MOSQUITO COMMUNITY AND WEST NILE VIRUS RISK ON A NATIONAL GUARD TRAINING BASE IN OKLAHOMA

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ABSTRACT. Military bases are important areas for mosquito surveillance to maintain active duty combat readiness and protect training exercises. The aim of this study was to assist Camp Gruber National Guard training facility personnel to assess their mosquito community and West Nile virus (WNV) risk using biweekly sampling of 50 sites. Between May and October 2018, 10,259 adult female mosquitoes consisting of 6 genera and 26 species were collected over 662 trap-nights using 2 trap types. The most commonly collected genus was Culex (72.2% of total), followed by Psorophora (13.3%) and Aedes (10.2%). Of note, most of the medically important species were collected in the area containing troop living quarters, including 1 WNV-positive pool of Culex tarsalis. Two specimens of Aedes aegypti were collected around a vehicle storage area. While smaller in land mass size than many other active military bases in Oklahoma, the diversity of species at Camp Gruber was comparable to collections from 4 larger bases in Oklahoma. These data demonstrate the need for regular season-long mosquito monitoring of training bases to protect the health of active duty and reserve military personnel.

KEY WORDS Aedes, Culex, military training sites, National Guard bases, surveillance

INTRODUCTION

Mosquito-borne infectious diseases are a major public health concern in the USA, with West Nile virus (WNV) currently the most significant in the USA (Curren et al. 2018, Rosenberg et al. 2018). The recent outbreaks of arboviral (dengue, chikungunya, and Zika viruses) diseases in Central and South America have caused US-based mosquito monitoring efforts to focus on Aedes aegypti (L.) and Ae. albopictus (Skuse). Surveillance efforts funded by the Centers for Disease Control and Prevention (CDC) have characterized mosquito communities in different regions (Goddard et al. 2017, Kovach and Smith 2018), but the Great Plains remains one of the least studied regions with regard to the ecology of mosquito vectors (Bradt et al. 2019).

Communities most at-risk of outbreaks of mosquito-transmitted diseases include areas of high human population movement (Sutherst 2004). Military bases and operations are characterized by rapid changes in human populations. These changes involve: 1) a rapid deployment of troops; 2) a return from regional or national deployment; 3) an influx of troops coming on base for training exercises and then leaving; 4) base staff who maintain operations; and 5) materials, primarily machinery that is driven or flown into the base from regional or national bases or even foreign countries. These factors could create conditions for a mosquito-borne disease outbreak, which could impair troop readiness (Moore 1999, Cofrancesco et al. 2007) and potentially introduce new species to a region. Because of these recognized risks, mosquito surveillance of active military installations has been conducted since World War II (Foley et al. 2011).

However, not all military installations are equally prepared for potential mosquito-borne disease outbreaks. Active military bases are full-time installations where military personnel work, train, and often live within the confines of the base. Active bases also have federal funds for arthropod monitoring and management programs run by full-time personnel, which increases the chances of detecting introduced or infected mosquitoes (McHugh and Vande Berg 1989, McHugh 1991, Moore 1999, McPhatter et al. 2012). In contrast, military installations used by reserve personnel, such as National Guard training facilities, usually have a small permanent operating staff and limited resources for arthropod monitoring and management. These training facilities are frequently occupied briefly by hundreds or even thousands of troops that come to the facility for 2–14 days before dispersing once their training period is finished. Because of the frequency of these movements of soldiers and equipment on military bases, these training facilities may be at greater risk for the establishment and population increase of imported mosquitoes and their associated diseases. Therefore, adequate funding and consistent surveillance on these facilities is necessary to protect base and transitioning personnel from mosquito-borne disease and to prevent the establishment of exotic species.

Oklahoma has both active bases and reserve training facilities. Camp Gruber in Muskogee County (east-central Oklahoma) is an Oklahoma Army National Guard training facility for weekend drills and 2-wk summer training exercises, which is also used for law enforcement training, pre- and post-

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deployment processing of troops, and for seasonal public hunting. To assist the training center in determining potential risk for mosquito-borne disease, the aims of this study were: 1) to assess the community of host-seeking adult female mosquitoes and nearby larval habitats on Camp Gruber; 2) to test for WNV infection in the main mosquito species involved in transmission in Oklahoma (Culex pipiens L. and Cx. tarsalis Coquillett); and 3) compare abundance of adult mosquito communities at Camp Gruber with 4 other active Oklahoma military bases.

MATERIALS AND METHODS

Study site

The primary mosquito collections in this study were carried out at Camp Gruber Training Center, an Army National Guard facility occasionally used by other military personnel and police task forces and seasonal public hunting. Located in Muskogee County, OK, Camp Gruber encompasses approximately 22,500 ha (55,680 acres). The town of Braggs, OK (population 257), is directly adjacent to the southeastern edge of Camp Gruber. The base has a cantonment area (250 ha) and firing ranges (185 ha), with the remainder a mixture of grassland and forest with streams and ponds with the southeastern corner bordered by Greenleaf Lake (370 ha). The western cantonment area has most of the living quarters, administrative headquarters, and service offices for personnel while the eastern cantonment area contains vehicle and equipment storage areas.

Sampling protocol

For the study, 50 collection locations approved by base personnel were selected using reports of mosquito activity, troop activity, and training area access. Between April and October 2018, a total of 23 collection trips (46 sampling dates) occurred biweekly. Trapping occurred using 2 types of traps: modified CDC light traps (Bioquip®, Rancho Dominguez, CA) and BioGents® (BG) Sentinel traps (BioGents, Regensburg, Germany). The CDC light traps, modified by removing the light source, were used to capture mosquitoes inside and outside military housing and training areas. BG Sentinel traps were placed on the ground in grassy areas and near vegetation, and the battery was placed inside the trap to protect from wildlife. Each time traps were checked, all captured mosquitoes were removed and placed into labeled containers and placed into a mobile freezer set at approximately –8°C. Upon return to the laboratory, mosquitoes were transferred to a –20°C freezer and stored until identification.

During the study period, traps were set on Friday, between 10:00 and 13:00 h, and then checked between 05:30 and 11:00 h each of the next 2 mornings. After the 1st night, dry ice was replenished for the CDC light traps. On the last day, traps were checked, all captured mosquitoes were removed and placed into labeled containers and placed into a mobile freezer set at approximately –8°C. Upon return to the laboratory, mosquitoes were transferred to a –20°C freezer and stored until identification.

During adult trapping at Camp Gruber, water bodies and containers holding water within 100 m of each trap were sampled. Samples were collected with a mosquito dipping cup (Bioquip) and larval mosquitoes were stored in 70% ethanol in 2-ml vials (VWR, Radnor, PA) after collection. All 4th instars were identified using larval dichotomous keys (Darsie and Ward 2005) and a Labom stereoscope. Species, collection location, and date were recorded.

Aedes aegypti confirmation assay

Samples of DNA were extracted from the head of a larva and legs of an adult tentatively identified as Ae. aegypti using a GeneJET Genomic DNA Extraction Kit (ThermoScientific, Grand Island, NY). The positive control was legs of an adult Ae. aegypti Liverpool strain. Then, 20 µl of Proteinase K and 180 µl of digestion solution were added to the 2 samples, and each was incubated overnight at 56°C on a shaker. The next day, extraction was completed following the manufacturer protocol and the extracted DNA samples were stored at –20°C. The extracted DNA was tested by polymerase chain reaction (PCR) using primers that amplify a 361-bp region of the DNA.
ND4 mosquito gene (da Costa da Silva et al. 2005): ND4-Forward primer (5′-ATTGCCCTAGGGCTCATGTAAG-3′) and ND4 Reverse (5′-TCCGGCTCTACTGCTTCA-3′). Positive amplicons were extracted from the 2% agarose gel using an Invitrogen PureLink Quick Gel Extraction Kit (ThermoFisher Scientific, Waltham, MA) and sent to the Oklahoma State University Core Facility to be bidirectionally sequenced. Resulting consensus sequences were compared with GenBank submissions using default conditions on NCBI BLAST (National Center for Biotechnology Information, Bethesda, MD) where the highest percent sequence identity was used to determine species similarity.

Comparison with other Oklahoma bases

The mosquito community at Camp Gruber was compared with data from an earlier study collected between May and September 2016 (Bradt et al. 2019) at 4 military installations in Oklahoma: Vance Air Force Base (Enid, Garfield County), Tinker Air Force Base (Oklahoma City, Oklahoma County), Altus Air Force Base (Altus, Jackson County), and Fort Sill Military Post (Lawton, Comanche County). Differing from the current study, Bradt et al. (2019) used CDC gravid traps baited with water infused with decomposed Bermuda grass (Cynodon dactylon [L.]) in addition to CDC light traps (CO2) and BG Sentinel traps. The CDC light traps collected an average of 23.70 mosquitoes per trap-night, while the BG Sentinel traps averaged 0.69 mosquitoes per trap-night. The majority of mosquitoes collected with CDC light traps were Culex spp. (73.0%), followed by Psorophora spp. (13.0%), and Aedes spp. (9.7%) in addition to Anopheles spp. and Uranotaenia spp. In contrast, the BG Sentinel traps collected primarily Aedes spp. (42.7%) in addition to Toxorhynchites spp. (Table 1).

**RESULTS**

**Adult mosquito collections**

A total of 10,259 adult female mosquitoes consisting of 6 genera and 26 species were collected at Camp Gruber between May and October 2018 over 662 trap-nights (Table 1). The most commonly collected genus was *Culex* (72.2% of total), with the most common species, *Cx. erraticus* (Dyar and Knab) (70.4% of all mosquitoes collected), averaging >10/trap-night. The 2nd most common genus was *Psorophora* (13.3%), followed by *Aedes* (10.2%), with *Ae. vexans* (Meigen) (72.7%) representing the majority of *Aedes* collected. The remaining 435 mosquitoes belonged to *Anopheles*, *Toxorhynchites* (*rutilus septentrioralis* Dyar and Knab, and *Uranotaenia sapphirina* (Osten Sacken)).

Of the mosquitoes collected during the study, 4,093 (39.9%) were collected in 18 (36% of total) traps distributed around the western cantonment area. The majority of mosquitoes of medical importance were also collected in this area: 96 *Cx. pipiens* (59%), 18 *Cx. tarsalis* (56%), 129 *Ae. albopictus* (79%), 643 *Psorophora columbiae* (Dyar and Knab) (71%), and 548 *Ae. vexans* (72%). The majority of *Cx. erraticus* (65%) were collected from one trap in the southern part of the base, but 2,342 (32%) were collected in the western cantonment area. The majority of *Anopheles quadrimaculatus* Say (88%) were also collected in the southern part of the base.

Modified CDC light traps captured 25 species, whereas the BG Sentinel traps captured only 14 species (Table 1). Of the 10,259 identified mosquitoes, 10,095 (98.4%) were collected from the CDC light traps, and the remaining 164 (1.6%) were from BG Sentinel traps. The CDC light traps collected an average of 23.70 mosquitoes per trap-night, while the BG Sentinel traps averaged 0.69 mosquitoes per trap-night. The majority of mosquitoes collected with CDC light traps were *Culex* spp. (73.0%), followed by *Psorophora* spp. (13.0%), and *Aedes* spp. (9.7%) in addition to *Anopheles* spp. and *Uranotaenia* spp. In contrast, the BG Sentinel traps collected primarily *Aedes* spp. (42.7%) in addition to *Toxorhynchites* spp. (Table 1).

**Seasonal trends**

Mosquito species peaked in different months throughout the sampling period. *Aedes albopictus* and *Cx. tarsalis* peaked in July and August (Fig. 1A), whereas *Cx. pipiens* increased as the sampling season ended in October. Similar to other species, *An. quadrimaculatus* and *Ps. columbiae* numbers both peaked in August while *Ae. vexans* peaked during September (Fig. 1B). *Culex erraticus* also peaked in August, increasing into the thousands during a short period of activity (data not shown).

**Larval counts**

A total of 743 larvae were collected of which 166 were identified to species (Table 1). The remaining larvae were not 4th instars and could not be positively identified using available keys. The identified larvae represented 4 genera and 10 species, with the most represented genus being *Psorophora* (4 species). No species was collected only as a larva although more Ps. mathesoni Belkin and Heinemann larvae (5) were collected than adults (2) (Table 1).

**WNV results**

A total of 90 pools (*n* = 194) of *Culex* mosquitoes were tested for WNV: *Cx. pipiens* (69 pools—162 mosquitoes) and *Cx. tarsalis* (21 pools—32 mosquitoes). One pool consisting of a single *Cx. tarsalis* was...
WNV-positive, producing a *Cx. tarsalis* prevalence of 3.1% (MIR of 31.2/1,000 mosquitoes). The positive pool was collected near the residential areas of the base in a group of eastern red cedar trees (*Juniperus virginiana* L.) on August 25, about 0.2 km from the elementary school in Braggs, OK.

**Aedes aegypti** confirmation assay

Two *Ae. aegypti* samples from Camp Gruber tested using PCR were confirmed using NCBI BLAST with 100% sequence identity with known sequences of *Ae. aegypti* (KX580042.1), whereas the positive control had 100% sequence identity with a known sequence of Liverpool strain (MF194022.1).

**Oklahoma military installations comparison**

Despite variable trap efforts and comparison of different years, all Oklahoma military institutions produced at least 4 genera and between 12 and 22 mosquito species. The 6 genera and 26 species collected at Camp Gruber are comparable with the 4 genera and 22 species collected at Fort Sill, with fewer species collected at Altus Air Force Base (4 genera; 16 species), Tinker Air Force Base (5 genera; 17 species), and Vance Air Force Base (4 genera; 12 species). Camp Gruber had the longest sampling period and produced the most diversity in genera and species (Table 2). *Culex pipiens* was the most abundant species collected on 3 bases (Altus, Fort Sill, Vance) while *Ps. columbiae* was most abundant on 2 bases (Camp Gruber and Fort Sill) (Table 2).

**DISCUSSION**

As the 1st study to characterize the mosquito community on a National Guard training facility in Oklahoma, the data reveal important information about the mosquito community and potential risks for mosquito-borne diseases on military training facilities. Camp Gruber has an abundant and diverse mosquito community. While the traps were more numerous and geographically more widespread than those placed on 4 active Oklahoma military bases for an earlier survey during 2016 (Bradt et al. 2019), Camp Gruber had similar diversity of mosquitoes (Table 2). The abundance of various species on Camp Gruber highlights important landscape factors that can be used for future mosquito control strategies. The presence of open, low-lying areas of grass that are prone to periodic flooding were common in training areas. The high capture rate for the 2 aggressive nuisance-biting floodwater species, *Ae. vexans* (50% of total) and *Ps. columbiae* (45% of total), occurred in these areas approximately 10–14 days after a heavy (12.5 cm) rain (Weather Underground; The Weather Company, San Francisco, CA).

While no larvae were collected, adult female *Cx. erraticus* was the most abundant species collected in

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**Table 1.** Summary of larval and adult female mosquitoes collected on Camp Gruber, Oklahoma, during 2018. Includes number of adult mosquitoes collected by BioGents Sentinel traps (BG) and Centers for Disease Control and Prevention light traps (CDC).

| Species                          | Larvae | Adults | BG   | CDC  |
|----------------------------------|--------|--------|------|------|
| *Aedes aegypti*                  | 1      | 1      | 0    | 1    |
| *Ae. albopictus*                 | 19     | 164    | 32   | 132  |
| *Ae. atlanticus*                 | 0      | 62     | 3    | 59   |
| *Ae. canadensis*                 | 0      | 1      | 0    | 1    |
| *Ae. epeius*                     | 0      | 17     | 12   | 5    |
| *Ae. fulvus pallens*             | 0      | 2      | 0    | 2    |
| *Ae. hendersoni*                 | 0      | 11     | 4    | 7    |
| *Ae. nigromaculis*               | 0      | 2      | 0    | 2    |
| *Ae. sollicitans*                | 0      | 4      | 0    | 4    |
| *Ae. triseriatus*                | 0      | 21     | 15   | 6    |
| *Ae. trivittatus*                | 0      | 2      | 0    | 2    |
| *Ae. vexans*                     | 93     | 763    | 4    | 759  |
| *Anopheles crucians*             | 0      | 47     | 0    | 47   |
| *An. punctipennis*               | 1      | 7      | 0    | 7    |
| *An. quadrimaculatus*            | 1      | 377    | 2    | 375  |
| *Culex erraticus*                | 0      | 7,218  | 28   | 7,190|
| *Cx. pipiens/quinquefasciatus*   | 2      | 163    | 17   | 146  |
| *Cx. tarsalis*                   | 0      | 32     | 0    | 32   |
| *Psorophora ciliata*             | 1      | 54     | 3    | 51   |
| *Ps. columbiae*                  | 1      | 377    | 28   | 375  |
| *Ps. cyanescens*                 | 0      | 99     | 4    | 95   |
| *Ps. discolor*                   | 0      | 273    | 10   | 263  |
| *Ps. ferox*                      | 4      | 28     | 0    | 28   |
| *Ps. mathesoni*                  | 5      | 2      | 0    | 2    |
| *Toxorhynchites rutilus septentrionalis* | 0  | 3   | 3    | 0    |
| *Uranotaenia sapphirina*         | 0      | 1      | 0    | 1    |
| **Totals**                       | 166    | 10,259 | 164  | 10,095|
this study. Its larvae are associated with the fringes of creeks and rivers as reported by Robertson et al. (1993). Primarily a bird-feeding species that transitions to mammals later in the season, *Cx. erraticus* has been documented with natural infections of eastern equine encephalitis (EEE) virus and WNV (Harrison et al. 2009, Mendenhall et al. 2012, Mukherjee et al. 2012, Godsey et al. 2013, Bingham et al. 2016).

*Culex erraticus* is a potential bridge vector (Cupp et al. 2003, 2004; Cohen et al. 2009; Lindsey et al. 2018) reported nonhuman cases of EEE in 4 counties and at least 1 human case along the Arkansas–Oklahoma border. They also reported nonhuman cases in McCurtain and Tulsa counties. More than 4,300 *Cx. erraticus* were captured on a single weekend (10–14 days after receiving 12.5 cm of rain), as well as high numbers of *An. quadrimaculatus* (53%), from an area disturbed by feral pigs in the Camp Gruber training area beside Greenleaf Lake in the southeast corner of the property. While this was not confirmed during this study, wild pigs have been implicated in facilitating mosquito breeding in other parts of the world (Nogueira-Filho et al. 2009).

The 2 main *Culex* species involved in WNV transmission in Oklahoma, *Cx. pipiens* and *Cx. tarsalis*, as well as *Ae. albopictus* were found throughout the training facility, but the majority of all 3 species were collected in the cantonment area. This is particularly important as this is the area in which the small cadre of camp personnel work daily and where training reservists stay on base and relax after training. These medically important species became more abundant in August into October, with *Ae. albopictus* (45%) and *Cx. tarsalis* (59%) numbers peaking in August and *Cx. pipiens* (46%) peaking in mid-October, each 2 wk after separate 12.5-cm rain events. The fact that the majority of all 3 species were collected in the cantonment area suggests a possible influence of anthropogenic factors including habitat for larvae and ample blood meals for host-seeking adult females. The risk of mosquito activity in this area was highlighted by collection of the only WNV-infected mosquito pool, <0.2 km from the local elementary school in the adjacent town of Bragg.

The discovery of 2 separate specimens of *Ae. aegypti* was also important. *Aedes aegypti* is a well-known vector for a variety of important arboviruses, including dengue, chikungunya, and Zika viruses. Not recorded in Oklahoma for >70 years, *Ae. aegypti* was recently collected in a variety of urban areas in southcentral and western Oklahoma (Bradt et al. 2019, Sanders 2019). Other than a few reports of *Ae. aegypti* on Tinker Air Force Base 2 decades ago (McHugh and Hanny 1990), this was the 1st documented collection of the species on a military base in Oklahoma. These specimens were collected almost 3 km from the cantonment area at a location of recently translocated heavy equipment (backhoes and front-end loaders) from out of state and an abandoned car used for training purposes. This further emphasizes the need for vigilance with regard to the ability of this species to establish on military bases unintentionally through equipment movement (Cofrancesco et al. 2007, Dallimore et al. 2017).

There was a disparity between the numbers of mosquitoes collected by the 2 different types of traps used in the study. This may have occurred because there were fewer BG Sentinel traps used than CDC light traps, a difference of 238 and 426 trapping events, respectively. However, even with these disparities, the composition of mosquitoes collected was notably distinct as BG Sentinel traps primarily collected *Aedes* spp., followed by *Culex* spp., while CDC light traps primarily collected *Culex* spp., followed by *Psorophora* spp. (Table 1). These distinctions are important when determining a sampling protocol for targeted mosquito surveillance on a military training base. While CDC light traps are known to collect a wide diversity of mosquito species (Reiskind et al. 2017), it may be necessary to use BG Sentinel traps in areas that are more concerned with arboviruses transmitted by *Aedes* spp.

Among Oklahoma military bases, Camp Gruber had the highest diversity of genera and species while no studied military facilities had the same 2 highest collected species. This was possibly due to a higher number of trap-nights, trap placement, and a longer sampling season compared with the 4 other military

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**Fig. 1.** Monthly abundance of (A) 3 important potential disease vectors, and (B) 3 important mosquito species on Camp Gruber, Oklahoma, during 2018. **JUNE 2020** **MOSQUITOES ON OKLAHOMA MILITARY BASES** 85

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bases (Bradt et al. 2019) in addition to ecological differences and local weather conditions at the time of each study. In terms of species, Camp Gruber had the greatest diversity of *Aedes* spp. and *Psorophora* spp. while Fort Sill had the greatest diversity of *Anopheles* spp. More *Culex* spp. were collected at all previously sampled bases when compared with Camp Gruber. However, *Cx. erraticus* was collected at Camp Gruber in greater numbers than any other species on any other base. Additionally, both *Tx. r. septentrionalis* and *Ur. sapphirina* were collected only at Camp Gruber.

In conclusion, larval and adult mosquito surveillance was conducted at Camp Gruber between May and October 2018, resulting in the collection of 6 genera and 26 species. The majority of the important vector species were collected in the cantonment area. Ninety pools (n = 164) of *Cx. pipiens* and *Cx. tarsalis* were tested for WNV and 1 pool of *Cx. tarsalis* tested positive. The knowledge that the cantonment areas containing living quarters for visiting soldiers produced the majority of medically important mosquitoes along with the only WNV-infected pool provided important information to the National Guard command to evaluate mosquito control protocols and change the landscape factors that are contributing to the production of mosquitoes. Detection of *Ae. aegypti* in a region of the state where it has not been collected since the 1940s highlights the need for vigilance using regular surveys. All of these components factor into providing safety for incoming and outgoing troops in any military training facility.

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