment Plant	18 April-20 November 2013	*Culex perexiguus* (821) *Culex pipiens* (252) *Culex sinaiticus* (301) *Aedes caspius* (32)	One pool of 25 *Culex perexiguus* from 2 October 2013 was positive for WNV virus.
Flight Line	8 April-6 November 2013	*Culex pipiens* (8)	WNV/Alphavirus Negative
Housing	16 May-29 August 2014	*Anopheles claviger* (1) *Culex perexiguus* (658) *Culex pipiens* (153) *Culiseta longiareolata* (1)	WNV/Alphavirus Negative
Dining Facility	2 July 2014	*Culex perexiguus* (1) *Culex pipiens* (3)	WNV/Alphavirus Negative
Sewage Treatment plant	23 April-29 August 2014	*Culex perexiguus* (327) *Culex pipiens* (90) *Aedes capensis* (9)	Two pools of combined *Culex* spp. (25 each) from 18 and 25 June 2014 tested positive for *Alphavirus*. One pool of 25 *Culex perexiguus* from 29 August 2014 was positive for WNV virus.
Housing	6 May-23 September 2015	*Culex perexiguus* (331) *Culex pipiens* (280) *Aedes caspius* (11) *Culiseta longiareolata* (14)	One pool of 25 *Culex perexiguus* from 16 September 2015 was positive for WNV virus.
Sewage Treatment Plant	1 May-16 September 2015	*Culex perexiguus* (831) *Culex pipiens* (198) *Aedes caspius* (2) *Culiseta longiareolata* (3)	WNV/Alphavirus Negative
Housing	12 April-12 August 2016	*Culex perexiguus* (11) *Culex pipiens* (33) *Culiseta longiareolata* (3)	WNV/Alphavirus Negative
Sewage Treatment Plant	31 March-16 October 2016	*Culex perexiguus* (225) *Culex pipiens* (182) *Aedes capensis* (3)	WNV/Alphavirus Negative

*Scientific species identification:

Aedes capensis Edwards
Aedes caspius (Pallas)
Culiseta longiareolata Macquart
Culex mimeticus Noè
Culex perexiguus Theobald
Cules pipiens L.
Culex sinaiticus Kirkpatrick

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Vector-Borne Diseases of Public Health Importance for Personnel on Military Installations in the United States

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Vector-borne diseases (VBDs) are among the leading causes of morbidity and mortality worldwide. The history of US military medicine dates from the formation of the Continental Army in the 1770s.[1] From the inception of our nation, the US military has combated infectious diseases. Today, scientific research programs at Walter Reed Army Institute of Research, the Uniformed Services University of the Health Sciences, and other institutions are major components in this effort. Additionally, the armed forces have worked to prevent the spread of disease through active public health measures such as the establishment of vector control programs at military bases, and international scientific collaborations aimed at increasing our prevention proficiencies.[2] While VBDs are of considerable concern for internationally deployed active military and civilian personnel, the concern for acquisition of VBDs in US territories and states has also been a reality for centuries. Endemic, emerging, and newly introduced VBDs have spread across the United States and its territories, creating foci of disease transmission. Military personnel are particularly susceptible to VBDs due to their increased contact with vectors during outdoor training exercises, military response missions, and occupational-specific exposures.

US Military Entomology Infrastructure

Vector control and pest management across the Department of Defense (DoD) involves a multitude of organizations both internal and external to the DoD. Born from a need to address the countless casualties and mission cost attributed to VBDs during World War II, the Army War Department established the Army Committee for Insect and Rodent Control (ACIRC) in 1944. In 1956, *DoD Instruction 5154.12* established the Armed Forces Pest Control Board replacing the ACIRC. By 1979, the title was amended to its current form, Armed Forces Pest Management Board (AFPMB), to better reflect the goal of balancing vector and pest control with environmental protection. The AFPMB is composed of pest management and medical entomologists from the Air Force, Army, Navy, and the Defense Logistics Agency (DLA). It has a strategic mandate from the Office of the Assistant Secretary of Defense for Acquisition, Technology and Logistics to recommend policy, provide guidance, and coordinate the exchange of information on all matters related to pest management throughout the DoD. Service entities including the Army Public Health Center, Navy Entomology Center of Excellence, Walter Reed Army Institute of Research, Walter Reed Biosystematics Unit, and Armed Forces Health Surveillance Center assist in the coordination and implementation of DoD and/or service-specific pest management policies and guidance. The AFPMB is the lead pest management agency for the DoD, coordinating with other US government (USG) agencies, non-USG organizations, and other foreign organizations as well, including the Environmental Protection Agency, US Department of Agriculture, US Centers for Disease Control and Prevention (CDC), North Atlantic Treaty Organization, and the World Health Organization.

Vector control programs employed by the US armed forces date from the late 1800s, when mosquitoes were first identified as vectors for human disease pathogens.[3] Vector management programs evolved into their modern form during the 1970s with the development of integrated mosquito control programs focused on a multifaceted approach designed to manage the target pest with integrated measures to mitigate risk of vector disease transmission while balancing risk of pesticide exposure and environmental risk. The introduction of West Nile virus into naïve US mosquito populations[4] and the recent chikungunya-Zika Latin American epidemics have created a shift in the paradigm of vector control programs.[5] Programs have now begun to balance the concern for environmental effects of pesticide use on a large scale with the threat of invasive mosquito species and their respective capacities to introduce emerging VBDs within our borders. Currently, several endemic VBDs continue to threaten our military and civilian populations residing

within US borders. Additionally, emerging arboviral diseases in Latin America threaten introduction into our resident mosquito populations,[6,7] and new pathogens are being identified on a regular basis through surveillance and innovative pathogen detection research investigations.[8-10] In this article, we present an overview of vector-borne diseases acquired by military personnel during field exercises and/or training evolutions within US borders and end with diseases of future concern and conclusions to reduce disease transmission in this population.

Mosquito-borne Diseases

West Nile Virus

West Nile virus (WNV) is transmitted by a variety of mosquito species found in the United States, primarily *Culex pipiens* Linneaeus in the north, *Cx. quinquefasciatus* Say in the south, and *Cx. tarsalis* Coquillett in the western states. Over 2,000 cases of WNV occur annually in the United States[11] with cyclic peak annual outbreaks.[12] Although many infected humans will not show signs of disease, 20% develop febrile illness, and 1% develop neuroinvasive disease (acute flaccid paralysis, meningitis, encephalitis or meningoencephalitis).[13] Neuroinvasive disease is a particularly concerning clinical manifestation, as these patients have the highest risk of long-term morbidity and death.[11] Diagnosis is made by detection of WNV viremia, or antibodies in the serum or cerebrospinal fluid. Acute disease diagnosis is complicated by the short duration of viremia antecedent to symptom onset and the sustained presence of IgM infection several months to years post-onset.[14] Evidence for persistent infection and/or sequelae exists, with patients continuing to report morbidity up to 11 years post-infection.[15,16] The economic burden from WNV infection is considerable with each individual case of acute neuroinvasive disease resulting in up to $51,240 in healthcare costs[17] and up to $400,000 in long-term loss of productivity wages.[18] Currently, there are no Food and Drug Administration approved treatments or vaccines for WNV infection; however, vaccine clinical trials are undergoing.[19] Surveillance has identified WNV activity at over 44 DoD sites,[20] resulting in nationwide vector control efforts.[21] The Armed Forces Health Surveillance Center reported 323 confirmed cases of WNV illnesses among Army, Navy, Marine Corps, Air Force, and Coast Guard personnel from 2006-2015.[22] An additional 245 cases were reported during that time, but the military branch of the patients was unknown and those cases could include contractors, civilians, and foreign nationals.

Dengue Virus

Dengue virus (DENV) is transmitted by *Aedes aegypti* (L.) and *Ae. albopictus* Skuse mosquitos found throughout the southern United States, with the *Ae. albopictus* vector geographic range reaching north to Minnesota and Maine.[23] Globally, 4 serotypes (DENV 1-4) have been well characterized, with a fifth serotype having recently been proposed in Malaysia.[24] Infection with one serotype does not provide cross-protection from other serotypes, and multiple serotype exposures increases one's odds of developing severe clinical outcomes, such as dengue hemorrhagic fever and shock syndrome. Symptoms of DENV fever are nonspecific (fever, headache, joint, muscle, and bone pain), with symptom onset typically occurring 4-7 days after vector transmission and lasting 3-10 days after symptom onset. As the fever is residing, warning signs for serious clinical manifestations can present, including capillary leakage, marked temperature change, thrombocytopenia, change in mental status, rapid weak pulse, and hemorrhagic manifestations that can rapidly progress to circulatory system failure and shock.[25] Although the majority of dengue cases in the United States are travel-related, autochthonous transmission has occurred along the Texas/Mexico border, as well as in Florida and Hawaii.[26-30] Diagnosis is determined by laboratory confirmation of viremia or antibodies. There is no specific treatment for DENV infection; however, fluid replacement treatment and pain relievers may improve outcomes among critically ill patients. Vaccine trials are underway[31]; but controlling mosquito populations in the interim is the best method for disease prevention. Between 2006 and 2015, approximately 700 cases of dengue fever were reported in military personnel.[32] Due to the rarity of the locally-acquired disease in most of the United States, one would assume most individuals acquired the infection during deployment to endemic regions. While only focal outbreaks of autochthonous transmission have occurred in the United States, military installations in regions where *Ae. albopictus* and *Ae. aegypti* exist should monitor for the potential of autochthonous infections.[26-30]

Other Endemic Arboviral Infections of Concern

St. Louis encephalitis (SLE) is a *Culex* sp. transmitted flavivirus infection of notable historical importance,[33] as this disease served as justification for the foundation of new vector control authorities across the country. While SLE is less prevalent due to the recent establishment of WNV, cases are still reported annually and it is a regular contributor to neuroinvasive disease nationally.[34] Alphaviruses are another group of positive-sense RNA viruses transmitted through the bite of an infected mosquito and are a member of the Togaviridae family.[35] They typically result in encephalitis or arthralgia. Western equine encephalitis (WEE), Eastern equine encephalitis (EEE), and Venezuelan equine encephalitis (VEE) viruses are encephalitic alphaviruses, while chikungunya

is an arthralgic alphavirus. *Aedes*, *Culex*, *Psorohpora*, and C*uliseta* are all known to transmit alphavirus infections. For the encephalitic alphaviruses, most infections are asymptomatic, but febrile illness can onset 2-10 days after infection and progress to encephalitis in approximately 5% of people.[35] Most EEE cases are reported in Florida, Georgia, Massachusetts, and New Jersey, but transmission is most common around the freshwater hardwood swamps in Atlantic states, Gulf Coast states, and the Great Lakes regions.[36] Most WEE cases occur in Texas, Colorado, Oklahoma, and New Mexico.[35] Most VEE cases occur in Central and South America, but spillover cases have occurred in Texas.[37] Complications of encephalitis can occur with lifetime care costs exceeding $4.6 million per patient.[35] According to the Armed Forces Health Surveillance Center, at least 107 cases of mosquito-borne viral encephalitedes (contributable to either WEE, EEE, or other less common arboviruses) were reported between 2005 and 2014, with cases originating annually from the Marines, Army, Air Force, and Navy,[38] further demonstrating their contributory role in serviceman illness.

Tick-borne diseases

Lyme disease

Lyme disease, also referred to as Lyme borreliosis, is caused by different genospecies of the bacterium *Borrelia burgdorferi* Johnson sensu lato.[39] This vector-borne infection is endemic to the majority of the northern hemisphere with active transmission ongoing in Europe, Asia, and the United States.[40] Multiple species of the *Ixodes* ticks are capable of propagating sylvatic transmission of this pathogen in the United States, but only two are implicated in human transmission: the black legged tick (also called the deer tick) (*Ixodes scapularis* Say), and the western black-legged tick (*I. pacificus* Cooley and Kohls).[41] Initial symptoms after exposure through the bite of an infected tick often include the development of a rash around the sight of inoculation referred to as erythema migrans, colloquially called a "bulls-eye" rash. In addition, many patients concurrently experience fatigue, headache, arthralgia, malaise, and myalgia. Rarely the disease can disseminate after initial infection and cause symptoms including carditis, neurologic complications, and arthritis.[42] Oral antibiotics such as doxycycline, amoxicillin, or cefuroxime axetil are commonly prescribed for treatment of Lyme disease.[43] Patients treated rapidly after onset of infection often recover completely. However, delayed diagnosis and treatment may lead to a higher likelihood of developing severe disease. Lyme disease is among the most commonly reported VBDs in the United States, with an annual incidence of around 300,000 cases.[44] It has long been proposed that individuals with occupations or hobbies that require extended time spent outdoors, such as military training exercises, in endemic areas are at elevated risk for contracting Lyme disease.[45]

Historically, the armed forces have struggled with Lyme disease infection in military trainees, active duty Soldiers, military dependents, and civilian contractors working on military bases and installations. Reports of infection date back to 2 years after the pathogen was first isolated. A naval base in New Jersey reported an incidence rate of 1,063 cases per 100,000 personnel between 1981 and 1982. Identification was based on clinical diagnosis as serologic testing was not yet available, introducing the possibility that this number underrepresents the true burden of disease due to misdiagnosis.[46] Further highlighting this issue, 2 case reports identified military personnel that were not identified until disseminated Lyme disease had developed, and presented with rare symptoms including carditis and neurologic complications.[47,48] The late 1990s was a period of low incidence. It was estimated that only 6 seroconversions occurred during military duty per 100,000 persons.[49,50] Reports of Lyme disease among all branches of the military steadily increased in the early 2000s.[51,52]

In 2006, an entire 110-person unit was preemptively treated for Lyme disease after a training exercise at Fort Dix, New Jersey. Between one and 2 weeks after the exercise, at least 5 personnel were diagnosed with erythema migrans. Watchful waiting was judged to be too high of a risk for this unit as they were preparing to deploy to an austere location in less than 2 weeks. Given the risk of cardiac and neurologic complications presenting in such circumstances, the entire unit received a 2-week course of doxycycline for early Lyme.[53] In 2011, a spike in cases was reported, at its peak 16 per 100,000 active duty personnel and 25 per 100,000 reservists were screened positive for *B. burgdorferi* exposure.[54] Incidence of military exposure appear to correlate with base location, with the highest incidence in the northeastern United States.[55] Reports indicate that a correlation exists between pathogen quantity in ticks removed from military personnel and human prevalence of disease at a given base, indicating that this may serve as an effective surveillance tool for detection of high risk areas and prevention of disease outbreaks.[56] This method might be particularly useful to distinguish between transmission sources for returning serviceman from European installations where transmission is also possible.[57]

Ehrlichiosis

First reported in the United States in 1986, human ehrlichiosis is caused by infection with either *Ehrlichia chaffeensis* Dumler (human monocyte ehrlichiosis) or *E.*

ewingii Dumler.[58] This zoonotic pathogen is an obligate intracellular bacterium that often infects monocytes, forming distinct *Ehrlichia* colonies. Cases are most often reported in the southcentral and eastern regions of the United States. This corresponds to the geographic region of naturally occurring lonestar ticks (*Amblyomma americanum* Linnaeus), the primary vector for both *Ehrlichia* species.[59] Within one to 2 weeks of exposure, patients typically develop general febrile illness and a subset present with a wide range of rash (maculopapular to petechial). If not identified and treated promptly, ehrlichiosis can cause more severe symptoms including difficulty breathing and abnormal bleeding, with a 1.8% case fatality.[60] The primary treatment is oral doxycycline for both adult and pediatric cases.[61] It is recommended that the antibiotic be prescribed even in suspected cases due to the severe and even fatal nature of this infection.

Among the first reports characterizing the causative agent of human ehrlichiosis includes a case-report of an Army reservist with tick exposure during a field exercise at Fort Chaffee, Arkansas.[62] Following this discovery, a prospective serologic investigation was launched to determine seroprevalence of *Ehrlichia* at Fort Chaffee and surrounding bases. Seroconversion was detected in 1.3% of the Soldiers with available pre-exposure samples (n=1,194) with 33.3% of seropositive personnel reporting a previous clinical history consistent with ehrlichiosis. Additionally, seropositive military personnel were significantly more likely to report history of tick attachment (RR=3.56, $P<.2$), indicating that active tick-borne transmission of *Ehrlichia* was ongoing during field exercises at bases within Arkansas.[63] A second outbreak was detected at a New Jersey base, where 12% of personnel screened seropositive (n=74) with all 9 cases recalling tick exposure during field exercises in knee-high grass.[64] Additional sporadic human case reports and *Ehrlichia* pathogen positive ticks collected from military personnel indicate this is an ongoing threat to military personnel performing field exercises in the eastern and southcentral portions of the United States.[52,65,66]

Rocky Mountain Spotted Fever

Rocky Mountain spotted fever (RMSF, infectious agent *Rickettsia rickettsia* Ricketts) is endemic to the United States,[67] and is sometimes referred to as spotted fever group rickettsiosis when including other less commonly transmitted rickettsial pathogens (*R. parkeri* Lackman and *Rickettsia* spp. 364D). Transmission has been noted throughout the contiguous 48 states, with a burden of disease occurring in the southcentral states. The primary vector for RMSF is the American dog tick (*Dermacentor variablilis* (Say)).[68] Symptoms of disease present 2 to 14 days after exposure and typically begin with a nonspecific fever and headache. Infection is often misdiagnosed at onset, due to the initial nonspecific nature of disease. Typically, it is not until the characteristic rash associated with infection forms 2 to 5 days following onset of symptoms, that the disease is positively identified.[68] Some individuals will have a more severe infection that can lead to vasculitis, and abnormal bleeding in the brain and/or vital organs. Those who experience severe disease symptoms often suffer from sequelae as a result of infection. Diagnosis of RMSF can be difficult as no serologic test are available to detect acute infection, and is largely determined by clinical symptoms and epidemiologic exposures. Once a case is identified, prompt doxycycline administration is critical to limit disease severity.[67] Given the frequency of movements of military trainees from one geographic location to another, this often presents well outside its usual geographic range. A thorough travel history is an essential component for the patient exam of any military personnel presenting with an acute febrile illness in order to consider geographically limited but life-threatening infections like RMSF.

Rocky Mountain spotted fever has affected military personnel and working dogs stationed across the United States since 1982. A serosurvey of dogs (N=467) housed at 4 different military bases across the country found 32% were seropositive, with a range of 4.3% to 63.4% depending on region. Additionally, a higher rate of RMSF exposure was reported (87%) in the working/sporting dogs breeds screened.[69] Prevalence in canine populations were seemingly in parallel with human seroprevalence in the same geographic region during this time period, suggesting that dogs may provide an efficient sentinel for infection.[69,70] At the same Arkansas base where Yevich and colleagues[63] reported *Ehrlichia* infections, a serosurveillance study detected 2.5% of military personnel (n=1,194) had seroconverted for RMSF. While less than a quarter of individuals that developed antibodies for RMSF had clinical symptoms (8/30), disease status was strongly associated with history of tick bite (RR=4.3 $P<.001$).[63] Reports continue to emerge linking cases of RMSF to field exercises in the United States,[71] detecting seroconversion in military troops,[52,72] and identification of pathogen from ticks implicated in human bites.[65]

Tick-borne Relapsing Fever

Tick-borne relapsing fever (TBRF) occurs when *Borrelia* spirochetes (predominantly *B. hermsii* Steinhaus and *B. turicatae* Steinhaus) are transmitted to humans by *Ornithodoros* ticks. These soft-shelled Argasidae ticks differ from hard Ixodidae ticks in several key characteristics: they have multiple nymphal stages; they feed

rapidly, typically between 15 and 90 minutes; as adults, they can feed and reproduce repeatedly, are capable of surviving for several years between blood meals[73]; and the spirochetes may colonize their salivary glands, rather than the midgut, allowing for rapid deposition after host attachment.[74] An infected human usually displays an influenza-like illness, often with some degree of altered mental status, after a mean incubation period of 7 days (range: 4-18 days). Although severe disease is atypical, acute respiratory distress syndrome and other serious sequelae have been reported. More commonly, the initial illness resolves in 3 days (range: 0.5-17 days), followed by an afebrile interval of approximately one week and then a relapse of fever. Since borreliae can vary their surface protein antigens repetitively, multiple relapses are possible. Febrile episodes typically become shorter and less severe over time. The mortality rate is well below 5%, with some fatalities attributed to a Jarisch-Herxheimer reaction after antibiotic initiation.[73] Diagnosis of TBRF may be confirmed by visualization of spirochetes on thick or thin smears using Giemsa or Wright stains during febrile episodes, although serologic and molecular techniques are becoming increasingly available.[75] The mainstay therapy for infected adults is a 7-10 day course of oral doxycycline (100 mg every 12 hours); other oral and parenteral antibiotics are also effective.[73]

Isolated cases or small clusters of TBRF are possible during military field training exercises in endemic regions of the country, most notably Florida, Texas, and the Pacific West. Sleeping on floors in close proximity to the natural habitat of *Ornithodoros* ticks, such as in limestone caves or rodent-infested cabins, is particularly risky.[76] In the summer of 2015, an Army Soldier contracted TBRF during a 30-day field exercise in northwestern Texas, likely while sleeping in an abandoned barn-like structure. He was hospitalized with fever and marked thrombocytopenia but recovered rapidly after initiation of doxycycline. Postexposure prophylaxis was provided to 10 soldiers in his detachment, none of whom became ill.[77] This case provided the first human isolate of *B. turicatae*[78] and should remind military public health personnel to emphasize tick control and personal protective measures during field exercises in TBRF endemic areas. When these measures cannot be followed, or when the risk remains high despite their implementation, postexposure prophylaxis with oral doxycycline should be considered.[79]

Other Tick-borne Diseases of Concern

Several additional endemic tick-borne diseases exist in the United States. Anaplasmosis (*Anaplasma phagocytophilum* Dumler rickettsial infection) is transmitted to humans by *I. scapularis* ticks and results in approximately 2,600 incident cases annually, primarily in northeastern and midwestern states.[80] A recent seroprevalence study of the DoD Serum Repository identified a 0.11%-2.6% *A. phagocytophilium* positive rate among a geographically diverse sample of servicemen, identifying this pathogen as an important cause of illness in military populations.[72] Colorado tick fever is another cause of undifferentiated febrile illness, occurring primarily at altitudes of 4,000 ft to 10,000 ft and transmitted by *D. andersoni* Stiles.[81,82] It can result in serious complications, including pericarditis, meningitis, and/or encephalitis. Tularemia infection (caused by *Francisella tularensis* Dorofeev) is a potentially life threatening illness that can be transmitted by *Dermacentor* sp., *Amblyomma* sp., and *Ixodes* sp. ticks, among other nonvector transmission sources. Tularemia has been reported from all contiguous states, but is most common in southcentral states and the Pacific Northwest.[83] Powassan encephalitis is a rare but serious viral tick-borne disease common in the northeastern United States, primarily transmitted by *I. cookei* Packard vector, although other *Ixodes* sp. and *D. andersoni* can be vectors.[81,82]

Other vector-borne diseases

Chagas Disease

An estimated 6 to 8 million people are infected with Chagas disease.[84] The disease results from infection with the protozoan parasite *Trypanosoma cruzi* Chagas is transmitted to humans through one of several routes: vector-borne, congenital, blood-borne, oral, and organ transmission. It is most commonly acquired via vectorborne transmission.[84] More than 130 triatomine species in the Americas can be infected by and transmit *T. cruzi*.[85] In the United States, the greatest diversity is found in southwestern states; *Triatoma sanguisuga* LeConte is the most widely distributed, yet *T. gerstaeckeri* Gerstaecker is the most commonly collected.[85] Autochthonous infection potential increases as triatomine species adapt to human dwellings, and as human living environments and military field activities expand into areas of sylvatic disease.[86]

Infection occurs when a *T. cruzi* positive triatomine feeds and excreta contaminates the bite wound or mucosal tissue, entering the bloodstream. After an incubation period of 1 to 2 weeks, an acute phase of 8 to 12 weeks follows. During the acute phase, patients may be asymptomatic, have mild symptoms, or local inflammation at the bite site; however, less than 1% will have severe acute disease that manifests as acute myocarditis and/or pericardial effusion.[87] Chronic infection occurs in 3 forms: indeterminate, cardiac, or gastrointestinal disease. Most infections remain in an indeterminate

phase for life and have positive anti-*T. cruzi* serology, but no clinical signs or symptoms.[86] Approximately 20% to 30% of indeterminate cases progress to cardiac and/or gastrointestinal disease years or decades later. Cardiac disease is detected by abnormal electrocardiogram, and symptomatic disease may present as aneurysm, thrombus formation, or congestive heart failure.[87] Less commonly, progression leads to gastrointestinal disease affecting the esophagus and/or colon, leading to motility disorders, megaesophagus, or megacolon.[88] Treatment with nifurtimox or benznidazole may be indicated for acute and indeterminate chronic disease to decrease symptoms and clinical course, but must be obtained from the CDC and administered under an investigational protocol.[88] Two case reports of servicemen with military and childhood exposures have been published, highlighting the potential for disease transmission among military personnel in the United States.[89,90]

Other Endemic Vector-borne Diseases of Concern

Leishmaniasis is a parasitic infection (20+ *Leishmania* sp.) transmitted by sand fly vectors (30+ *Phlebotomus* sp.), and can manifest clinically as either cutaneous or visceral forms of disease. Two genetic lineages exist that correspond to either Old World infections (Asia, Middle East, and Africa) or New World infections (Western Hemisphere). While vector-borne transmission is most common, anthroponotic transmission has occurred,[91] heightening concern of this disease among our armed forces personnel living in close quarters. While military cases have not been reported, autochthonous leishmaniasis human cases have been reported from Oklahoma and Texas,[92] implicating these states as possible transmission risk areas for military operations and training. Trench fever (*Bartonella quintana* Schmincke infection) is transmitted by the human body louse (*Pediculus humanus humanus* Linnaeus), and manifests clinically as nonspecific febrile illness, bacillary angiomatosis, or endocarditis.[93] While trench fever was historically a major concern in World War I, it has yet to be reported among contemporary service personnel; however, high rates of infected body louse among American homeless populations[94] indicates a potential risk to military personnel in contact with these populations, ie, those serving in natural disaster response operations. Murine typhus is a typhus group rickettsiosis transmitted by rat fleas (*Xenopsylla cheopis* Rothschild) and cat fleas (*Ctenocephalides felis* Bouché), which can present as nonspecific febrile illness, conjunctivitis, and/or heptosplenomegaly. Murine typhus is a reemerging disease with geographic distribution in Hawaii,[95,96] Texas,[97] and California.[98]

Diseases of Future Concern

> *Should the disorder infect the Army, in the natural way, and rage with its usual virulence, we should have more to dread from it, than from the Sword of the Enemy.*
>
> Letter from General George Washington to the Continental Army Surgeon General, Dr William Shippen, February 6, 1777.[99]

Whether engaging in stateside-based training activities, traditional major combat operations, or humanitarian assistance missions, US armed forces military personnel have always had to grapple with ubiquitous vector-borne diseases which have rivaled bayonets, bullets, missiles, and mortars throughout history as the causes of morbidity, mortality, disability, and diminished operational effectiveness. While certain diseases have lost their military importance (yellow fever,[100] plague,[101] and epidemic typhus,[102,103]), others remain of concern (dengue fever,[102,104] leishmaniasis,[102,105-107] West Nile encephalitis,[108] and malaria[100,109]), and emerging diseases have recently occurred (Zika virus[110] and chikungunya fever[111]) that affect operational forces.

Leishmaniasis, characterized by the CDC as a "neglected tropical disease," persists as a pestilence of future concern. The facts that the incidence of cutaneous leishmaniasis among US military during operations in Iraq and Afghanistan was substantial,[112] and it is endemic in 88 countries[113] are reasons to keep this condition on military preventive medicine's radar. Notably, there has also been an upsurge of cutaneous leishmaniasis among Syrian refugees in traditionally nonendemic locations,[114] including Europe.[113,115] Furthermore, leishmaniasis has been identified in Texas and other areas of the southern United States,[116,117] complicating the ability to accurately identify origin of infection in returning military personnel.

Mayaro virus, of the genus *Alphavirus* in the family Togaviridae, is a close relative of chikungunya that produces an analogous debilitating arthralgic disease in South America. Mayaro could be endemic in regions across the continent but camouflaged by the unspecific symptoms it shares with other mosquito-borne viruses. First isolated from febrile forest workers in Trinidad in 1954,[118] the etiologic agent of Mayaro fever has been identified in French Guiana, Suriname, Venezuela, Peru, Bolivia, Brazil,[119] and Haiti.[120] The apparent primary vectors, *Haemagogus* mosquitoes, inhabit rural settings, a reason that may justify the relative paucity of cases and inhibited endemicity. Conversely, *Ae. aegypti* mosquitoes have been shown to be competent vectors of Mayaro virus,[121] signifying that an urban-dwelling arthropod could potentially be a vector of this virus on

a global scale. Because Mayaro virus symptoms can resemble those of both chikungunya and dengue, it may be underdiagnosed. While the Mayaro virus has not been linked with fatal human disease like dengue, primary infections are often more debilitating, with loss of productivity for weeks or even months due to severe arthralgia.

Military preventive medicine personnel should also be on guard for the Oropouche virus,[122] the Amazonian cousin of the Mayaro virus, which is spread through *Culex* sp. mosquitos and *Culicoides paraensis* Goeldi midges.[123] Both vectors are known to have a broader distribution[124] than the *Aedes* sp. mosquitoes that carry Zika virus.[125] Lastly, Ross River virus, which was previously thought to be indigenous to Australia and Papua New Guinea by sustaining itself in marsupials,[126] has been documented in Pacific Island travelers. The spread of these new geographic regions suggest the potential for further geographic expansion and global epidemic potential. Collectively, these arboviral pathogens not only infect people via enzootic spillover, but they use humans as amplification hosts and represent a tremendous risk for urbanization.[127] The latest epidemic activity of Zika and chikungunya should underscore the need to consider these diseases in febrile US service members returning from endemic areas and serve as a caution that presumably obscure viruses like Mayaro virus, Oropouche virus, and Ross River virus should not be underestimated as potentially emerging human pathogens.

Conclusion

Vector-borne diseases have been an important cause of morbidity and mortality since the inception of our nation. In line with the general population, military and civilian personnel acquire diseases while on stateside military installations, active missions, and training exercises. Military personnel with occupational duties resulting in extended time outdoors are potentially at an increased risk for VBD transmission. Development and implementation of integrated vector management plans can be useful tools to reduce vector exposure and transmission risk during disease outbreaks, in endemic disease areas, or in the event of emerging VBDs. As identified by the Armed Forces Health Surveillance Center, our military personnel routinely acquire a wide range of VBDs spread through mosquitoes, ticks, triatomines, and other arthropods. Our current review identified the VBDs of greatest public health concern to serviceman taking part in military missions and training in the United States. Vector surveillance, insecticide applications, and personal protection measures are warranted to prevent future infections.

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Blast-Associated Traumatic Brain Injury in the Military as a Potential Trigger for Dementia and Chronic Traumatic Encephalopathy

Jamal Hasoon, MD

Abstract

Traumatic brain injuries (TBIs) resulting from blast exposures have significantly affected US military personnel throughout the world, particularly in Iraq and Afghanistan. From 2000-2016, more than 350,000 military service members were diagnosed with a TBI. Since the majority of blast-related TBIs are classified as mild with no definitive findings on physical examination or diagnostic studies, it is difficult to accurately diagnose individuals inflicted with such injuries. There are likely far more mild TBIs that remain undiagnosed in the military. Traumatic brain injury is a serious public health concern since it can lead to long-term neuropsychiatric changes such as posttraumatic stress disorder and various forms of dementia. Blast-related TBI has also been linked with neuropsychiatric dysfunction that is commonly seen in athletes that have suffered chronic traumatic encephalopathy. Further research is needed to aid in prevention, diagnostic studies, and care of military service members and veterans who have suffered a TBI.

Traumatic Brain Injury in the United States

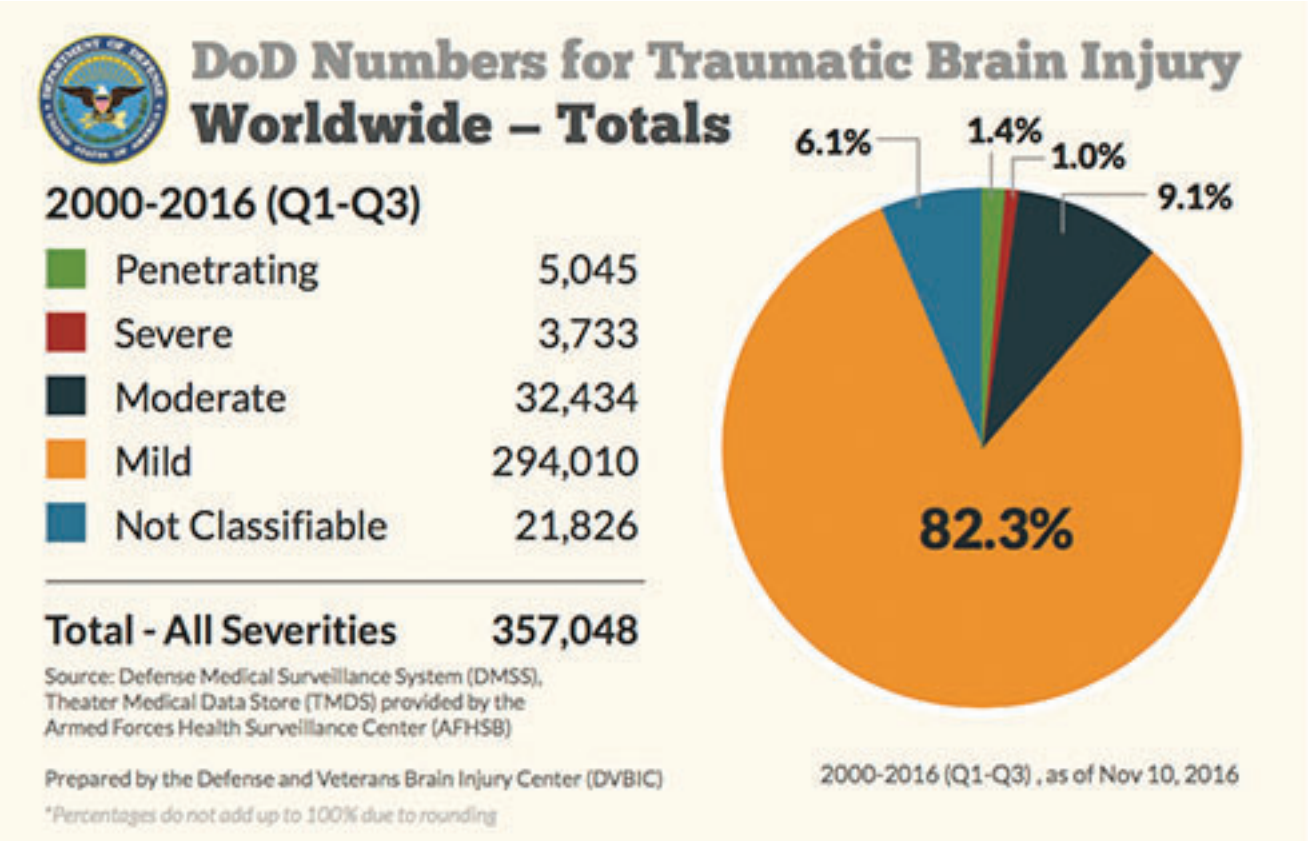
Traumatic brain injury (TBI) is a disruption in brain function or pathology due to an external force that exceeds the protective capacity of the head and causes neuropsychiatric impairment, either permanent or temporary. Complications of TBI include seizures, spasticity, gait abnormalities, agitation, depression, headaches, insomnia, cognitive decline, dementia, gastrointestinal and urogenital complications, and chronic traumatic encephalopathy. In the United States alone, an estimated 1.7 million people suffer a TBI annually, of which 275,000 require hospitalization.[1] Falls and motor vehicle accidents are the major causes of TBI in the United States.[1] Traumatic brain injury is a leading cause of death and disability for individuals between the ages of one and 44 years, and it is estimated that almost 2% of the population currently live with complications and disabilities related to a prior TBI.[2]

Traumatic Brain Injury in the US Military

Traumatic brain injury is a major health concern for the US military. With the widespread use by enemy combatants of improvised explosive devices in recent conflicts, there have been a higher proportion of explosive-related head injuries in comparison to previous wars.[3,4] Data from Operation Enduring Freedom, Operation Iraqi Freedom, and Operation New Dawn show that blast-related injuries are a major cause of TBI for military troops.[5] As illustrated in the Figure, from 2000-2016, there were 357,048 medical diagnoses of traumatic brain injuries in the US military with 82.3% of these injuries classified as mild TBIs.[6] A mild TBI is characterized by the Department of Defense as a confused or disoriented state that lasts less than 24 hours; or loss of consciousness for up to 30 minutes; or memory loss lasting less than 24 hours. Mild TBI is difficult to detect as there is often a lack of any external evidence of damage to the head. A CT scan is generally not indicated for these patients, and if obtained, is often found to be normal.[6] Currently, there are no specific laboratory markers or imaging studies that are routinely performed that can detect any long-lasting damage to these patients.[7] Due to the difficult nature in diagnosing a TBI, there are likely far more mild TBIs that remain undiagnosed in the military.

Traumatic Brain Injury and a History of Neuropsychiatric Dysfunction in War: Shell Shock, Posttraumatic Stress Disorder, and Dementia

The development of neuropsychiatric symptoms in military veterans exposed to high-intensity explosives has been recognized since World War I (WWI). Military troops involved in trench warfare were often barraged with artillery, with many Soldiers developing the neuropsychological impairment known as "shell shock."[8,9] Symptoms of shell shock included fatigue, confusion, nightmares and impaired sight or hearing. Soldiers were often diagnosed once they became unable to function/perform their duties, usually without any obvious cause that could be identified. Since Soldiers often lacked physical signs of disease, they were often accused of cowardice or malingering. It was later recognized that



shell shock shared similar symptoms to posttraumatic stress disorder (PTSD), which would later be recognized during the Vietnam War.

Since the conflicts in Vietnam, Iraq, and Afghanistan, there has been more insight into the neuropsychological dysfunction of military personnel who suffer from PTSD. Similar to the shell shock observed in WWI, PTSD symptoms include disturbing thoughts, flashbacks during trauma-related cues, cognitive and behavioral changes, depression, anxiety, and a higher risk of suicide. Although there is tremendous overlap between PTSD and shell shock in several ways, it is important to acknowledge that patients can also develop PTSD without evidence of TBI or head trauma. Examples include victims of rape, natural disaster survivors, or individuals who have suffered severe psychological stressors. However, evidence shows a TBI alone can be a potential trigger for PTSD as well. A survey of 2,525 US Army Infantry Soldiers deployed to Iraq for one year demonstrated that 44% of Soldiers who suffered a mild TBI with subsequent loss of consciousness met the criteria for PTSD.[10] Another study found blast-related injuries on mice under anesthesia produced PTSD behavioral changes in the absence of psychological stressors.[11] Traumatic brain injury appears to be a very strong risk factor for the development of PTSD. It is also very likely that the majority of Soldiers who suffered from shell shock in WWI would fit the diagnosis of PTSD in modern times.

Traumatic brain injury has also been recognized as a potential risk factor for the development of dementia and neurodegeneration.[12] Individuals who suffer from dementia typically demonstrate a decline in cognitive ability that is often severe enough to interfere with daily life. These individuals may demonstrate memory loss, cognitive decline, and behavioral or psychological changes. A study of US Navy and Marine Corps veterans hospitalized during WWII demonstrated an association between TBI in early to midlife with the development of dementia later in life.[13] Veterans who sustained a TBI that resulted in loss of consciousness or posttraumatic amnesia longer than 30 minutes but less than 24 hours were more than twice as likely to develop dementia.[13] Veterans who sustained a TBI severe enough to cause loss of consciousness or posttraumatic amnesia greater than 24 hours were more than 4 times likely to develop dementia compared to the control counterparts.[13] These findings demonstrate that TBI-related complications may manifest much later in life, and our veterans may actually have a higher risk of developing dementia compared to the civilian population.

Traumatic Brain Injury and Chronic Traumatic Encephalopathy

Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease that is thought to develop secondary to repetitive head trauma, including concussive and subconcussive injuries. The clinical presentation of CTE varies, and often overlaps with other neuropsychological disorders including Alzheimer's, Parkinson's, and PTSD. Patients with CTE often exhibit both cognitive and behavioral impairment including chronic headaches, memory difficultly, poor impulse control, aggression, depression, suicidal tendencies, and also dementia.[14,15] Behavioral changes are typically seen earlier in the disease, and these patients may often be diagnosed with PTSD. Later in the disease process, patients show symptoms such as memory loss and cognitive decline consistent with dementia.

Chronic traumatic encephalopathy is a newly recognized disease with few confirmed diagnoses. The disease is a clinical diagnosis that can only be confirmed postmortem. It is often regarded as a disease that develops from repetitive head trauma in athletes, such as football players and wrestlers, who suffer multiple concussive injuries. However, new data suggests military personnel may be at risk for developing CTE, and a single blast-related TBI may be sufficient to trigger the neuropathologic changes seen in CTE.[16] Brain pathology of postmortem athletes who have suffered repetitive concussive injuries have demonstrated distinct neuropathologic changes that differentiate CTE from other known forms of dementia.[15-17] Comparison of brains from postmortem military veterans exposed to blast injuries demonstrated neuropathology that was indistinguishable from the neuropathology of young athletes with CTE.[16] Furthermore, experiments on rodents exposed to an isolated blast-related injury demonstrated neuropathology similar to that of patients found to have CTE from repetitive head trauma.[16] This study in rodents also showed that TBI from blast-related head injuries resulted in progressive neurodegeneration that continued for more than one year after the initial injury. These studies demonstrate that isolated blast-associated TBIs could trigger the development of CTE-like symptoms and neuropathology.[16]

Comment

Traumatic brain injury is a serious public health concern in both civilian life and in the US military. Today, our military troops have a greater risk of TBIs due to the increased use of improvised explosive devices and subsequent blast-related injuries. Evidence shows that a mild TBI is a risk factor for developing long-term neuropsychiatric conditions such as PTSD, dementia, and even CTE. Since CTE is a newly discovered disease that can currently only be confirmed postmortem, CTE may be overlooked as a differential diagnosis and mistaken for other neuropsychiatric diseases. Chronic traumatic encephalopathy should be considered in patients that have a previous history of a TBI and are being evaluated for a neuropsychiatric disorder. Furthermore, the diagnosis of CTE may better explain the development of symptoms in veterans found to have shell shock, PTSD, or other forms of dementia. Any patient with a history of TBI should be counseled and closely followed to ensure appropriate care and social support. With increasing awareness that military service members are at risk for TBI-related neurodegenerative diseases, further research into diagnostic studies, preventive strategies, and eventually therapeutic interventions is essential for the wellbeing of our veterans.

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Challenges of Practicing Environmental Epidemiology in the US Military

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When service members return to their normal routines following the end of a war, some of them develop illnesses. These may be associated with heredity, personal risk factors such as smoking, normal aging, or the result of their occupational exposures. When someone develops a serious illness, it is common for them to review their past to look for a potential cause. This is human nature and part of the "why did this happen to me?" assessment. For many, their military deployments are likely to be considered. Deployments represent changes to routines such as eating and sleeping and lack of control over daily life, and may also include changes in hygiene and unpleasant exposures to things such as trash burning; old, abandoned warehouses and industrial sites; and sources of radiation. For many, a deployment may be the most unusual and noteworthy experience in their memory. Therefore, it is not uncommon for returning service members to ask their health care providers whether or not the deployment was a factor in their illness. Associations between exposures to burning trash, blowing sand (particulate matter), old chemical weapons, and subsequent illnesses have been considered following Operations Iraqi Freedom, Enduring Freedom, and New Dawn. Generally, few clear associations have emerged. The challenges of practicing environmental epidemiology in a deployed military force are identified and reviewed.

Approaches in Environmental Epidemiology

Epidemiological studies may be conducted to assess potential relationships between exposures during deployment and health outcomes. *Epidemiology* is a field concerned with the identification of factors associated with disease and development of public health measures for disease reduction. *Environmental epidemiology* is the study of the effect on human health of physical, biologic, and chemical factors in the external environment, broadly conceived.[1] It examines associations between exposures and outcomes to determine if an association places a segment of the population at increased risk of certain outcomes, usually a disease. The magnitude of the association measures how much increased risk of an outcome can be associated with the exposure. Avoidance of the exposure should then result in a proportionally lowered risk for the outcome. Thus, one aim of public health is to optimize health by limiting hazardous exposures. There are several different types of studies which may be conducted, the selection of which depends on the study question(s) being addressed, the frequency of the exposure(s), and outcome(s) of interest, as well as the expected strength of association between them, the current state of knowledge related to the topic at hand, issues regarding efficiency and validity, and matters of practicality and ethical considerations.

Cross-sectional Studies

A cross-sectional study attempts to assess exposure and outcome at the same time. It provides a snapshot view (cross-section) of disease patterns in the population of interest while assessing the degree of presumed or documented exposure.[2] The main difficulty with this type of study is that the "snapshot" captures only one moment in time, and by looking at exposure and outcome at the same time, it is not always possible to say with certainty that the exposure *preceded* the outcome. This is a noteworthy limitation of cross-sectional studies due to the fact that in order to draw conclusions about exposure-outcome causation, epidemiologists must be able to first establish correct temporal sequencing. Also, because cross-sectional studies are a snapshot, cases of illness can be counted only if present at the time of the look, which presents another disadvantage of this study design: it tends to identify prevalent cases of long duration and may miss capturing individuals who are lost to follow-up shortly after the outcome is established or acute outcomes among individuals who recover quickly. Advantages of cross-sectional studies include high generalizability and minimal resource requirements, relatively speaking, due to the fact that they can be conducted quickly.

An example of a cross-sectional study would be testing pulmonary function in service members exposed to particulate matter levels in the desert at the time of exposure. The aforementioned limitation would be highlighted if a unit demonstrated some impairment in pulmonary function, as it would not be possible to say for certain that it was due to in-theater particulate matter exposure. It is

possible that some unit members had another type of exposure prior to deployment that influenced their pulmonary function. Individuals who were so affected that they had been evacuated from theater, or who were not onsite at the time of the testing would not be included.

Cohort Studies

Cohort studies assemble an exposed group and an unexposed group, follow them forward in time for the occurrence of health effects, and compare the rates in the 2 groups. This study type is suitable when the at-risk population is well-defined, the disease is common, or the exposure is rare or specific. When the at-risk population is known and there is a specific or rare exposure, it is easy to identify the exposed versus the unexposed. The advantages of this method are that accurate measures of exposure are possible before the outcome occurs, and that multiple outcomes can be monitored. A source of bias in these studies is that the knowledge of exposure status may alter the follow-up and subsequent diagnosis. Other limitations include the fact that many years may pass between exposure and outcome, which makes the study costly, increases the likelihood of losing people to follow-up, and is particularly impractical when looking at rare diseases or diseases with long latency periods, such as cancer. An unexposed population is used to provide a comparison rate for the health effects of interest. However, it is critical that the exposed and unexposed populations differ only with respect to exposure status, something that is not always easily accomplished. To alleviate some of the limitations, *nonconcurrent* or *retrospective cohort* studies are often performed. This type of study reconstructs exposed and unexposed groups based on exposure in the past. These groups are then compared with respect to the rate of the outcomes of interest. The chief advantage is that since exposure occurred in the past, sufficient time for the outcome to develop may have already passed. The disadvantage occurs when exposure is not well quantified at the time of occurrence.[3] For example, to study the effects of burn pit exposure on the respiratory system, health outcomes for individuals identified as having been at base camps with burn pits were compared to individuals who were at base camps without burn pits. One key limitation was that exposures associated with the burn pits had not been well characterized.[4]

Case-control Studies

The case-control method consists of identifying people with the disease of interest (cases), and people without the disease (controls). The unknown variable to be ascertained is the type, frequency, and duration of past exposure(s). Critical to the validity of a case-control study is careful selection of controls, in that they come from the same population as the cases and exposed and unexposed controls have the same likelihood of being selected for inclusion.[5] This type of study is useful when the population at risk is not well-defined, when the disease is rare and the exposure common, and although only one disease can be investigated at a time, *multiple* risk factors can be assessed. The major weakness of this method is that exposure is investigated after disease status is established. This leads to recall bias, where those who are diseased are more likely to remember and report exposures than are those in good health. Furthermore, accuracy can be particularly challenging when conducting case-control studies due to the fact that relevant exposures may have occurred many years prior to data collection, which can be difficult to ascertain and define correctly. To assess the potential association between deployment and the development of specific cancers, one approach would be to compare cancer cases with controls who have no such diagnosis. The main variable to be assessed would be whether or not the cases had a stronger history of deployment.

Factors Which Affect the Success of Studies

Given that these study designs each have strengths and weaknesses, other factors affect the ability of any given study to accomplish what it sets out to do—support or refute an association between an exposure and an outcome. Unfortunately, these limitations are common in environmental epidemiology studies. Illustrating this point, the US Department of Veterans Affairs asked the Institute of Medicine (IOM) to form a committee, its main charge being a determination of the association between exposure to burn pits during deployment in support of Operations Enduring and Iraqi Freedom and subsequent long-term health effects.[6] Although the IOM task was to respond to a specific exposure, the committee's conclusions regarding feasibility and design issues apply on a much broader scale. They identified the major challenges of any epidemiologic study to be both exposure assessment and outcome ascertainment. Regardless of type of study selected, the committee reiterated the elements characteristic of any well-designed epidemiological study, including selection of a relevant study population of adequate size, comprehensive assessment of exposure, careful evaluation of health outcomes, reasonable methods for controlling confounding and minimizing bias, appropriate statistical analyses, and adequate follow-up time.

Magnitude of the Disease Risk

If an exposure is strongly associated with an outcome, following a group of people who are exposed and comparing their incidence of illness with that of a group without the exposure allows one to calculate the excess

risk which can be attributed to the exposure. This is the cohort study design. For example, nonsmoking physicians in England were compared with smoking physicians, and followed over time to count the number of lung cancers in each group. It became clear that cigarette smoking was associated with a risk of lung cancer, perhaps 10 times greater than that seen in nonsmokers.[7] In this study design, accurate information about the magnitude of exposure is collected for all individuals and all individuals should be followed for a period of time sufficient to allow the outcome to occur. If an exposure produces a risk which is 4, 5, or even 10 times greater than in people without the exposure, it is relatively straightforward to sort this out and quantify the risk. In contrast, lung cancer risk from passive smoke exposure is thought to be elevated to less than 2 times that of nonexposed individuals.[8] This small difference is difficult to detect. When an exposure is associated with only a slightly elevated risk, that risk sometimes "blurs into the baseline" upon investigation. This is due to the fact that in order to detect a slightly elevated risk, one must be able to distinguish it from the baseline, or "usual incidence" of the outcome without any exposure. This is the role of control groups. However, small risks require that the studies include large numbers of people so that the study has sufficient statistical power. Since large numbers of service members deployed in the most recent conflict, this may not be a problem, but might be if the outcome is particularly rare. Typically, the increased risk associated with environmental exposures is estimated to be small. It has been estimated that the probable range of increased risk for an outcome due to chronic, low level exposure is less than 2 times that of someone without the exposure.[8] This has implications for the number of people necessary in each group in order to detect a difference. This is especially so when competing exposures such as smoking may lead to a much higher elevation of risk, so the role of smoking would have to be thoroughly assessed.

Magnitude of the Exposure

Detection of effects associated with exposure is also greatly simplified when the magnitude of the exposure is great. Excess numbers of leukemias were easily detected following the exposure to radiation from atomic bombs dropped on Hiroshima and Nagasaki because the magnitude of exposure was so large.[9] High doses of radiation are strongly associated with leukemia as an outcome. In contrast, environmental exposures are classically small—doses lower than those known to produce effects in animal studies. Often the exposures may be chronic low-level exposures, occurring over weeks or months, but which may not have been quantified since they were not recognized.[10] Although some studies ask individuals about whether or not they were exposed, they do not always know. This can lead to *misclassification bias*, where it becomes difficult to categorize individuals as exposed or nonexposed, and thus difficult to make causal interpretations about their outcomes. One study evaluated health outcomes for service members who were present at the time of a sulfur mine fire that burned for several weeks in Iraq.[11] The "exposed" group included individuals within a 50 km radius of the site, based on satellite images of the plume and rosters of units in the area. However, some individuals may not have been present, and it was difficult to assess the level of exposure.

Dose-response Relationship

An axiom of toxicology states, "The dose makes the poison." The implication of this is that the larger the dose, the larger the effect. Associations between exposures and outcomes are strengthened when this relationship can be shown to be logical and uniform. This does not necessarily mean that the greater the exposure, the sicker the individual, but that more outcomes (eg, more cases of cancer) occur in the higher exposure groups as compared with those less exposed. For example, more lung cancers would be expected in groups of individuals who smoke 3 packs of cigarettes a day for 20 years versus one pack a day for 5 years. This introduces again the requirement for accurate, quantitative exposure data. Unfortunately, we can rarely reconstruct individual exposures in deployed settings. For example, the study looking at health outcomes associated with having deployed to a base camp with a burn pit was reviewed by the IOM for their report, Long Term Health Consequences of Exposure to Burn Pits in Iraq and Afghanistan.[4] One of their criticisms was that the study did not identify those with the highest exposures, such as those who guarded the burn pits, compared to those with lesser exposures.[6] However, this information on individuals was not available. In another example, the study of the health outcomes associated with those who were "under the plume" of the sulfur mine fire was not able to distinguish individuals who were at locations with higher levels of exposure versus those at locations which were briefly under the plume.[11] This is because the information on exact daily locations of individuals was not available. Efforts must be made to accurately measure contaminants at the individual level, but having sufficient sampling capabilities at the location of an exposure, particularly an unplanned one, is a daunting task.

Controlling for Confounding

Confounding occurs when an association between exposure and outcome is over or underestimated due to a third factor. It is best explained by example. Studies have shown that smoking causes an increase in pancreatic

cancer. An investigator attempted to ascertain the relationship between coffee consumption and pancreatic cancer. He questioned cases with cancer and controls about coffee consumption and found that cases had a greater history of exposure to coffee. He concluded that coffee consumption was associated with an increased risk of pancreatic cancer. Critics challenged his conclusion by pointing out that those who drink coffee are more likely to smoke as well, and that by not questioning subjects about smoking behaviors and adjusting for it, he was in effect seeing the association between cigarettes and pancreatic cancer, not coffee and pancreatic cancer.[12] Cigarette smoking, the unmeasured "true" risk exposure, was the confounder. In many studies of deployment and health outcomes, individual smoking status is unavailable. Furthermore, the rate of smoking has been shown to increase on deployments. Since outcomes such as respiratory conditions and cancer are strongly associated with smoking, it is an important variable to consider.

Latency Period

The job of the epidemiologist is easier when the time from exposure to outcome is short. This time is called the "latent period." An exposure to high levels of chlorine gas leads quite quickly to respiratory distress. Inferring the association between exposure and outcome is relatively clear. The same is true in that exposure to someone with measles is quickly followed by measles in a susceptible person, and ingestion of contaminated food quickly leads to gastrointestinal illness in those who ingest it. An outbreak or cluster of outcomes is clearly defined, and searches for "exposures" and associations ensue.

Unfortunately, in environmental scenarios, most of these conditions are not met. Cancer outcomes typically have latent periods on the order of decades. This presents difficulty in that the exposure can go unrecognized or forgotten because it does not lead to an acute outcome; and if it eventually leads to an outcome, sufficient time may have elapsed that the individual no longer recalls the exposure, or that the past "dose" or magnitude of the exposure can only be estimated by memory.[10] This, as mentioned, leads to recall bias.

Specificity of the Health Effect

Phocomelia, the congenital absence of the proximal part of a limb, is an uncommon defect in infants. When infants were seen to have this defect in greater than expected numbers, mothers were questioned about the use of medications during pregnancy. It became apparent that infants with the defect were more likely to be born to women who had taken Thalidomide, compared with women who had not.[13] In this instance, the outcome was specific enough to allow for the assembly of a group of these mothers to compare with mothers who had normal outcomes, and compare their prenatal exposures. Another classic example was when occupational health physicians noticed an increased incidence of an unusual liver cancer called angiosarcoma in certain workers and determined that they had an increased history of exposure to vinyl chloride. The key was that the outcome was one with few other causes. Legionnaire's disease was discovered and described because an unusual pneumonia developed with high frequency in a group of men at a convention in a single hotel, and was linked to the air conditioning system.[14] Hantavirus illness in the southwestern United States was characterized after a physician became alarmed that 2 cases of fatal respiratory illness occurred one week apart. Suspicion was raised that there was some common exposure.[15]

When the effect of a low-level hazardous exposure is undefined, those exposed may attribute multiple health effects from varied causes to the exposure. Individuals who have registered for the burn pit registry have reported diverse medical conditions.[16] This is likely because they are reporting all health conditions that they may have. Without a comparison group, it is difficult to determine which effects might be associated with an exposure. The lack of a comparison group made it difficult to draw conclusions from clinical evaluations of redeployed service members who were veterans of the first Gulf War, when there was a concern about potential health effects resulting from their exposures in 1991.[17]

Evaluating the relationship between health effects that are known but nonspecific and environmental exposures can also be challenging. Exposures to solvents, which are metabolized in the liver, can lead to elevations of liver enzymes in the blood, but so can a host of other chemicals including alcohol and acetaminophen (*confounding*). Respiratory symptoms may follow exposure to burn pit smoke, dust storms, or other factors. The investigations which attempt to relate exposures to effects must take into account other possible exposures and question the individuals about these and control for these in the data analysis.

Measurement of Health Effect

When studying health effects, it is important to consider how the effect is measured. In some studies, health effects were measured as symptoms reported by the study participants. Reporting bias may occur when people with concerns report their symptoms.[1] Studies which use databases that report disease by diagnostic codes are subject to problems with miscoding. Analyses of healthcare encounter diagnostic codes following redeployment

are subject to unique limitations. For example, acute changes in health status during deployment due to exposures in theater could be missed. Likewise, changes in health status that occur over the long-term could be delayed beyond the available follow-up period and are therefore unobservable. This would be demonstrated, and particularly problematic, in evaluations of cancers with longer latency periods. Using medical encounter diagnostic codes to define cases may result in false positives, capturing as cases those individuals for whom health care providers entered codes for conditions that were being considered but not yet rejected or confirmed.

Multiple Hypothesis Testing

Since health effects are not always known, a common strategy employed in investigations is to question patients about multiple health effects. Questionnaires may ask about respiratory symptoms, gastrointestinal symptoms, neurologic symptoms, and vague, nonspecific symptoms. This can result in the calculation of a significantly high disease rate due to chance alone. Statistical "rules" for study precision generally allow for 5% error due to chance. This means that if 100 symptoms were asked about, 5 of the 100 could appear to be increased due to chance alone.[3]

Power and Sample Size

The power of a study is its statistical ability to detect a difference between 2 groups if it truly exists (for example, an elevated cancer rate) between the exposed and the unexposed. The power of a study is intimately related to the sample size, which is the number of people in each of the 2 groups. It is also related to the prevalence of the disease of interest in the population. In order to detect significant patterns, rare diseases such as cancer in relatively young populations, are studied using a case-control approach, or require large populations. More common diseases can be studied in smaller populations. However, to the extent that multiple causes (or exposures) are involved, as they are with most chronic diseases, larger populations are generally required in order to obtain significant results in studies of more common diseases as well. Refining the measures of diseases and the assessment of exposure can improve the power of a study to detect an association.

Conclusions

Some situations lend themselves to an epidemiologic investigation of the strength of association. The strength of an association, while not proof of causation between the exposure and the outcome, gives one confidence that the two may be related. These situations occur when the exposure is large and leads to a greatly increased risk of a specific outcome. The exposure must have occurred before the outcome, and ideally, the outcome follows soon after the exposure. Ideally, current and past exposures to chemicals would be precisely quantified, and testable hypotheses of specific health effects would be generated. An accurate assessment of adverse health effects in exposed and unexposed populations would be made, and account would be taken of all potential confounders. The sample size would be adequate, and irrelevant hypothesis would not be included. Because all studies have limitations, these conditions are never achieved. However, practitioners of military preventive medicine must continue to work to quickly identify potentially hazardous exposures, precisely measure the exposures, and document those who were exposed and the extent of their exposures to facilitate these evaluations.

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Indirect Military Occupational Lead Exposure to Children at Home: A Case Report

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Historically, lead has been an important commodity for industry, including metal alloys, cosmetics, medicinal preparations, and paint pigments. Concomitantly, lead poisoning has been well described since early Greek history (2nd century BC), and, more recently (19th century), adverse health outcomes were well defined in workers and children.[1] By the 20th century, more in-depth studies revealed the cumulative toxic effects of lead exposure, especially among the vulnerable population of children.[1,2] Today in the United States, there are an estimated 4 million households with potential childhood lead exposures, and nearly half a million US children (ages 1-5 years) are known to have blood lead levels (BLLs) above 5 µg/dL, the recommended public health action level of the Centers for Disease Control and Prevention (CDC).[3,4] Although BLLs have demonstrated an overall dramatic decline in the past few decades, lead exposures among high risk populations (low-income, African American, urban, and rural mining communities or developing countries) still exist.[5]

Interestingly, lead toxicity can affect nearly every body system. Because subclinical presentations are common among lead exposed patients, this public health risk frequently goes unrecognized. Throughout the world, children remain a persistent at-risk population due to prevalent hand to mouth behavior, close proximity to lead exposures, and physiologic higher gastrointestinal absorption per unit body weight and increased respiratory rates.[5] Regardless of the route of entry, the toxic effects of lead exposures remain uniform.[1] Specifically, lead binds to erythrocytes which can then transfer to multiple organs (brain, liver, kidneys, spleen, muscles, lungs, and heart), and the majority of the lead can absorb onto bones and teeth after several weeks.[1] Further, exposures to lead at low levels (BLL less than 10 µg/dL) in childhood has also been shown to contribute to deficits in central nervous system functioning and cognition.[6,7] Children may exhibit "pica," a unique manifestation of elevated BLL which involve abnormal eating habits with soil or paint chips.[5]

Within a child's environment, main lead exposures derive from diet, contaminated soil, paint in homes built before 1978, water pumped through leaded pipes, imported clay pots, certain consumer products such as candies, make-up, jewelry, certain imported home remedies, electronics, and toys.[1,3,5] Indirect exposures from adult recreational activities such as take-home lead dust from firing ranges have also been documented.[8,9] More importantly, certain parental occupations (ship yards, manufacturers, and handlers in lead alloys including batteries and ammunition) can also pose considerable risk to the child.[10] Therefore, occupational lead exposures can indirectly expose the employees' families and children under the age of 6 years by take-home lead dust on clothes, boots, hands, and face.[3,11-13]

In 1991, the CDC responded to this growing public health concern by issuing new guidelines.[1,3] This included an emphasis on the child's environmental history, parent education, and follow-up for children with BLLs of 10 µg/dL or greater.[1] More recently, the American Academy of Pediatrics emphasized lead screening history in a 2005 guidance statement, and the CDC recommended initial and follow-up screening (within 1 to 3 months) of pregnant and lactating women, neonates, and infants of women with BLLs of 5 µg/dL or greater.[4] In May 2012, the Advisory Committee on Childhood Lead Poisoning Prevention recommended the use of a reference range for an elevated BLL based on the growing body of literature that levels less than 10 µg/dL do indeed adversely affect children.[1,3-5,11,14] Thus, the current value (5 µg/dL) identifies children with elevated BLLs.[3,15] Understanding the complex toxic effects of lead exposures among children underscores the importance of primary prevention and vigilant surveillance and screening.

Case Study

An 18-month-old female was seen in the pediatric clinic for complaints of "pica." She was known to eat unusual items such as cat food, wood chips from the stair railings, chalk, and paint off the walls. The birth history revealed a normal term delivery via emergency C-section with breech presentation without complications or hospitalizations. The mother of the child denied any past surgical history, but the child's past medical history was significant for chronic constipation treated with diet and medications. Also, her mother expressed concerns of child's development delays in speech, but she denies any

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neurologic deficits; skin manifestations; bone, teeth, or nail deformities. Family history was unremarkable and there are no siblings or other family members in the living environment whether foreign or domestic. There was no history of travel or visitors from outside the country.

Since September, the family lived on the military installation with well-developed housing (post-1980s) and no history of lead in paint, soil, or water systems. The child was formula fed with the introduction to whole milk at 9 months old, and currently she is solely on whole milk with a normal diet of solid foods. Her mother denied any foreign-made toys, ceramics, or products that would contain lead. The mother consistently was a "stay at home" mom. Her recreational activities included crochet and bow hunting, but she denied any participation of the latter since they moved to the current duty station. At the time, The child's father was a Soldier in the US Army; his occupation (Field Artillery) was in small arms munitions and Howitzers, but mostly he worked in the motor pool. The mother stated that he worked with lead products and had coveralls at the workplace; however, he did not change his boots from work to home. Although he washed his hands upon entering the home, he denied taking any showers prior. According to the mother, his boots and duty uniform was typically located in a common place, the laundry room, and were not segregated. The child has had full access of the contaminated clothing, and was known to play with the work boots constantly. The father's recreational activities included hunting with a rifle, but he has denied any shooting range activities since arrival at this post. Both father and mother drove separate cars, and the child only traveled with the mother in her vehicle. The mother denied any history of cross contamination between the car seats.

A review of the medical history showed the 12-month visit had normal results on the Ages and Stages Questionnaire (ASQ) and lead screening. Although the subsequent 15-month visit still revealed a negative lead screen, the mother reported significant chronic constipation with decreased ASQ scores in fine motor skills. All growth parameters were appropriate for age. A follow-up visit with the primary care provider revealed the diagnosis of "pica," although there was still a negative lead screening. Laboratory results included a normal hematocrit/hemoglobin (12.5/36.8) for her age group. However, serum lead levels were elevated (6 µg/dL). Public health officials were notified and an evaluation of the work and home environment was conducted.

The installation Industrial Hygiene (IH) Department conducted a home visit using Lead Check swabs (3M, St Paul, MN). Initial qualitative findings revealed positive lead contamination only on the father's uniform and boots, with negative results in other high risk areas within the home. The IH team then performed a health hazard survey of the father's workplace (Field Artillery motor pool) including lead surface sampling. The workers in the motor pool provided routine wheeled vehicle and armaments maintenance. Although lead exposure can occur both via ingestion and inhalation, airborne levels of lead in the motor pool were anticipated to be very low risk, whereas the presence of residual lead on Field Artillery equipment from firing activities could lead to exposure through eating, drinking, and tobacco use. One important concern was take-home lead dust on uniforms and boots which could contaminate privately owned vehicles and home environments. All quantitative samples were collected using Ghost Wipes with a 100 cm^2 template. The samples were then sent for analysis to the US Army Public Health Command by ASTM Method E1613.* The samples were reanalyzed by the laboratory using Environmental Protection Agency (EPA) Method 200.8† to better quantify lead at the lower levels. To note, the IH Group at Brookhaven National Laboratory has established industrial guidelines for lead dust based on standards developed by the Department of Housing and Urban Development and the EPA. Based on these guidelines, dust wipe samples standards included 40 µg/ft^2 for nonlead operational areas. Final sampling above the 40 µg/ft^2 cutoff revealed positive for the Soldier's front of uniform pants, top and bottom of boots, and front of the mechanic coveralls, shown in the Table.

Results of analyses of the home and workplace sampling for possible lead contamination.

Item	Lead Concentration (µg/ft^2)	Positive (>40 µg/ft^2)
Front of uniform pants	69.7	Yes
Top and bottom of boots	82.7	Yes
Mechanic desk area	<LOQ	No
Mechanic work table	<LOQ	No
Top of mechanic uniform	23.2	No
Mechanic desk area	<LOQ	No
Front of mechanic coveralls	45.5	Yes

LOQ indicates limit of quantitation.

Primary exposures to lead were associated with routing maintenance activities. The lead sampling indicated the presence of lead in quantities sufficient to warrant precautionary procedures to minimize risk of lead exposure to Soldiers and family members. These procedures included enforcing the use of dedicated coveralls in the workplace and changing areas, not wearing uniform boots into the home environment with segregation of any potentially contaminated clothing items transported

* https://www.astm.org/Standards/E1613.htm

† https://www.epa.gov/sites/production/files/2015-08/documents/method_200-8_rev_5-4_1994.pdf

in a plastic bag, prohibited eating, drinking, or using tobacco products in the maintenance section, and integrated washing areas for hands, face, and shower activities before interacting with the home environment.

Comment

Indirect occupational exposure to lead in the home environment is a military-relevant topic with enormous public health risks. In the mid-1900s, certain US policies were enacted to protect children from environmental and industrial lead exposures which led to the sharp reduction in children's BLL (1976 thru 1989).[2,16] Thus, legislative actions have proven the effectiveness of public health interventions in light of emerging research that elevated BLLs in children can cause serious adverse outcomes.[17-19] Although the follow-up lead levels, particularly for this child, revealed a definitive decline below threshold limits of lead levels after workplace interventions, improvements in early detection, surveillance, and prevention are still needed to protect any long term effects to a child's development in the military home. Future studies in population risks in the environmental and occupational setting among the military are warranted.

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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 Benjamin Cameron Shaffer was selected as the best submission of the 2016 competition.

Enduring for the Patients' Sake: The Emotional Experiences and Endurance of American Ambulance Drivers in World War I

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I. Introduction

The Canadian poet and adventurer Robert W. Service could not have imagined the emotional journey he was about to embark on when he enlisted in an "American Ambulance Unit" in 1915 in Paris. Though unenthusiastic about combat, he did not mind taking "a chance of being killed" while helping others. Only a few weeks after enlisting in the ambulance service, the middle-aged poet found himself "driving an ambulance under fire" in Flanders. Despite the explosions all around him and the wounded men in the rear of his car, he remarked that the thrill of being under fire had been pleasurable. However, after his thrill seeking brought him to a station closer to the front lines, his sense of adventure was extinguished by the horrors of war. After noting a French soldier died in the rear of his ambulance, he wrote that "…I prefer to forget that…Those who went through the horrors of war never want to talk about it."[1]

Despite Service's contention that he did not want to talk about war's horrors, his writing became his venue for exploring his emotional reactions to combat. What is most striking about his autobiographical writing is the fact that Service developed both adaptive and idealistic endurance strategies to survive the horrors of war. On the one hand, he wrote that "…we became very callous, grumbling if brains or guts soiled the car. We were sorry for the poor devils but saw so many they were like shadows."[2] In essence, he learned to treat his wounded charges as 'shadows' in order to avoid absorbing the emotional impact of carrying the dying on a daily basis. Conversely, Service also found solace in his mission. In his 1921 compilation of poetry and fictional prose, *Ballads of a Bohemian*, Service's narrator is an ambulance driver like the author. The protagonist notes that "My only consolation is that the war must soon be over, and that I will have helped."[3]

Thousands of ambulance drivers developed similar survival strategies as they volunteered in a war-torn Europe. Droves of Americans volunteered in varying capacities in different fronts in the years preceding their country's declaration of war on Germany in 1917. Individual reasons for this massive volunteerism varied widely. Historian George Plimpton categorizes volunteer ambulance drivers in three groups: humanitarians, pacifists, and adventure seekers.[4] Historian Arlen J. Hansen asserts that many of these 3,500 documented volunteer ambulance drivers that enlisted before the American entry in the war were Ivy Leaguers who joined European armies out of a romantic affection for France, or even to be closer to family and friends in Europe. He asserts this volunteer fervor later spread throughout the middle class as well.[5]

Whatever their reasons for enlisting before 1917, American ambulanciers faced untold horrors in what was a

new kind of war. In addition to the more than 8 million deaths in the conflict, 21 million soldiers were wounded. On average, about 6,000 men died every day.[6] While common soldiers experienced the carnage of machine guns and gas attacks first hand, the men evacuating them from the field also dealt with shellfire, the stress of rescuing casualties and the shocking aftermath of the bloodshed.

In the face of carnage, American ambulance drivers had disparate emotional and psychological reactions to their hellish surroundings. Ana Carden-Coyne, a historian of the British medical services, argues that morbid wounds "deeply affected even the most hardened surgeons who contemplated their patients' grim futures…" Despite the horrors British medical officials faced, Carden-Coyne asserts that the only way they could deal with the suffering around them was "simply to get on with their jobs, allowing work to subsume their feelings."[7] Despite her focus on British medics, Carden-Coyne's argument for the persistence of British doctors and nurses easily applies to the thousands of American ambulance drivers in the Allied armies. The concept of forbearance through wartime trauma is evident in scores of published journals, poems, memoirs, and even autobiographical novels written by American medics both during and after the conflict. Ultimately, American ambulance drivers in the First World War not only faced extreme emotional trauma and physical dangers, but developed adaptive and idealistic forbearance strategies to survive their ordeal. Whereas ambulanciers learned to inure themselves to the horrors of war, they also developed positive survival strategies which centered on the critical value of their work in saving the wounded and dying.

II. Historiography of "Wartime Endurance" and Methodology

This article is inspired by two current trends in the historiography of the First World War: the study of "troop endurance," and the study of the cultural and emotional aspects of military medicine during the Great War. A recent anthology produced by World War I scholars in New Zealand—*Endurance and the First World War: Experiences and Legacies in New Zealand and Australia*—is a concise study on the topic of 'endurance' during the Great War.[8] In the volume, historian David Monger asserts that endurance's presence has "occupied a quiet, yet underlying and central place in the historiography of" the war.[9] Out of the several authors Monger lists that have directly and indirectly covered endurance, Stephen Loveridge—author of the article "Seeing Trauma as Sacrifice: The Link between 'Sentimental Equipment' and Endurance in New Zealand's War Effort,"—Michael Roper—author of *The Secret Battle: Emotional Survival in the Great War*—and Alexander Watson—author of *Enduring the Great War: Combat, Morale and Collapse in the German and British Armies, 1914-1919*—have all particularly inspired this current study on the endurance of medics. Watson's method of tracing battlefield endurance among troops amidst a sea of calamities will be repeated here.[10]

At the same time, this article is also influenced by current studies on the emotional world of Allied medics[11] during World War I—particularly the work of Christine Hallett, author of *Containing Trauma: Nursing Work in the First World War.* While Hallett's stated purpose is to broadly "uncover the hidden world of nursing work" in the war, her contention that nurses were forced to "contain their own emotions" lest they fail to be "of much use to [their] patients" is particularly relevant to this study.[12] Finally, Arlen J. Hansen's suggestion that ambulance drivers' resilience was partially based in the "intrinsic worthiness" of their job will be explored throughout this study.[13] Out of all the scholarly work consulted in this study, Hansen comes to the closest to actually analyzing the endurance strategies of American ambulance drivers. All in all, this study seeks to extend groundbreaking arguments for World War I endurance to ambulanciers on the front line.

III. The Life of an Ambulancier

Ambulanciers were among the first Americans involved in the Great War. These idealistic young men mostly came from Ivy League universities, and included future authors of the Lost Generation such as Ernest Hemingway and John Dos Passos. These men typically served in one of three ambulance corps: the "Harjes Formation" of banker H. Herman Harjes, Richard Norton's "Anglo-American Volunteer Motor-Ambulance Corps"—a unit that later merged with Harjes's formation under the control of the American Red Cross—and A. Piatt Andrew's "American Ambulance Field Service."[14] Although there are incomplete records of American women who served as ambulance drivers, the preponderance of drivers were men.[15]

While these sections served with distinction throughout the conflict in France, Belgium, and even eastern regions such as Greece, the structure of most of these organizations shifted dramatically when the United States entered the war. The United States Army Ambulance Service—what Jeffrey C. Larrabee calls the "vanguard of the American army"—arrived in France before most American troops in the summer of 1917. While this organization was created by Washington to help the French Army, the American Expeditionary Force's ambulance system was separate, and not nearly as effective as the

USAAS.[16] Despite heavy protest from former section leaders—especially from Richard Norton—the three main ambulance groups were absorbed by the USAAS. One of the biggest complaints from the formerly-civilian drivers who continued on was the destruction of the "volunteer spirit" that came with army enlistment.[17]

The experiences and trials of the men in any ambulance section varied year by year, and from station to station. Wherever they served, they excelled in their primary task: quickly transporting the wounded to aid stations and evacuation centers. One unit of Andrew's American Field Service (formerly the American Ambulance Field Service) was reported to have evacuated over 56,000 wounded troops between 1915 and the war's end in 1918.[18] Though experiences and procedures in hospital transport obviously varied from location to location, Hansen maintains the American volunteers typically had two tasks: "jitney duty" and front line duty. Initially, these volunteers were permitted to take part only in "jitney" transport, which involved the transport of *blessés* (the French term for wounded soldiers) from hospital to hospital or from transport trains and ships to hospitals. As the war progressed, ambulance drivers began "working the front lines." After a soldier was wounded, he was typically taken by his comrades to a first aid post, and from there stretcher-bearers carried him to an "advanced dressing" clinic where doctors performed basic operations. From here, ambulance drivers carried the *blessés* to triage stations and evacuation hospitals.[19]

Ambulancier James R. McConnell gives a snapshot of the dangerous duties these drivers had on an average day during the Lorraine Offensive in 1915. McConnell wrote: "It is difficult to take any one day's work and describe it...for with us all days are so different...but the action and experiences, which add the color, are never alike..." He then laid out a prototypical schedule, beginning with breakfast and a discussion of German shrapnel and shell that came in the night before. Then the men take up their various duties. While some men are held in reserve, others evacuate the wounded from various posts both near and far. At the *poste de secours* (dressing station), McConnell would pick up French *blessés*. Artillery fire was intermittent at these posts, with light damage from shrapnel and heavy casualties from shells. He wrote that his section had several close calls despite not having lost any drivers yet.

The last of the *postes* was "in the very line of fire," where drivers would have to watch out for craters in the road and frequently take cover in "bomb-proofs." With the wounded in the car on the trip to the evacuation hospital, drivers started to take notice of shell damage, but never dwelled too long on the risks they took.[20] Despite the intensity of these duties, Hansen contends that relatively "little of the drivers' time was spent under fire at the front." Young ambulance drivers spent upwards of 75% of their time 'En Repos.' Activities while 'En Repos' ranged from car repair to adventuring around Europe. Another favorite activity was writing letters or in journals.[21] While the drivers spent most of their time off the battlefield, it was during these short missions that drivers faced many of the same anxieties and emotional crises as the soldiers in the trenches.

V. Emotions and Sources

In the wake of constant personal danger, the emotional reactions of American ambulance drivers to their environments varied by personal experience and location. Here it will be necessary to address a few major themes in emotional studies of men during the war and of emotions in wartime memoirs. Perhaps the first consideration that must be made is that the ambulanciers grew up in a society that valued stoicism and a "stiff upper lip" when undergoing trauma. Ana Carden-Coye writes that physical "endurance was built into military and civilian codes of masculinity—whether located in the British 'stiff upper-lip' or new world images of American, Australian, and Canadian frontiersmen whose hardy bodies had supposedly toiled with stoic silence." She also notes that among individual British doctors, "triumph over adversity was the main narrative of British medical and surgical innovation."[22] While Carden-Coyne focuses on physical pain, the same stoicism can be applied to men's emotions as well.

Carden-Coyne and other historians have also noted the ability of soldiers to share their emotional responses to horrors in various capacities. In her study on British soldiers and masculinity during the war, Jessica Meyer argues that while British soldiers' writings rarely "comment directly on masculinity as a specific aspect of identity," their accounts reflect on how they understood themselves to be men, both physically and emotionally."[23] While this article does not extensively examine the gendered experiences of ambulanciers, it is important to note how reactions to combat could play into their perceptions of manhood—and ultimately their will to persevere through trauma.

Michael Roper contends that although British soldiers experienced a multitude of emotions, fear and dread were constantly on the mind of most participants. Roper writes that in response, these men were caught between unprocessed emotions and the aforementioned rule of the "stiff upper lip." As we will see, the emotional responses of medics to combat were also very complex

and largely rooted in fear. In terms of sources, Roper warns that retrospective memoirs "are generally more reflective about the emotional experience of the war" than letters or journals.[24] Some of the following accounts are postwar memoirs, but the raw emotional experiences recorded in the memoirs often match the more contemporary accounts.

One problem that does arise with wartime publications is the issue of subjectivity and writing for an audience. Arlen J. Hansen notes the compilations, *With the American Ambulance Service in France*[25] and *Friends of France*, were both recruitment books inspired by the administration of the American Field Service. This genre tended to "make ambulance work sound gloriously exciting and grandly humanitarian" for recruitment and funding purposes.[26] Nevertheless, these sources provide valuable insights into the emotional journeys of ambulanciers. Authors such as Jessica Meyer have examined the emotional world of soldiers categorically in chapters with titles such as "Fear," "Discomfort," etc. Some of these emotional categories will be repeated here. Broadly speaking, some of the most intense emotions ambulance drivers experienced during their service included: intense fear, exhaustion, and severe emotional trauma.

Fear

Fear is one of the most primal and intense emotions that ambulance drivers, doctors, nurses, civilians and soldiers experienced together. The closer to the front an individual was, the more intense their record of fear will be. Here it is important to distinguish between 'fear' and 'anxiety' in battle. According to military psychologist and Vietnam veteran Walter F. McDermott, fear comes from an "external threat" (i.e. a foe). However, after the battle is over, anxiety—an internal form of fear—arises from the memories of combat. McDermott contends that Post Traumatic Stress Disorder, though partially psychological, often emerges from the body's "stress response system continually being overstimulated," and forms of 'anxiety' often emerge as symptoms.[27]

Fear under fire is clearly seen in Robert Whitney Imbrie's 1918 memoir, *Behind the Wheel of a War Ambulance*. Imbrie joined Andrew's AFS at the end of 1915, when his section joined the French Army at Beauvais. Imbrie's first experience with shell fire came in the early winter of 1916 in the village of Julzy. In a journal entry from 10 February, he wrote that "I am unable to...describe my sensations and I question whether a trained psychologist would be much better off. There is something disturbing about shell fire...I do not believe I was especially frightened; my feelings were more of curiosity." He later wrote that "Perhaps I was not frightened by those first shells...but as time went on, and I saw the awful, destructive power of shell fire...the realization of their terribleness became mine." Even after a "year and a half of war" he was "no more reconciled to shellfire than at first," and did not think one could become inured to a fear that grew worse each time.[28]

Fear was also recorded by ambulance drivers after the United States entered the war in 1917. In his introduction to the diary of USAAS driver Guy Emerson Bowerman, Jr., historian Mark C. Carnes notes that Bowerman had evolving notions of fear and courage. Carnes contends that Bowerman wanted to appear brave before his comrades. On the other hand, Bowerman—like Imbrie—came to realize no one could become fully inured to German shells constantly flying in.[29] For instance, in March of 1918 he reported boldly listening to his Victrola record player while "waiting either for a shell to hit" his house or a "call to come." He also remarked that it was niceto know the Germans might be as "badly scared" of French shells as he was of German artillery.[30] That Bowerman could attest to passively waiting for shells while also being mortified can be substantiated by a postwar attempt at writing a fictional autobiography, in which he declared that true bravery is "being afraid but carrying on just the same."[31] In essence, no amount of exposure to shells could completely eradicate fear. Perhaps some level of fear of shell fire was healthy in order to ensure self-preservation, but intense fear could have psychological ramifications.

Fatigue and Mental Disturbances from War's Horrors

Aside from fear, battle fatigue was a frequent companion of ambulance drivers. According to Jessica Meyer, in the case of British soldiers' postwar memoirs, "exhaustion remained a central memory of trench life for most men." Not only did fatigue "[sap] men's morale," but even "undermined memoirists' perceptions of themselves as men both physically and emotionally."[32] Sleeplessness was a major factor in fatigue. AFS driver Graham Carey, one of the first American volunteers to work as a driver at the Ambulance Military Hospital at Neuilly, France, complained of the lack of sleep in a letter to his mother. He wrote: "At four I was so sleepy that I got into a railway carriage that we have on a siding there and immediately went sound asleep. At six-thirty, however, Richardson and I were waked up and given two sitting cases" to transport.[33] As one could expect, the rampant sleeplessness drivers faced led to extreme mental fatigue. While taking a break from the front in Paris, AFS driver Phillip Sidney Rice described feeling too tired and dejected to even regret not going out with his friends. He was particularly upset he was too poor to afford cigarettes, the only remedy he had to the "nerve-racking strain of war."[34]

Exposure to the horrors of war was the primary culprit behind this "nerve-wracking" strain. According to Michael Roper, many soldiers who were exposed to the war's horrors "suffered from periods of what has been called 'battle stress,' although never becoming incapacitated to the point where they were withdrawn from the line."[35] More often than not, ambulance drivers also suffered emotional strain without being diagnosed as a "shell shock" case. Historian Alexander Watson notes that "hunger, homesickness, exhaustion and continual danger" were common amongst men in the trenches, alongside a growing sense of the "purposelessness of combat." However, Watson later argues that the most psychologically damaging aspect of trench warfare was artillery fire. This danger, as we have seen, was common for ambulance drivers.[36]

A driver's most obvious exposure to the horrors of war was through the transport of wounded men. In a poem by driver Emery Pottle, the narrator tries to comfort a wounded man in his ambulance: "*Courage, mon brave*! We're almost there!"/ God, how the fellow groans—/ And you'd give your heart to ease the jolt/ Of the ambulance over the stones." The driver laments that only God knew how he would "go on, through the dreadful night," and notices his charge had died. He could not wake him. Towards the end of the poem, he laments: "it's just another *poilu* [French soldier] that's dead;/You've hauled them every day/Till your soul has ceased to wonder and weep/ At war's wild, wanton play."[37] The narrator's horror can be seen in his attempts to wake up the wounded *blessé* and admission that he wept when first exposed to the carnage.

Perhaps one of the most well-known results of emotional trauma during the Great War was the phenomenon of shell shock. The term, created in 1914 by British psychologist Charles Meyers, was used to cover a wide range of disturbing physical and emotional symptoms after combat (i.e. vomiting and vivid nightmares). By 1916, the term "shell shock" was dually applied to two different sets of symptoms: physical effects from the shell impact and "hysteria" caused by the horrors of combat. The nature of the disease was so controversial that doctors vehemently disagreed whether physical or emotional trauma led to shell shock.[38] It was not until the 1980s that doctors realized the permanent emotional effects combat had on soldiers and formulated the idea of post-traumatic stress disorder.[39]

Ambulance drivers were vulnerable to shell shock as well, Phillip Sidney Rice being a prime example. After one particularly exhausting mission in Verdun in 1917, Rice's commander sent him to Paris for treatment for "battle fatigue."[40] In his memoir, Rice noted that he was not alone. During the battle he noticed worn out stretcher-bearers with their "burdens" and that his own face "seemed to pinch at the cheek bones" with fatigue. He wrote that a "couple of our men had taken sick—had broken down under the strain and been sent in to Paris." His symptoms had been worse than theirs, however. Towards the end of the battle he noticed he was "playing out very rapidly," was unable to relax and could not sleep. After his mission, he awoke in the middle of the night because of a "terrible nightmare" in which he "went through the whole experience" of battle again.[41]

VII. Endurance

To survive innumerable tribulations, ambulanciers were forced to develop mental endurance strategies. Despite some similarities in coping techniques, soldiers and ambulanciers typically developed emotional survival strategies particular to their jobs. For instance, Hansen makes the case that American ambulanciers were both "in and out of the war." They experienced the mangled bodies of their charges and dangerous enemy fire, but also had the ability to drive away from the battlefield—a "double perspective" that alleviated some of their emotional trauma. Hansen contends that the drivers' belief in the worthiness of their jobs prevented them from falling into despair. However, Hansen goes too far in asserting that drivers rarely resorted to fatalism like their comrades in the trenches, and that they rarely succumbed to self-doubt. As we have seen, drivers consistently doubted their ability to persevere through trials.[42] Hansen also notes that ambulance drivers found a sense of strength in the perceived hardiness of their French allies.[43] Conversely, drivers such as Service developed a certain callousness to the war's horrors in order to survive their jobs. In the following examination, these two types of endurance will be categorized as adaptive and idealistic endurance.

Adaptive Endurance

Alexander Watson notes how British and German soldiers were able to inure themselves to shell fire through constant exposure. On the other hand, to survive, soldiers had to "learn to judge risk without being overwhelmed by it" and to make judgements based on their chances of survival.[44] Phillip Sidney Rice—the aforementioned ambulance driver who suffered shell shock—certainly never learned to get used to shelling, but he was able to evaluate the benefits and consequences of enduring a trial as opposed to withdrawing in fear. During a bombardment in Verdun in September of 1917, Rice reasoned with himself that "if I do go on and am hit, the agony will be over within a few minutes, but if I

turn back, the agony will be with me the rest of my life. So I put on my gas mask and drove on."[45] A sense of shame and duty forced him to keep driving.

Such inurement strategies were not universally effective. Norton-Harjes driver Edward R. Coyle also initially adopted an adaptive approach to overcoming his fear of shell fire. Coyle wrote that "…conditions are met in a more or less matter-of-fact way when one is continually forced to accept them. Life seems a matter of fate and little attention is paid to bursting shells."[46] According to Coyle, drivers had to passively accept the harsh reality of shelling in order to carry on with their tasks. Despite this initial adaptive approach, Coyle was ultimately crippled by fear during one particularly heavy bombardment in Verdun.[47]

While Coyle realized the futility in questioning the presence of shelling in his routine, other drivers also learned to develop a 'thick skin' to overcome their fear of artillery. During a bombardment in the summer of 1915, AFS driver Leslie Buswell was requested to transport three wounded soldiers. Buswell wrote that he "had to grin and bear it, but it is a horrid feeling to have to go out into a little street where shells are falling regularly... and run a few yards down the street to a poste de secours where a shell has just landed and another is due any moment."[48] Just as with the others, Buswell realized the inevitability of German shells, and forced himself to carry on out of necessity rather than desire.

Ambulanciers also had to harden themselves to the constant presence of death and disfigured corpses. Joshua G. B. Campbell wrote about his unit's emotional response when hospital trains came in with gas victims for the first time. These trains came in at the same time as "a hail of shrapnel," and tested the men's emotions severely despite the fact that in "months of [his company's] work [they] had become so accustomed to dreadful sights and to suffering as to be little affected by them."[49] Although his company became emotional when seeing gas victims, he did admit some level of adaptation to the horrors of war in Flanders fields.

Idealistic Endurance

Despite the prevalence of adaptive coping strategies, ambulanciers did develop positive endurance strategies. New Zealand historian Stephen Loveridge writes that "…conceptions "that one's circumstances are associated with some larger or redeeming purpose, however that is defined, offer the human psyche some capacity to reframe and control apparently impossible situations."[50] On the other hand, Arlen J. Hansen notes that despite the "shock of the war's horrors," drivers did find a sense of adventure and education in their work.[51] Drivers internalized their mission to help the wounded, and even used their belief in their jobs to help alleviate personal horrors. Of course, self-effacing service was not always healthy for individual drivers. Driver Leslie Buswell wrote that "...of course one can never allow even a shadow, much less a mention of one's own problems to appear. The personal equation practically doesn't exist here."[52]

Despite having to neglect personal needs, the ambulance service did provide a form of escapism for the drivers. In a reflective essay that he wrote during a spell of pre-mission anxiety, Phillip Sidney Rice described seeing the horrors that the German Army wrought upon France, and decided he was "glad to go on living and loving France."[53] While one could dismiss this essay as mere propaganda, it is worth noting that Rice penned this patriotic essay while having premonitions of his own demise. Even in his fear, he was able to focus on the larger mission at hand.

Even after the United States entry into the war, American drivers continued to adopt idealistic endurance strategies. In June of 1918, Guy Emerson Bowerman, Jr. reflected on his participation in the war in the year since his enlistment. He wrote that although "…war is a terrible thing it still has its compensations for those who live." Among the benefits he listed included learning "true human values…[having] more self confidence initiative and courage…" He admitted the "…whole business is unromantic," but appreciated that there were "many here with me doing the same thing that it is just as if…this war was a perfectly natural mode of life. Thus are we able to adapt ourselves…"[54] In essence, despite the horrors of war, Bowerman found purpose in his work and comradery with other drivers.

Perhaps the most succinct example of finding purpose in one's larger mission comes from a letter Leslie Buswell wrote in August of 1915. He wrote that the "…horror of the whole war is growing on me day by day, and sometimes when I have got into my bed or am trying to get a few hours' sleep on a stretcher...the horrors of blood... haunt me, and I feel I can hardly go through another day of it." In the wake of these horrors, Buswell realized "all that is soon forgotten when a call comes, and you see those bandaged soldiers waiting to be taken to a hospital. I almost love my old car..."[55] In essence, the call to duty distracted Buswell from even the most traumatic memories.

VIII. Conclusion

In conclusion, American medical personnel in World War I experienced varying emotional responses to the

circumstances around them, and developed adaptive and idealistic endurance strategies to survive the war. While adaptation to trauma merely helped these medics to survive the horrors around them, idealistic endurance allowed ambulanciers to find pride in their missions that transcended their trials. Some might contend that accounts published during the war were mere propaganda, or that postwar accounts were too far removed from the battlefields to be reliable. While these accusations are reasonable, they cannot negate the existence of sundry emotional accounts and survival strategies found within these medics' journals, letters, and memoirs.

Unfortunately, the emotional experiences of American ambulance drivers and physicians have been neglected by historians for too long. As the centennial of the conflict has arrived and every last veteran of the conflict has passed away, it falls upon current social and medical historians to not only expand the scholarship on World War I endurance, but to reexamine the emotional and social experiences of the American medics who volunteered in foreign armies and the United States military during the Great War.

End Notes

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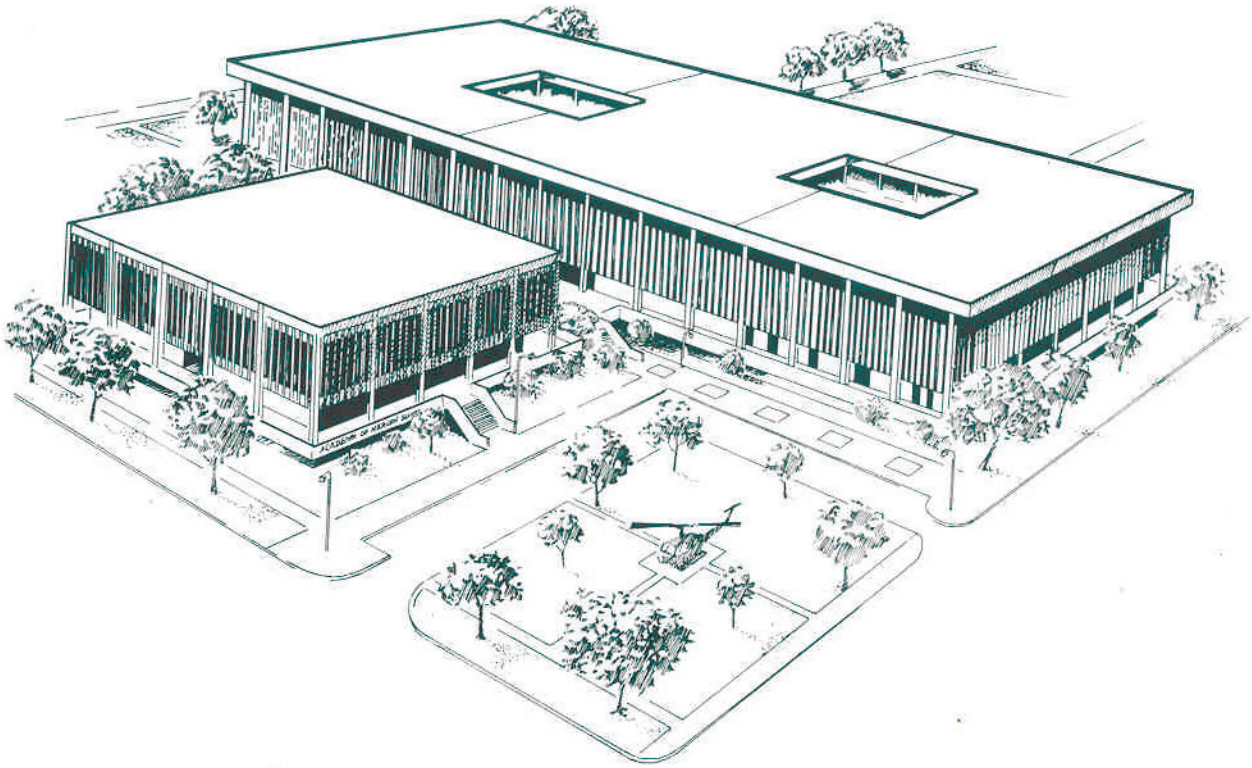

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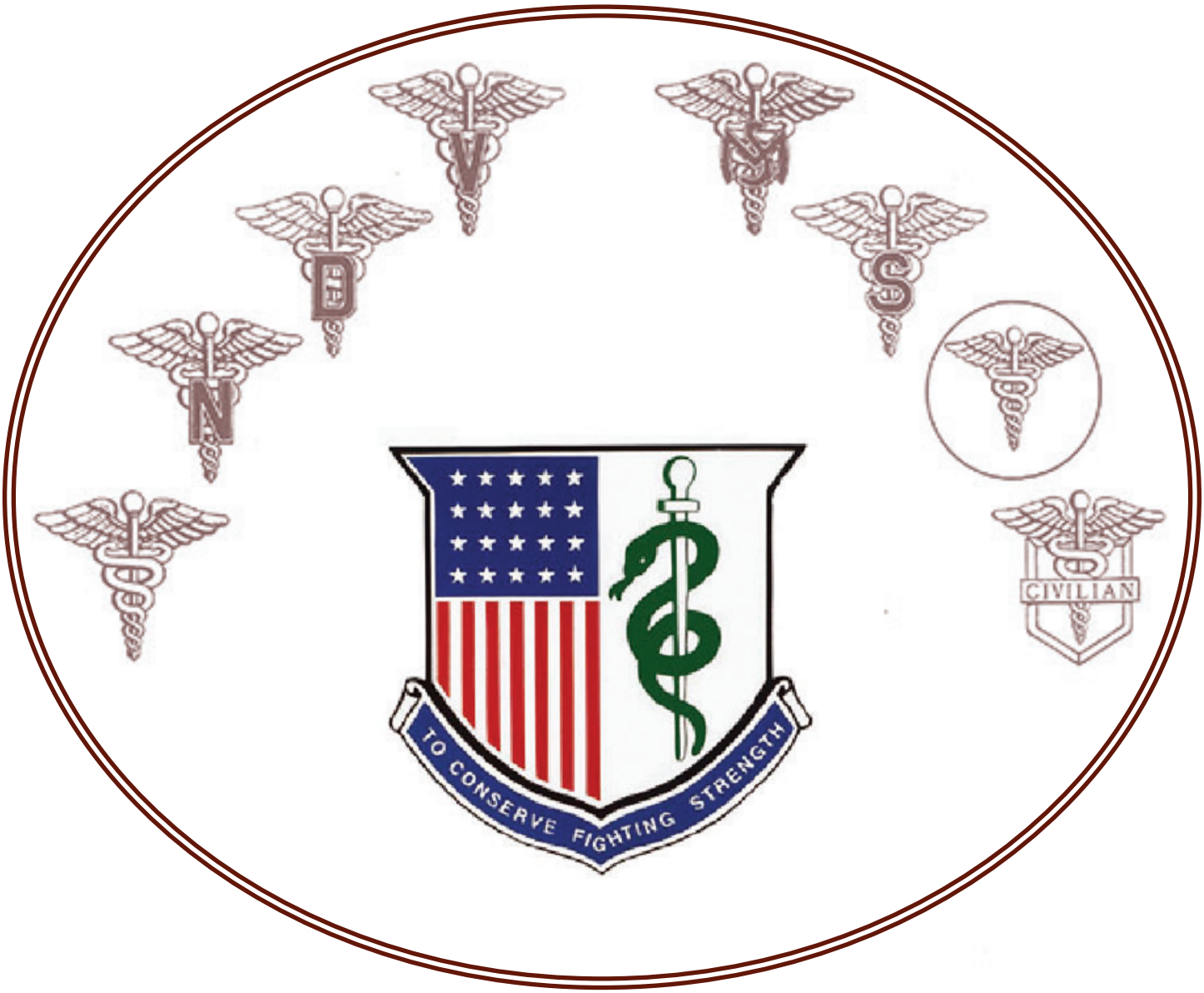

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