Texas A&M Institute of Renewable Natural Resources

Final Chagas Disease Report

Agreement W912G-14-2-0011

Chagas Disease in Mammals on Joint Base-Lackland Training Annex and Camp Bullis Military Training Reservation, San Antonio, Texas

R. R. Lopez, M. M. Kramm, and I. D. Parker

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EXECUTIVE SUMMARY

Chagas disease, also known as American trypanosomiasis, is caused by the etiological flagellate protozoan *Trypanosoma cruzi* (*T. cruzi*). It is a significant health concern in South and Central America where millions of people are infected or at risk of infection, and an emerging health concern in the United States. The prevalence of Chagas disease in natural environments is supported by mammal host species but those primary species may vary based on geographic location. In South Texas, the primary host species for the disease is poorly understood requiring a field study to determine the spatial distribution of *T. cruzi* prevalence in free-ranging mammals.

Chagas disease can reduce force readiness and cause life-long incurable health effects. The persistence and maintenance of the disease parasite in the areas along the southern U.S. threatens essential military weapon detections system (K-9 military working dogs) and personnel as warfighters. Biosurveillance to quantify Chagas disease distribution in DoD environments is important for protecting the health of military communities through an understanding of disease and disease vector presence. Determining the incidence of the parasite and disease on DoD installations, will provide transmission risk assessment to military personnel and allow them to implement management and control initiatives to suppress.

Project objectives were to (1) identify and estimate relative density of triatomine insects in various vegetation types, (2) determine spatial distribution and prevalence of *T. cruzi* parasites in free-ranging mammalian species, and (3) determine relative mammal abundances by vegetation type and season. Triatomine distribution analyses included light traps and CO_2 traps placed throughout Joint Base San Antonio (JBSA) – Lackland Training Annex (2012–2014). This was supplemented by infection data collected from meso-mammal sampling. From 2012–2016, blood and tissue samples from geo-located free-ranging wildlife mammal species were analyzed using antigen strips and polymerase chain reaction (PCR) methods to detect protozoan *T. cruzi* DNA. Mammal

abundance was determined using mark-recapture methodology and recorded capture-site characteristics such as vegetation structure.

Triatomine distribution calculations were hampered by low capture rates using light traps or CO_2 traps. This is not unusual in triatomine capture efforts throughout the world. However, mammal infection rates were highest in wooded areas when controlling for relative mammal abundances. This indicated triatomines were primarily feeding on fossorial mammals found in wooded areas. This was supported by both the literature and a concurrent U.S. Army triatomine distribution findings. Chagas disease infection rate in meso-mammals were significantly higher in deciduous forests compared to grasslands or semi-improved woodlands. A disproportionate percentage of infections were also found in lower elevation floodplain and riparian areas (57% positive) compared to upland areas (27% positive). There was high prevalence in small and meso-mammals, but much lower prevalence found in large mammals. This project suggests that common free-ranging fossorial mammal wildlife populations support *T. cruzi* in natural environments and are a potential public health concern in South Texas. Preventative or mitigation strategies should consider a range of management activities to include, habitat management, selected application of insecticide, and changes in human behavior and personal protection in high-risk areas.

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LIST OF ACRONYMS, ABBREVIATIONS AND SYMBOLS

Acronyms, Abbreviations, Symbols	Definition
%	Percent
χ^2	Chi-square
ac	Acre
AFB	Air Force Base
CDC	Centers for Disease Control and Prevention
df	Degrees of Freedom
DITC	Digital Infrared Triggered Cameras
DNA	Deoxyribonucleic Acid
DoD	Department of Defense
EPA	U.S. Environmental Protection Agency
°F	Degrees Fahrenheit
Fig.	Figure
ft	Feet
GB	Gigabyte
GIS	Geographic Information System
IACUC	Institutional Animal Use and Care Committee
in	Inch
INRMP	Integrated Natural Resources Management Plan
IRNR	Texas A&M Institute of Renewable Natural Resources
JBSA	Joint Base San Antonio
lbs.	Pounds
mi	Mile
MWD	Military Working Dog
n	Number
P	P-value
PCR	Polymerase Chain Reaction
spp.	Species (plural)
T. cruzi	*Trypanosoma cruzi*
USAF	United States Air Force

1.0 INTRODUCTION

1.1 PURPOSE AND NEED

Chagas Disease, also known as American Trypanosomiasis, is caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*). This incurable disease affects humans and other mammals such as domestic dogs (*Canis lupus familiaris*), raccoons (*Procyon lotor*), striped skunks (*Mephitis mephitis*), and Virginia opossums (*Didelphis virginianus*). Chagas disease is considered a significant human health problem in Central and South America where 8 –11 million people are infected. The primary vector of Chagas disease are triatomines (commonly known as kissing bugs but also known as reduviid bugs, assassin bugs, vinchuca, or blood suckers), and found from South America through the southern United States. Eleven species of triatomines are known to occur in North America (Bern et al. 2011), with the most common vector species being *Triatoma rubida* and *Triatoma protracta* in Arizona and California and *Triatoma gerstaeckeri* and *Triatoma sanguisuga* in Texas and New Mexico (Fig. 1).

Triatoma sanguisuga *Triatoma gerstaeckeri*

Figure 1. South Texas Triatomine species (Photo courtesy of Sonia Kjos).

Kissing bugs are blood-feeding insects, which obtain the parasite from an infected host. The parasite carries out part of its life cycle in the insect's digestive tract. Parasite

transmission occurs when fecal material from infected kissing bugs containing the *T. cruzi* protozoa trypanosome is rubbed or introduced into the feeding bite wound or mucous membranes or when infected feces contaminate food or water. Triatomine insect species use both domestic and free-ranging mammal species as hosts. Free-ranging mammals are bitten typically on the ventral or belly side and feeding occurs for a few minutes typically unnoticed with no observed pain or discomfort. Additionally, the disease pathogen can be transmitted through contact with infected blood and tissue, transplacentally, through carnivory, and through direct consumption of infected triatomines by mammals (Fig. 2).

In the United States, although there have been few reported cases, the Centers for Disease Control and Prevention (CDC) estimated that >300,000 immigrant and homeless people are infected or have been infected. Chagas disease with high parasitemia, initiated by the acute phase of the malady, is identifiable in humans by conjunctivitis (Romaña sign), skin rash, headaches, lymphadenophay, fever and other disorders, but it is difficult to visually isolate in animal species without serological and/or molecular (e.g., Polymerase Chain Reaction [PCR]) confirmation. The chronic or long-term phase of the disease may be characterized by little or no obvious symptoms but may also result in cardiac issues such as enlarged heart, cardiac arrest, and heart failure. Additional long-term problems may include enlarged esophagus or colon and problems eating or passing stool. JBSA-Lackland AFB is home to the Military Work Dog (MWD) program and trains approximately 700 dogs annually for security, anti-narcotics work, and explosives detection. Early analyses indicated at least 8% of military working dogs at JBSA-Lackland AFB show *T. cruzi* antibody prevalence indicating infection (M. Kramm, unpublished). Military working dogs are impacted in a variety of ways during the acute and chronic stages. During the acute phase dogs may experience lethargy, liver and spleen enlargement, diarrhea, neurological issues, or sudden death. Chronic problems may include weakness, exercise intolerance, fluid retention, and death.

Chagas disease can reduce force readiness and cause life-long incurable health effects. Installations in the southern U.S. are now more vulnerable to Chagas disease

as climate change has supported the spread of important disease vectors. The persistence and maintenance of the disease parasite in the areas along the southern U.S. threatens essential military weapon detections system (K-9 military working dogs) and personnel as warfighters. Military working dogs are vital for detecting explosive devices and protection in support of military members and allies. JBSA-Lackland AFB is the sole training facility supporting ~40,000 basic trainees annually, and responsible for providing K-9 working dogs to all military forces, Department of Homeland Security, and other government agencies. Chagas disease has the potential to interfere with the operational impact of the warfighter by permanently removing experienced combat personnel and K-9 military working dogs from the theater. Biosurveillance to quantify Chagas disease distribution in DoD environments is important for protecting the health of military communities through an understanding of disease and disease vector presence. Determining the incidence of the parasite and disease on DoD installations provides transmission risk assessment to military personnel and allow them to implement management and control initiatives to suppress. Installations require up-to-date information and cutting edge research to address the dynamic issues caused by climate change.

The United States Air Force (USAF) is required under Department of Defense Instruction (DoDI) 4150.07 and Air Force Instruction (AFI) 32-1053 to “prevent or control pests and disease vectors that may adversely impact readiness or military operations by affecting health of personnel, or by damaging structures, materiel, or property …” (JBSA 2015). This project is also within the purview of the policies set forth in the Sikes Act Title 16, Section 670, DoDI 4715.3, *Environmental Conservation Progr*am, AFI 48-102, *Medical Entomology Program*, AFI 32-7064, *Integrated Natural Resources Management*, AFI 32-1053*, Integrated Pest Management Prog*ram, National Environmental Policy Act (42 USC 4321, 4331 through 4335, 4341, and 4347); Public Law 93-452, *Conservation Programs on Military Reservations* (16 USC 670a through 670f).

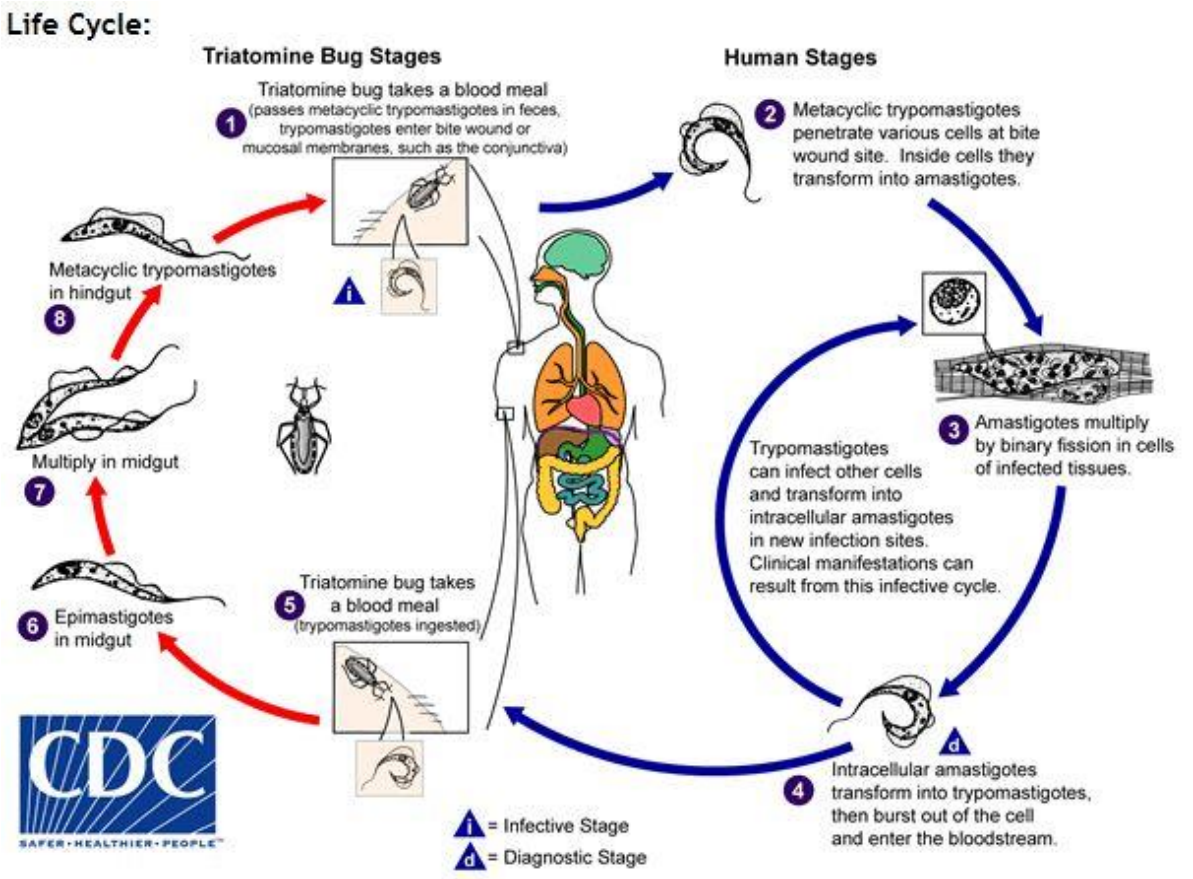

Figure 2. *Trypanosoma cruzi* (Chagas disease) life cycle (CDC 2016).

The distribution of *T. cruzi* prevalence in mammal species is an important component in disease transmission to people. Research indicates triatomines are located in a variety of habitats including grasslands, woodlands, and human-dominated areas such as houses to prey on mammals (Bern et al. 2011, Kramm 2015).

1.2 GOALS

The goal of this project was to provide foundational information for prevention and control of Chagas Disease on JBSA. The prevalence of Chagas disease in natural environments is supported by mammal host species, but those primary species may vary based on geographic location. In South Texas, the primary host species for the disease is poorly understood requiring a field study to determine the spatial distribution of *T. cruzi* prevalence in free-ranging mammals. The goal of this project was to understand the parasite-vector-host interaction in the surrounding environment

of the MWD facilities at JBSA. This project clarified the spatial distribution and relative densities of triatomine insects (vector) and possible free-ranging wildlife mammalian species (hosts) by season and vegetation type. The project objectives were to determine (1) spatial distribution and relative densities of triatomines, (2) spatial distribution and prevalence of *T. cruzi* parasites in free-ranging mammalian species, and (3) relative mammal abundances by vegetation type. This project is in accordance with tripartite agreement outlined in the JBSA Integrated Natural Resources Management Plan (INRMP). The INRMP was "developed to provide an interdisciplinary approach for the management of natural resources" and "promote responsible stewardships of the land and waters" (JBSA 2015). The Chagas Project supports these broad INRMP objectives and directly aids efforts to improve personnel health.

1.3 STRATEGY

Mission success on JBSA requires flexible use of the facilities to train combat ready forces in English-language training, technical training, and basic military training for all enlisted people entering the U.S. Air Force, U.S. Air Force Reserve, and U.S. Air National Guard. The on-the-ground reality is that much of the area is impacted by mission requirements. Support activities (e.g., vegetation control) and structures such as roads, training areas with built structures, and other facilities add complexity to disease control efforts. These activities and support structures are critical to mission success and unlikely to change significantly. However, reducing risks to personnel and canines requires careful consideration of vegetation management, meso-mammal management, alteration of personnel activity regimes, and area use. In general, these require minimizing contact between installation personnel and canines and triatomines. This will require consideration of native wildlife (e.g., maintenance of mixed grass/shrub areas), use of mechanical and chemical treatments to control exotic vegetation, and alteration of range use to avoid times of high-triatomine activity.

2.0 PROJECT AREA

2.1 GENERAL DESCRIPTION

Joint Base San Antonio missions are broad to include over 200 partners dedicated to training and maintaining a quality workforce, providing timely support for worldwide contingencies and sustain a quality working, training, and living environment (JBSA 2015). These missions require a healthy personnel population and detailed knowledge of potential disease vectors. Much of JBSA is located in the urban or urbanizing San Antonio metropolitan area (Bexar County, Texas) and deals with urban encroachment which potentially impacts mission success (Fig. 3). The San Antonio – New Braunfels Metropolitan Area covers 7,398 mi^2 with a population of 1.3 million people in 2010 (JBSA 2015). This growing population is at or nearing the installation fence lines leading to increasing interactions between military missions (e.g., installation security) and off-installation civilians.

Primary work occurred on JBSA-Lackland Training Annex and JBSA-Camp Bullis Military Training Reservation (hereafter JBSA-Camp Bullis, Fig. 4). The JBSA-Lackland Main Base is a 4,000 ac military installation under the jurisdiction of the 802nd Mission Support Group, Air Education and Training Command. Lackland AFB houses approximately 42,000 people and supports multiple uses that include military mission and training activities. It is divided into three primary areas including the Main Base, Lackland Training Annex, and Kelly Field Annex (total 8,698 ac). The Lackland Training Annex is relatively rural and surrounded by undeveloped forest to the north, west, and south (JBSA 2015). As a whole, JBSA-Lackland is comprised of developed areas with grasslands, managed woodlands, and deciduous riparian upland woodlands.

JBSA-Camp Bullis (27,990 ac) houses approximately 900 permanent personnel, 180,000 personnel annually and is a U.S. Army training area primarily used as a large-scale maneuvering area for multiple military branches. It also serves as a center of medical personnel training for all branches. In total, JBSA educates more students and

has more active runways than any other installation and supports the largest Department of Defense hospital (JBSA 2015).

All of JBSA lay at the intersection of the Post Oak Savannah, Blackland Prairie, Edwards Plateau, and South Texas Plains ecoregions. These are characterized by mixed prairie grasslands and deciduous forests underpinned by karst formations and the Edwards Aquifer. JBSA-Camp Bullis is characterized by extensive karst formations formed from the underlying limestone and dolomite. Karst formations do not occur at other JBSA installations. The topography of the project area is relatively flat with elevations ranging from 630–760 ft above sea-level.

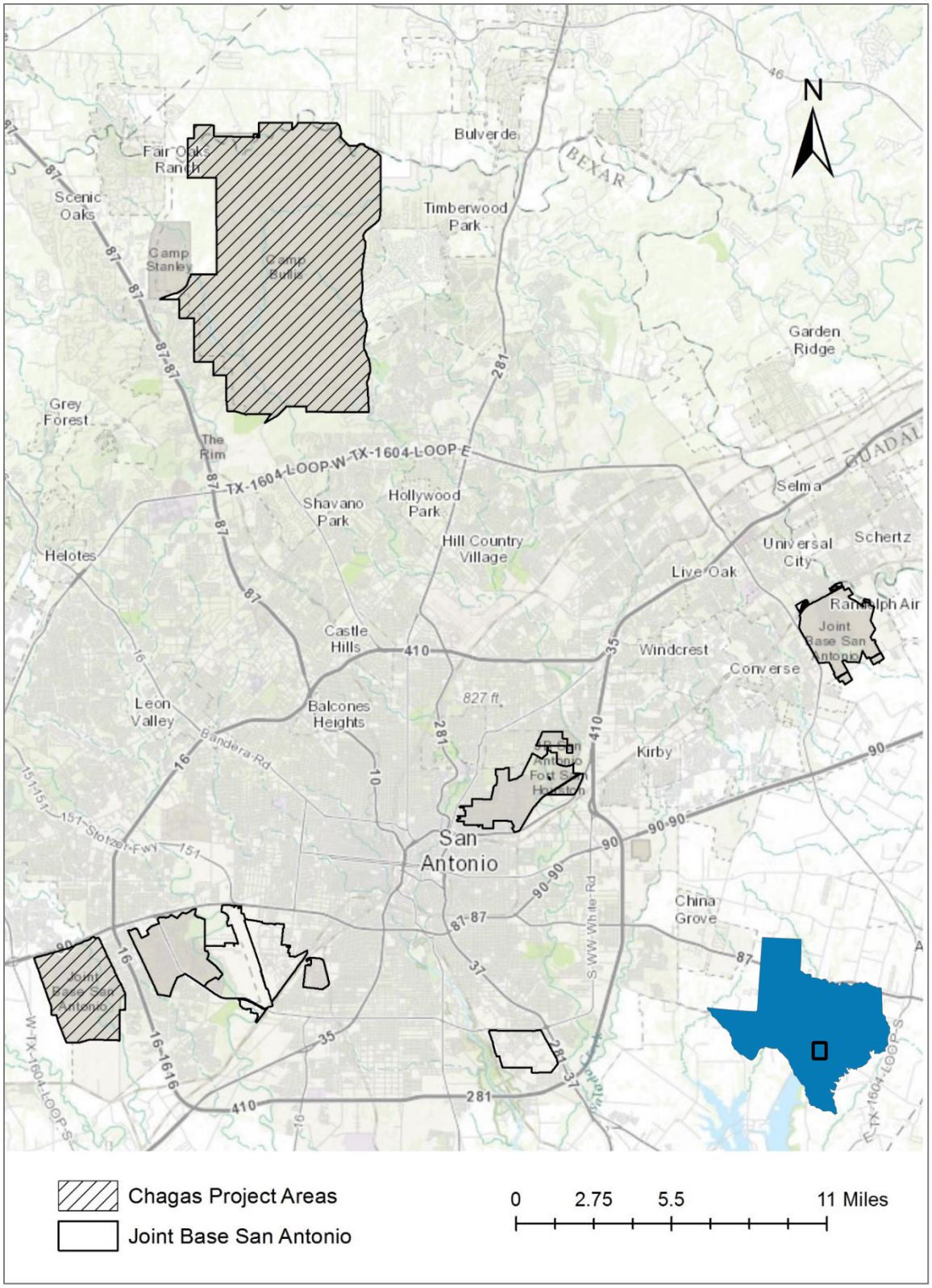

Figure 3. Joint Base San Antonio installations with Chagas Project areas highlighted, Joint Base San Antonio, San Antonio, Texas, 2012–2016.

Figure 4. Chagas disease project locations (JBSA-Lackland Training Annex and JBSA-Camp Bullis), San Antonio, Texas, 2012–2016.

2.2 CLIMATE

The climate is subtropical with mild winters and hot summers (mean temperature = 57.2 and 80.6°F, respectively). Precipitation is variable but averages 33 in annually with peaks in Spring and Fall (JBSA 2015). Temperatures below freezing are rare annually (approximately 20 days). Conversely, extended warm temperatures are common during the spring, summer, and early fall months with highs exceeding 100°F.

2.3 VEGETATION

Herbaceous cover primarily includes King Ranch bluestem (*Bothriochloa ischaemum var. songarica*), buffalograss (*Buchloe dactyloides*), and Texas wintergrass (*Stipa leucotricha*). Managed woodlands are similar to deciduous woodlands but differ in the relative openness of the areas due to periodic mowing and selective harvest of canopy species (JBSA 2015). Woodland plant species include mesquite (*Prosopis glandulosa*), sugarberry (*Celtis laevigata*), cedar elm (*Ulmus crassifolia*), annual sunflower (*Helianthus annuus*), and ashy sunflower (*Helianthus mollis*). Riparian woodland plant species include black willow (*Salix nigra*), green ash (*Fraxinus pennsylvanica*), basswood (*Tilia caroliniana*), sugarberry (*Celtis laevigata*), chinaberry (*Melia azedarach*), giant ragweed (*Ambrosia trifida*), and morning glory (*Ipomoea* sp). JBSA-Lackland Training Annex has relatively more grassland and developed areas than JBSA-Camp Bullis (Fig. 5). JBSA-Camp Bullis is heavily wooded with intermixed shrublands and smaller amounts of grasslands (Fig. 9).

3.0 METHODS

The primary location for data collection was JBSA-Lackland Training Annex. This included all spatial analyses, triatomine distribution determination, and mammal density analysis. JBSA-Camp Bullis was included in 2016 for the sole purpose of determining *T. cruzi* prevalence in mammals on that installation.

3.1 TRIATOMINE RELATIVE ABUNDANCE (JBSA-LACKLAND TRAINING ANNEX)

Triatomine distribution was estimated through trap efforts, disease prevalence and literature review. Trap efforts included construction and deployment of multiple types of CO_2 traps and light traps. Project personnel used portable black light traps set up at random locations along each of the 9 transects surrounding the kennels on JBSA-Lackland Training Annex. Traps consisted of a plastic bucket with a hard white colored structure above it in an X configuration. A light fixture with a black light was placed at the center of the trap, and activated before dusk each night and checked again the next morning at dawn to collect any insects. Insects were identified by species and sex and placed into a glass vial for further examination; preserved specimens were placed in a 70% isopropyl alcohol solution. Light traps were used throughout the year to determine the ideal trap time for triatomines (Klotz et al. 2010).

Trap efforts for triatomines are notoriously difficult. As a consequence, indirect relative abundance was estimated from meso-mammal distribution and infection rates to provide secondary abundance estimates. This provided a rough picture of triatomine presence.

3.2 *T. CRUZI* SPATIAL DISTRIBUTION (JBSA-LACKLAND TRAINING ANNEX)

Two potential indicators on *T. cruzi* spatial distribution based on vegetative types and floodplain extent were identified. The JBSA-Lackland Training Annex was characterized by three distinct vegetative communities: (1) grasslands dominated by herbaceous species and absence of canopy cover, (2) semi-improved woodlands (i.e., park-like) made up of widely-dispersed canopy trees, and (3) deciduous woodlands defined by largely unmanaged tree stands. The study area on JBSA-Lackland Training Annex was divided by Medio Creek running north-south with an approximately 2000-ft wide floodplain. A total of nine transects with three transects in each vegetative

community (Fig. 6, 7, 8) were used. Four of these transects were in the floodplain and riparian areas and five of the transects were in upland areas.

T. cruzi distribution on JBSA-Camp Bullis was not analyzed. This installation was added as a study location for the 2016 calendar year with a goal of determining *T. cruzi* presence and prevalence in meso-mammals and large mammals.

3.3 MAMMAL SAMPLING (JBSA-LACKLAND TRAINING ANNEX AND JBSA-CAMP BULLIS)

Project personnel conducted seasonal (December 2012–2016) large and meso-mammal (body mass of 5.5–55 lbs) population surveys and trapping to determine *T. cruzi* incidence and estimate mammal abundance in various vegetative community types (i.e., grasslands, semi-improved woodlands, deciduous woodlands) and topographies (i.e., riparian/floodplain, uplands). On JBSA-Lackland Training Annex, personnel placed cameras along 9 different 328-ft transects for approximately 60 weeks prior to mammal trapping efforts (3 transects in each of the 3 vegetative communities). Cameras were set to take one picture followed by a 30 second video on a 15 second delay. Data were collected on 2GB standard digital cards and animals were individually identified when possible to help determine frequency of use. Each camera was located using a handheld global positioning system and then uploaded into a Geographic Information System. DITC were baited with aromatic lures, to include, skunk scent, liquid apple scent, oats and wet or dry cat food to attract diverse mammal species.

Seasons were defined as fall (September-November), winter (December-February), spring (March-May), and summer (June-August). Population estimates derived from mark-recapture data were limited to fall seasons due to small sample sizes. Mammalian relative abundance data were collected using 2 primary approaches: (1) digital infrared-triggered cameras (DITC), and (2) mark-recapture analyses derived from camera and live-capture data. First, 9 Cuddeback DITC (Non-typical Inc., Park Falls, WI) were placed at transect locations (one randomly placed along each transect at 1.6 ft above

ground, *n* = 9 total cameras) to determine presence of mammal species and provide relative abundance of large and mid-size mammals.

Relative abundance of small and meso-mammals was calculated using mark-recapture methods (White et al. 1982). Project personnel captured small mammals using 10 Sherman live traps (3 in x 3.5 in x 9.1 in; H.B. Sherman Traps, Tallahassee, FL) placed at 32.8-ft intervals along the 9 individual 328-ft transects (*n* = 90 total traps) described previously (Fig. 6, 7, 8). Meso-mammals were trapped using 5 Tomahawk Live Traps (18.9 in x 5.9 in x 5.9 in; Tomahawk, WI) randomly placed along each 328-ft transect (*n* = 15 total traps). Traps were baited with oats and cat food and set between dusk and dawn for 4–5 consecutive nights. Trapping events were conducted 2–4 times per season annually. Project personnel identified mammals by species, sex, age, weight, vegetation type captured in, and transect/trap number. Individuals were ear-tagged or paint marked for recapture identification.

Project personnel determined *T. cruzi* incidence and prevalence on JBSA-Camp Bullis using small and meso-mammal trapping and hunter check stations (white-tailed deer [*Odocoileus virginianus*] and feral hogs [*Sus scrofa*]). Trap locations were arranged to sample the broadest extent of JBSA-Camp Bullis (Fig. 10). Individual trap locations were based upon reports of high meso-mammal activity and trapper experiences. All small and meso-mammal capture locations on JBSA-Camp Bullis were in variants of forest, shrubland, or urban development due to availability and focus on potential capture success. Small and Meso-mammal trapping relied upon the same Tomahawk live traps used at JBSA-Lackland Training Annex. White-tailed deer and feral hog samples were obtained from carcasses brought to hunter check stations on JBSA-Camp Bullis during the late 2016 hunting season.

3.4 DISEASE DATA COLLECTION AND PREVALENCE ANALYSIS (JBSA-LACKLAND TRAINING ANNEX AND JBSA-CAMP BULLIS)

We collected blood and tissue samples from small and meso-mammals during density estimation capture and additional lethal captures on JBSA-Lackland Training Annex (medical-grade CO_2, Institutional Animal Care and Use Committee (IACUC) #2011-294). Additionally, we secured blood and tissue samples from concurrent white-tailed deer research and feral hog control efforts conducted on JBSA. We extracted femoral artery blood from live-captured and released white-tailed deer. We opportunistically collected whole blood and tissue samples from euthanized feral hogs caught in circular style corral traps by installation personnel. Blood samples were collected from anesthetized meso-mammals captured on JBSA-Camp Bullis in 2016. The meso- and small mammals were revived and released after sample collection. Blood was collected from white-tailed deer and feral hog carcasses brought to hunter check stations. Collected whole blood specimens were placed in BD Vacutainer® tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and tissue excisions, which included heart, liver, lung and muscle, were placed in Ziploc® plastic bags; specimens were stored in a cooler with dry ice for transport to the laboratory.

Parasitic behavior and different parasitemia levels occur between the trypomastigote (extracellular non-dividing form) acute and amastigote (binary fission replicative form) chronic disease phases, make detecting protozoan gene DNA sequences or host antibodies challenging depending on analysis techniques (Brasil et al. 2010). Polymerase chain reaction (PCR) investigations were applied in our study to detect specific gene sequence (parasite) molecules from meso-mammal blood and tissue samples, and to serve in further validation. To determine *T. cruzi* infection on Lackland Training Annex, both mammal whole blood and tissue samples were submitted to determine acute versus chronic infection. We extracted whole blood and tissue from mammalian samples. PCR analyses were performed by the Department of the Army Public Health Command Region – South, JBSA – Ft. Sam Houston, San Antonio, Texas as described by Kramm (2015). Samples were designated as *T. cruzi* positive based off

2 separate PCRs coinciding with expected 330 bp kDNA and 188 bp nDNA (Reisenman et al. 2010, Kramm 2015, Kramm et al. 2016). JBSA-Camp Bullis analyses were simpler and based upon both an on-site antigen strip and a laboratory PCR analysis of blood samples taken from anesthetized animals

3.5 DATA ANALYSES

We calculated species presence and selection of vegetation communities using DITC photographs (white-tailed deer, feral hogs) and trapping (small and meso-mammals). Densities of small and meso-mammals were calculated using the Schumacher-Eschmeyer mark-recapture analysis (Silvy et al. 2005). Triatomine distribution was determined through capture efforts, infection locations, and literature review. We compared *T. cruzi* prevalence rates between vegetative communities and between different topographies (i.e., floodplain versus upland) both using chi-square tests ($\alpha = 0.05$). We used descriptive statistics to determine *T. cruzi* prevalence on JBSA-Camp Bullis.

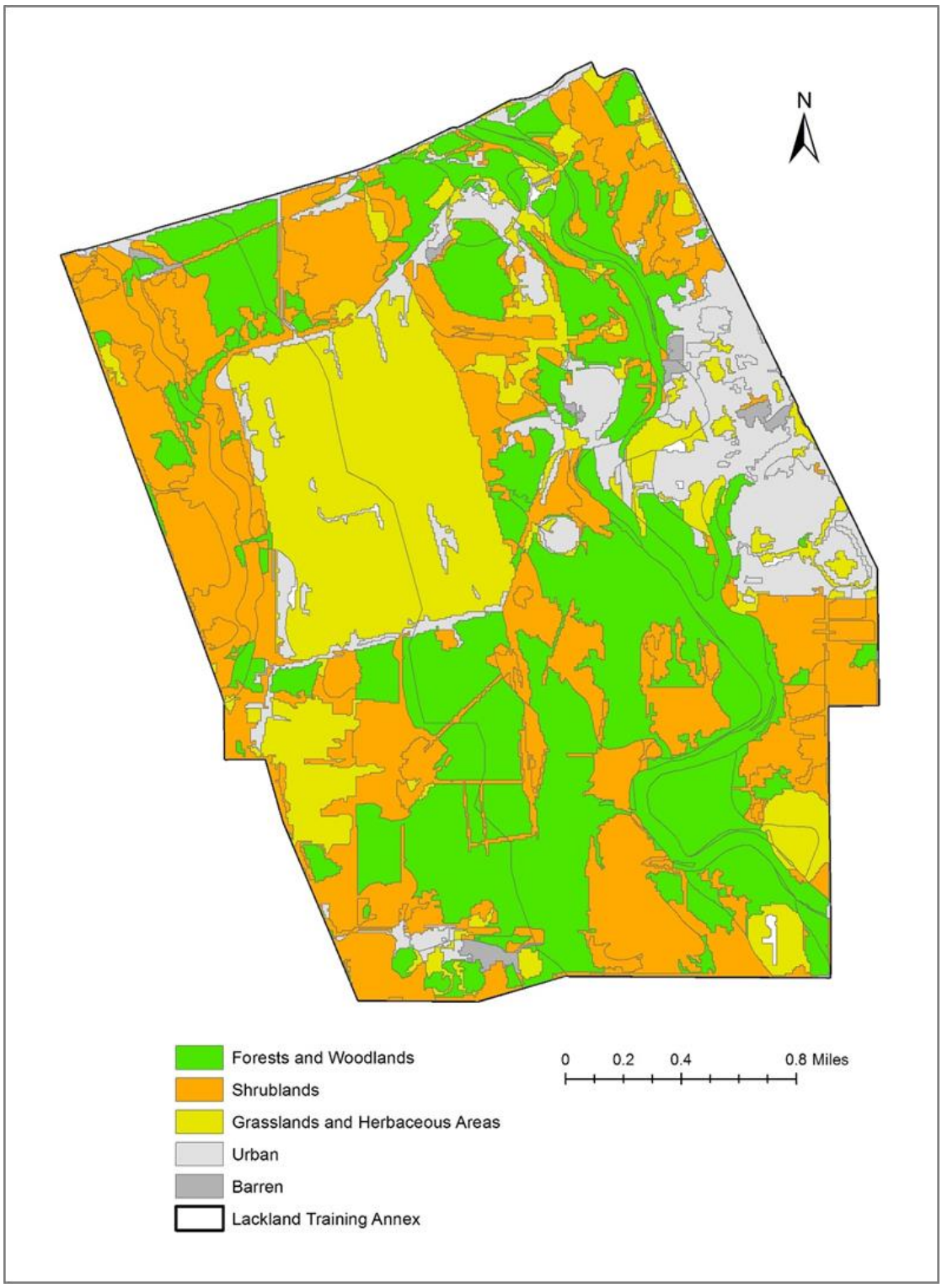

Figure 5. General land cover of JBSA-Lackland Training Annex, San Antonio, Texas.

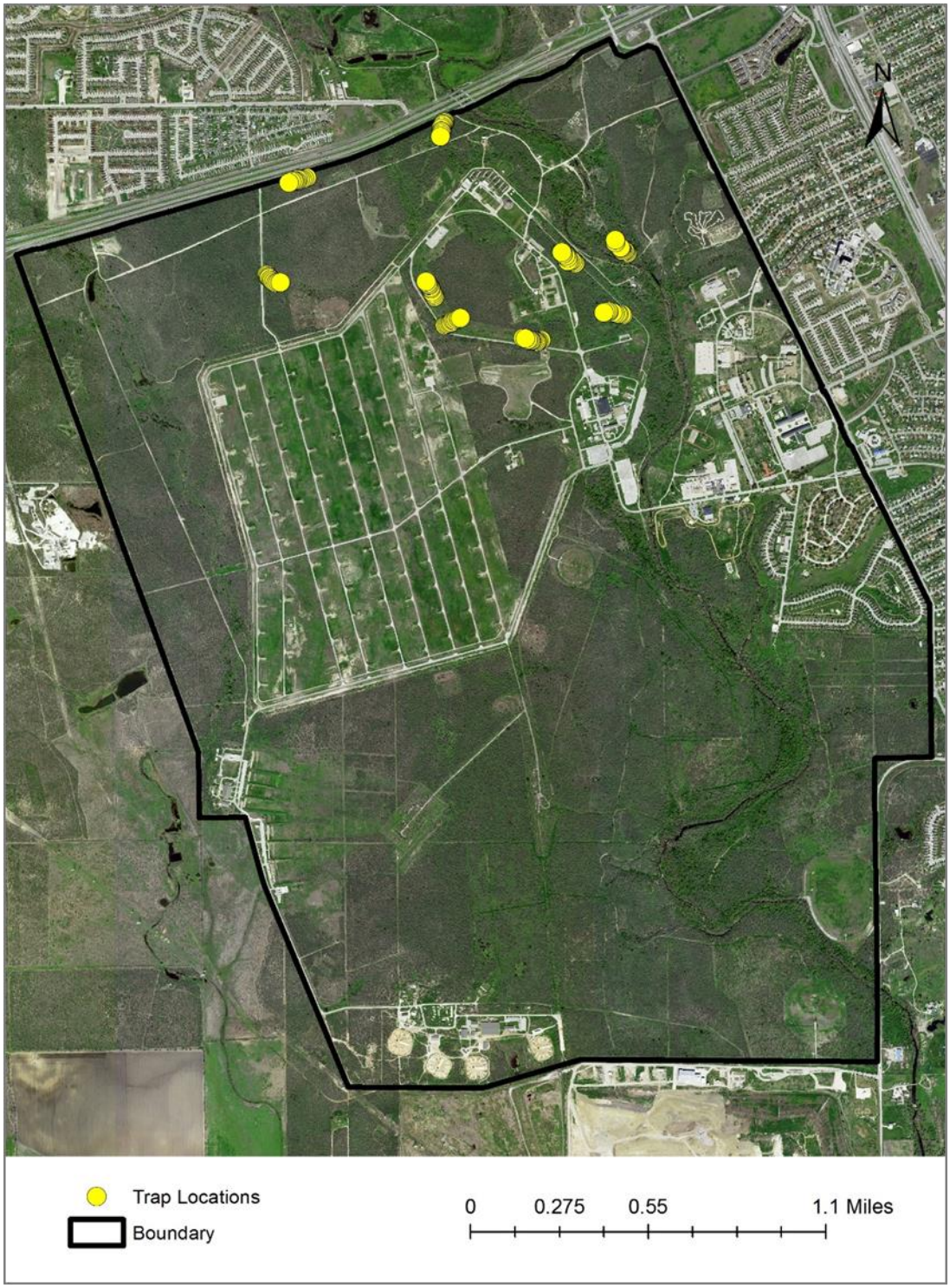

Figure 6. Chagas disease trap locations on JBSA-Lackland Training Annex, Texas, 2012–2015.

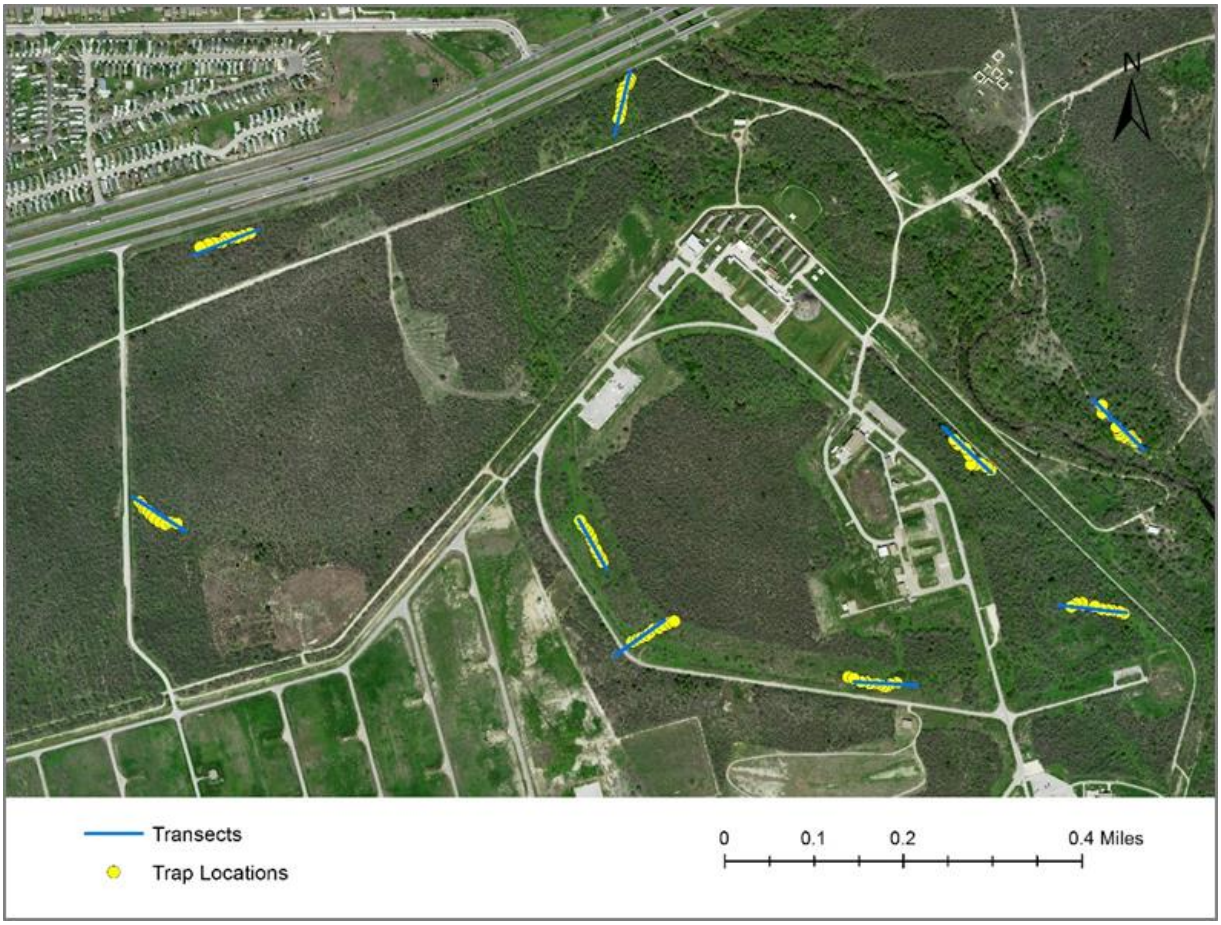

Figure 7. Overlay of transects and trap locations for the Chagas disease project on JBSA-Lackland Training Annex, Texas, 2012–2015.

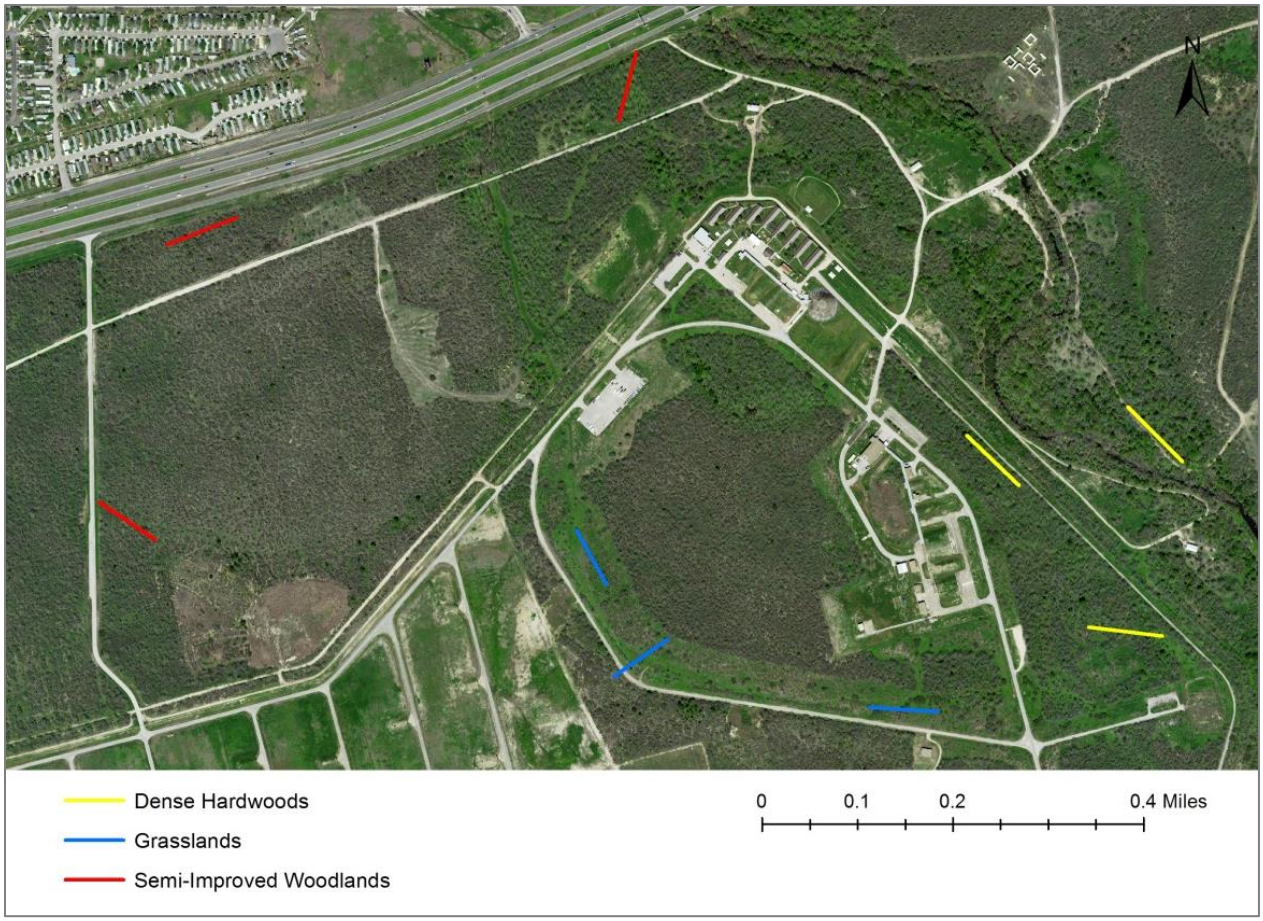

Figure 8. Chagas disease mammal capture transects on JBSA-Lackland Training Annex separated by vegetation type (dense hardwoods, grasslands, and semi-improved woodlands), San Antonio, Texas, 2012–2015.

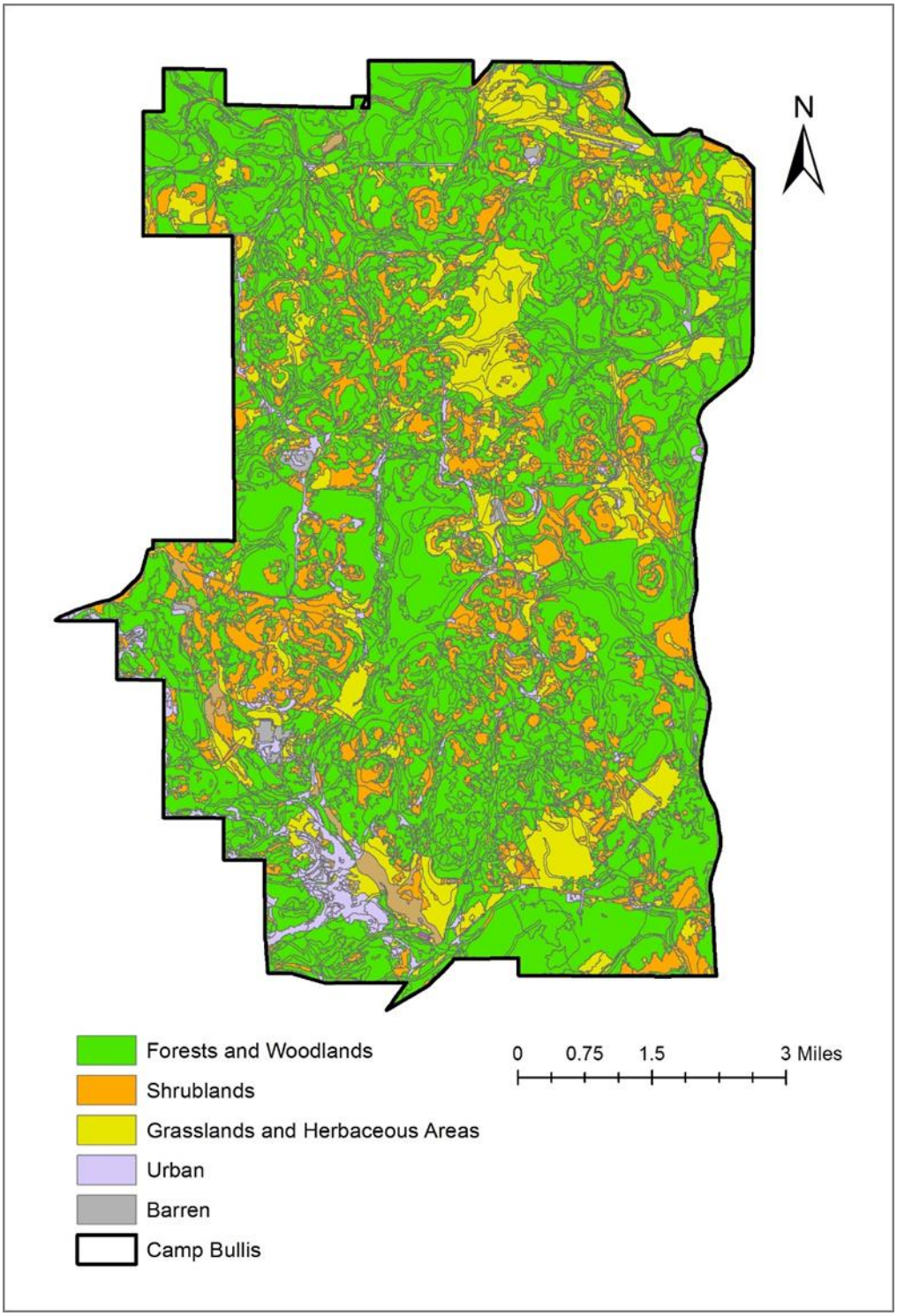

Figure 9. General land cover of JBSA-Camp Bullis, San Antonio, Texas.

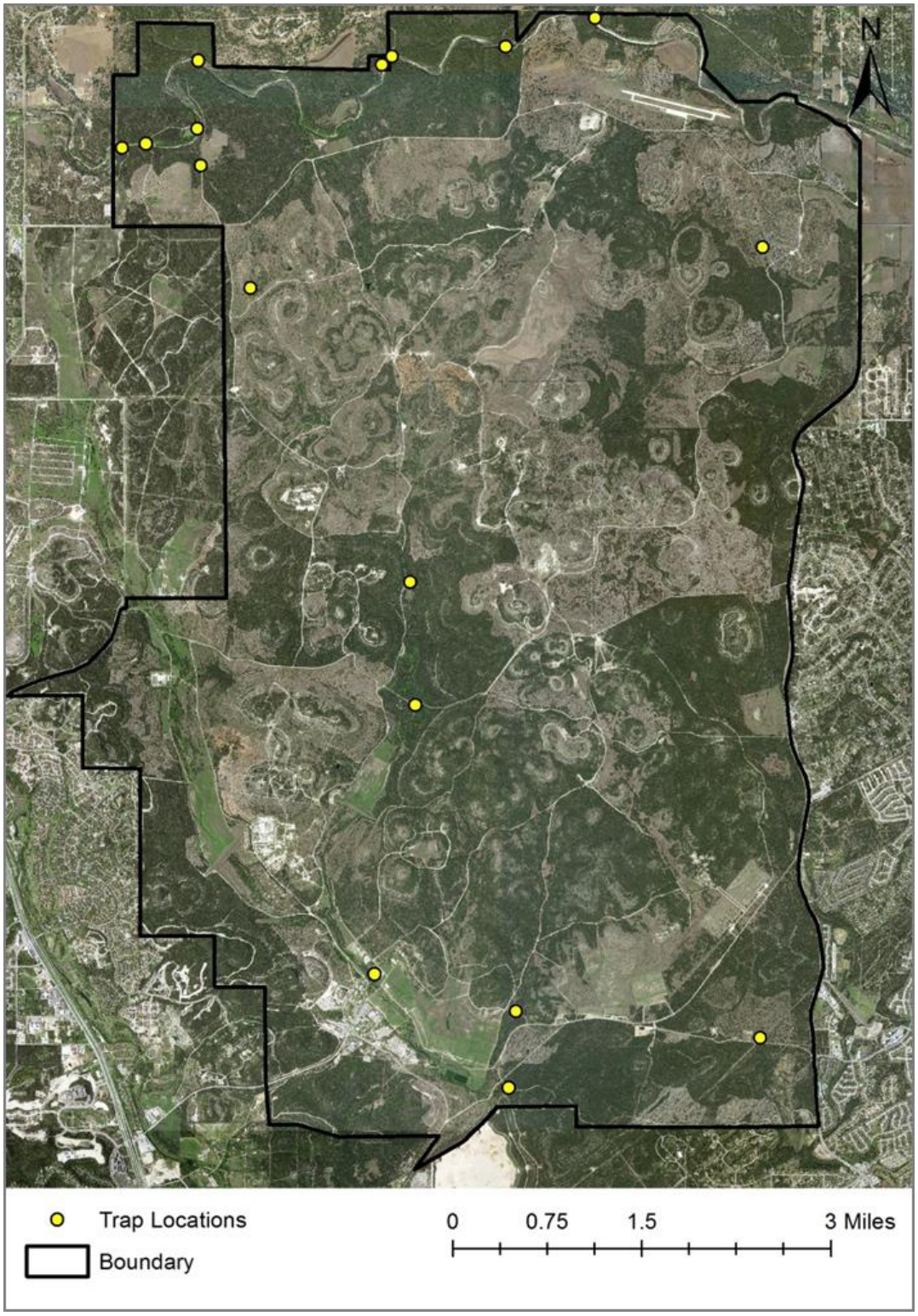

Figure 10. Chagas disease project trap locations on JBSA-Camp Bullis, San Antonio, Texas, 2016.

4.0 RESULTS

4.1 TRIATOMINE RELATIVE ABUNDANCE

Triatomine captures were conducted 2012–2015 on JBSA-Lackland Training Annex at 9 separate trap locations. Determination of distribution based on capture was not possible due to low capture success (*n* = 4). Neither light traps nor CO_2-based capture was successful. However, infection rates skewed higher in the forested areas; though this was impacted by host-species ecology. This was supported a concurrent U.S. Army study focusing on *T. cruzi* distribution and occurrence that relied primarily upon shovel-based excavation of suspected habitat (McPhatter et al. 2012). They found triatomines primarily in hollow logs or dead dry yucca plants indicating forest or successional forested areas. This follows general ecological knowledge of triatomines as inhabitants of mammal nests, within trees, or in urban environments (Lorenzo et al. 1998, Noireau et al. 1999, Grijalva and Vallacis 2009).

4.2 *T. CRUZI* SPATIAL DISTRIBUTION AND PREVALENCE

4.2.1 JBSA-Lackland Training Annex

DITCs recorded 15 mammal species in a total of 2,065 photographs (Fig. 11). White-tailed deer had higher relative abundances in photographs compared to other mammalian species (Fig. 2.2). White-tailed deer were photographed in much higher numbers than raccoons throughout all vegetative communities (white-tailed deer, *n* = 620 total photographs; raccoons, *n* = 493 total photographs), but selection of vegetative communities differed significantly between the 2 species ($\chi^2 = 44.008$, $P < 0.001$). Other species recorded from DITCs included coyote (10%), eastern cottontail rabbit (*Sylvilagus floridanus*) (8%), Virginia opossum (*Didelphis virginiana*) (6 %), javelina (*Tayassu tajacu*) (5%), striped skunk (*Mephitis mephitis*) (4%), nine-banded armadillo (*Dasypus novemcinctus*) (4%), wild hog (*Sus scrofa*) (3%), gray fox (*Urocyon cinereoargenteus*) (3%), bobcat (*Lynx rufus*) (2%), eastern fox squirrel (*Sciurus niger*)

(0.04%), ringtail (*Bassariscus astutus*) (0.2%), and wood rat (*Neotoma micropus*) (0.05) (Fig. 12, 13). Densities of *T. cruzi* positive species on JBSA-Lackland Training Annex was relatively high (Table 1). Sufficient data was present to estimate population densities for 3 species. However, these species also had high prevalence of *T. cruzi*.

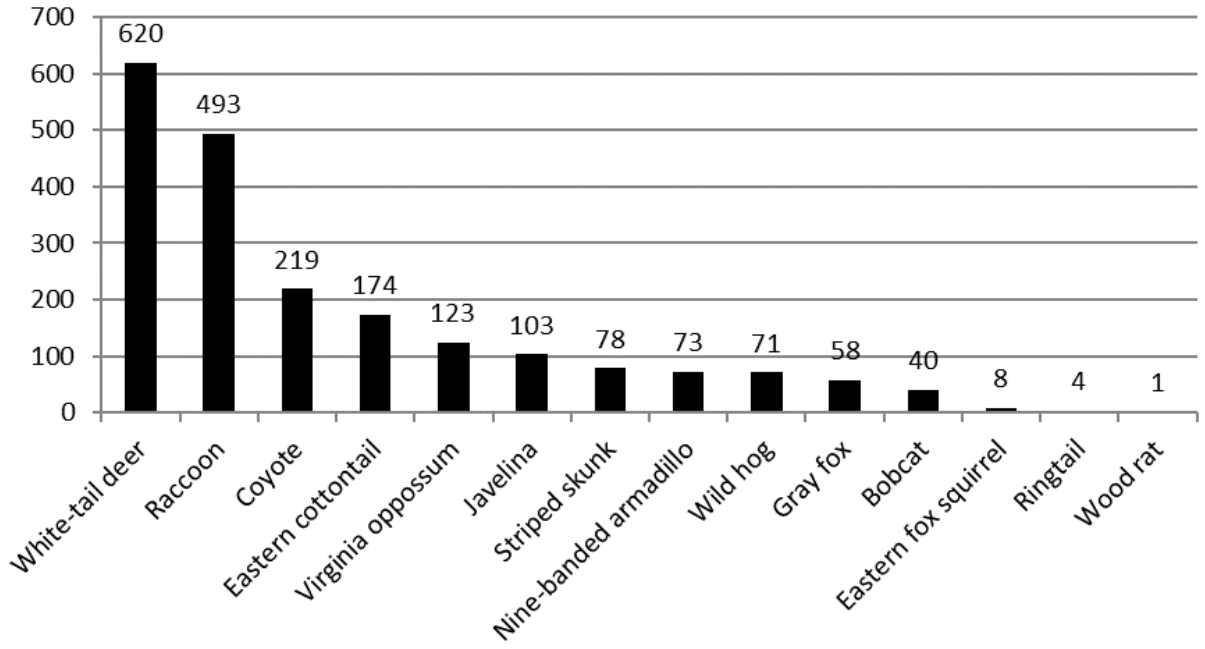

Figure 11. Photographs of species recorded via digital infrared-triggered cameras at JBSA-Lackland Training Annex, Bexar County, Texas, 2012–2015.

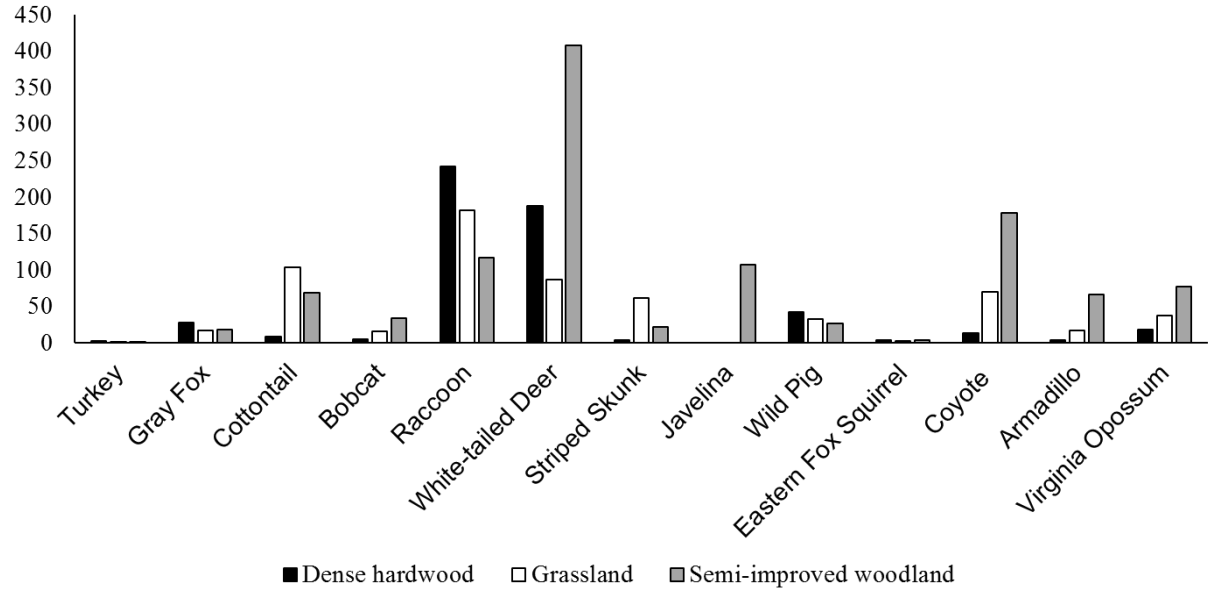

Figure 12. Photographs of mammals in each vegetation type recorded via digital infrared-triggered cameras, JBSA-Lackland Training Annex, San Antonio, Texas, 2012–2015.

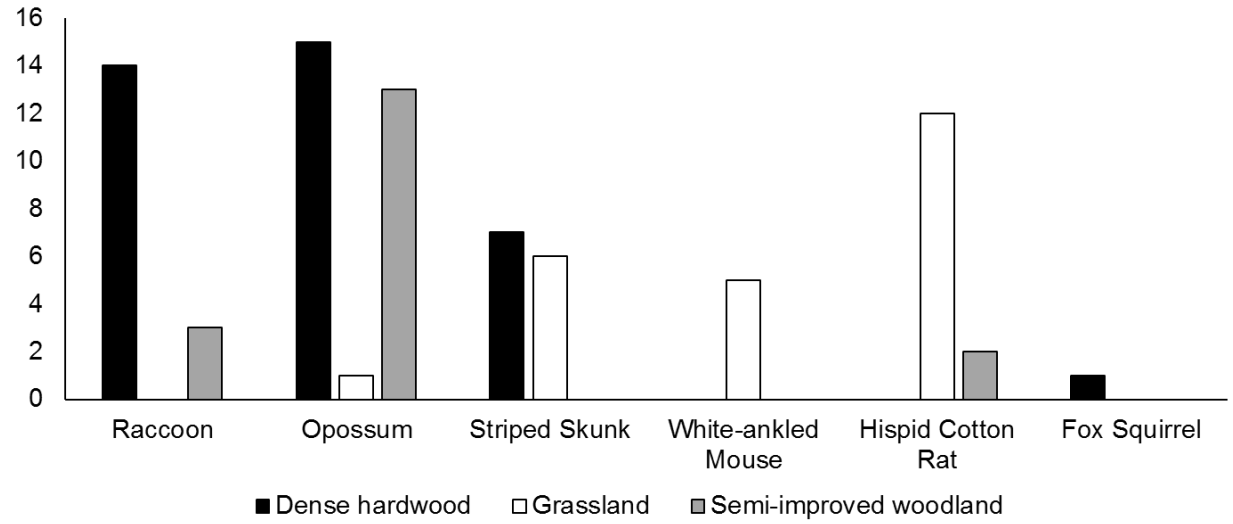

Figure 13. Mammal captures in each vegetation type, Joint Base San Antonio-Lackland Training Annex, San Antonio, Texas, 2012–2015.

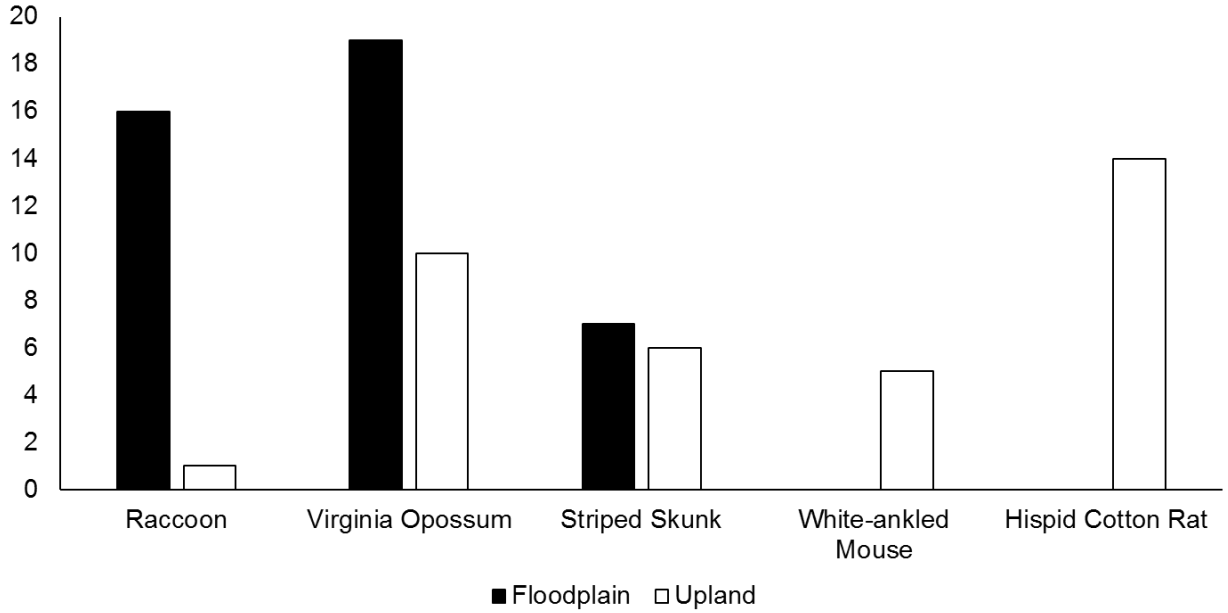

Figure 14. Mammal captures based on topography, JBSA-Lackland Training Annex, San Antonio, Texas, 2012–2015.

Table 1. Meso-mammal density estimates derived from mark-recapture data (9 transects) for JBSA-Lackland Training Annex, San Antonio, Texas, 2012–2015.

Species	Season	Density (km^2)	CI-Low (95%)	CI-High (95%)
Raccoon	Fall 2012	41.9	28.6	78.9
Virginia Opossum	Fall 2012	43.5	30.5	75.7
Virginia Opossum	Fall 2013	42.8	29.6	77.6
Striped Skunk	Fall 2012	49.9	31.3	123.0
Striped Skunk	Fall 2013	55.7	33.6	161.4

We tested 192 free-ranging meso-mammals and large mammals on JBSA-Lackland Training Annex for *T. cruzi* (captured, *n* = 79 tested; opportunistically collected (provided by concurrent study), *n* = 113 tested). We captured a total of 86 individual small and meso-mammals including 17 raccoons (*Procyon lotor*), 29 Virginia opossums, 13 striped skunks (*Mephitis mephitis*), 14 hispid cotton rats (*Sigmodon hispidus*), 1 southern plains wood rats, and 5 white-ankle mice (*Peromyscus pectoralis*), 4 plains harvest mice (*Reithrodontomys montanus*), 2 northern pygmy mice (*Baiomys taylori*) and 1 fox squirrel (*Sciurus niger*). We could not test 7 individuals due to various circumstances. Additional meso-mammal and large mammal samples (*n* = 3 hispid cotton rats, *n* = 14 raccoons, *n* = 20 striped skunks, *n* =18 white-tailed deer, *n* = 41 feral swine, *n* = 11 Virginia opossums, *n* = 4 plains harvest mice, *n* = 1 northern pygmy mouse, *n* = 1 fox squirrel) were obtained from unrelated but concurrent management efforts and also sampled for *T. cruzi*. We did not have spatial data for these opportunistically collected samples, which were not included in the spatial analyses.

As expected, captured species demonstrated strong preferences for different vegetation communities and topographies (Fig. 2, Fig. 3, Fig. 4). We found *T. cruzi* in 4 species including captured and opportunistically collected samples with significant differences in *T. cruzi* prevalence among species (χ^2 = 18.113, df = 3, P<0.001; Virginia opossums, *n*=26 positive, 65% of sampled; striped skunks, *n*=9 positives; 27% of sampled; raccoons, *n*=14 positives; 45% of sampled; and hispid cotton rats, *n*=2 positive; 12% of sampled). No other species tested positive for *T. cruzi*. The preponderance of *T. cruzi* infections found in verified locations (from captured animals) occurred in the lower elevation floodplain (62% positive, *n* = 46 captures) as compared to the higher elevation uplands (27% positive, *n* = 33 captures; Table 1). These also represent significantly higher infection rates in the floodplain than in the uplands (χ^2 = 9.849, df = 1, P = 0.002). Additionally, we found more infected animals in the dense hardwoods vegetation community (*n* = 22), than semi-improved woodlands (*n* = 11), or grasslands (*n* = 4 positives). A greater percentage of captured animals were infected in the semi-improved woodlands (*n* = 11 positives, *n* = 18 captures, 61%) when compared to the other vegetative communities (dense hardwoods, *n* = 22 positives, *n* = 37 captures,

60%; grasslands, *n* = 4 positives, *n* = 24 captures, 17%; Table 1). Grasslands had a significantly lower infection rate when summed across all species compared with dense hardwoods and semi-improved woodlands (χ^2 = 13.078, df = 2, *P* = 0.001). Dense hardwoods and semi-improved woodlands did not differ significantly in infection rates (χ^2 = 0.495, df = 1, *P* = 0.481).

Table 2. Captured mammals tested and incidence rate of *Trypanosoma cruzi*, JBSA-Lackland Training Annex, San Antonio, Texas, 2012–2014.

		# Positives Samples - Vegetation			**# Positive Samples - Topography**	
Mammal Species	Total Tested	Grasslands	Semi-Improved Woodlands	Dense Hardwoods	Upland	Floodplain
Hispid cotton rat	14	1	0	0	1	0
White-ankle mouse	5	0	0	0	0	0
Fox squirrel	1	0	0	0	0	0
Virginia opossum	29	0	9	12	5	16
Raccoon	17	0	2	5	0	7
Skunk	13	3	0	5	3	5
Total	**79**	**4**	**11**	**22**	**9**	**28**

4.2.2 JBSA-Camp Bullis

Project personnel captured and sampled a total of 47 small and meso-mammals on JBSA-Camp Bullis (Table 3) over 289 trap nights (May–June 2016) and collected samples from 12 large mammals (*n* = 5 white-tailed deer, *n* = 7 feral hogs) at hunter check stations in 2016 (October – November 2016). A total of 34 small and meso-mammals (72%) tested positive (either antigen strip, laboratory analysis or both) for *T. cruzi*. Virginia opossums and raccoons were the predominant captured species representing 36 out of the total 47 captures (77%). Both species had very high *T. cruzi*

prevalence (Table 3; Raccoons, prevalence = 93%; Virginia Opossums, prevalence = 78%). Only 2 positives (*n* = 1 fox squirrel (*Sciurus niger*), *n* = 1 striped skunk (*Mephitis mephitis*)) were detected out of the remaining 11 captured animals. No *T. cruzi* was detected in any of the large mammals sampled on JBSA-Camp Bullis.

Table 3. Mammals sampled for *T. cruzi* on JBSA-Camp Bullis, San Antonio, Texas, 2016.

Species	**# Sampled**	**# T. cruzi Positive**
Raccoon	27	24
Virginia oppossum	9	7
Striped skunk	1	1
Fox squirrel	9	1
Nine-banded armadillo	1	0
White-tailed deer	5	0
Feral hog	7	0

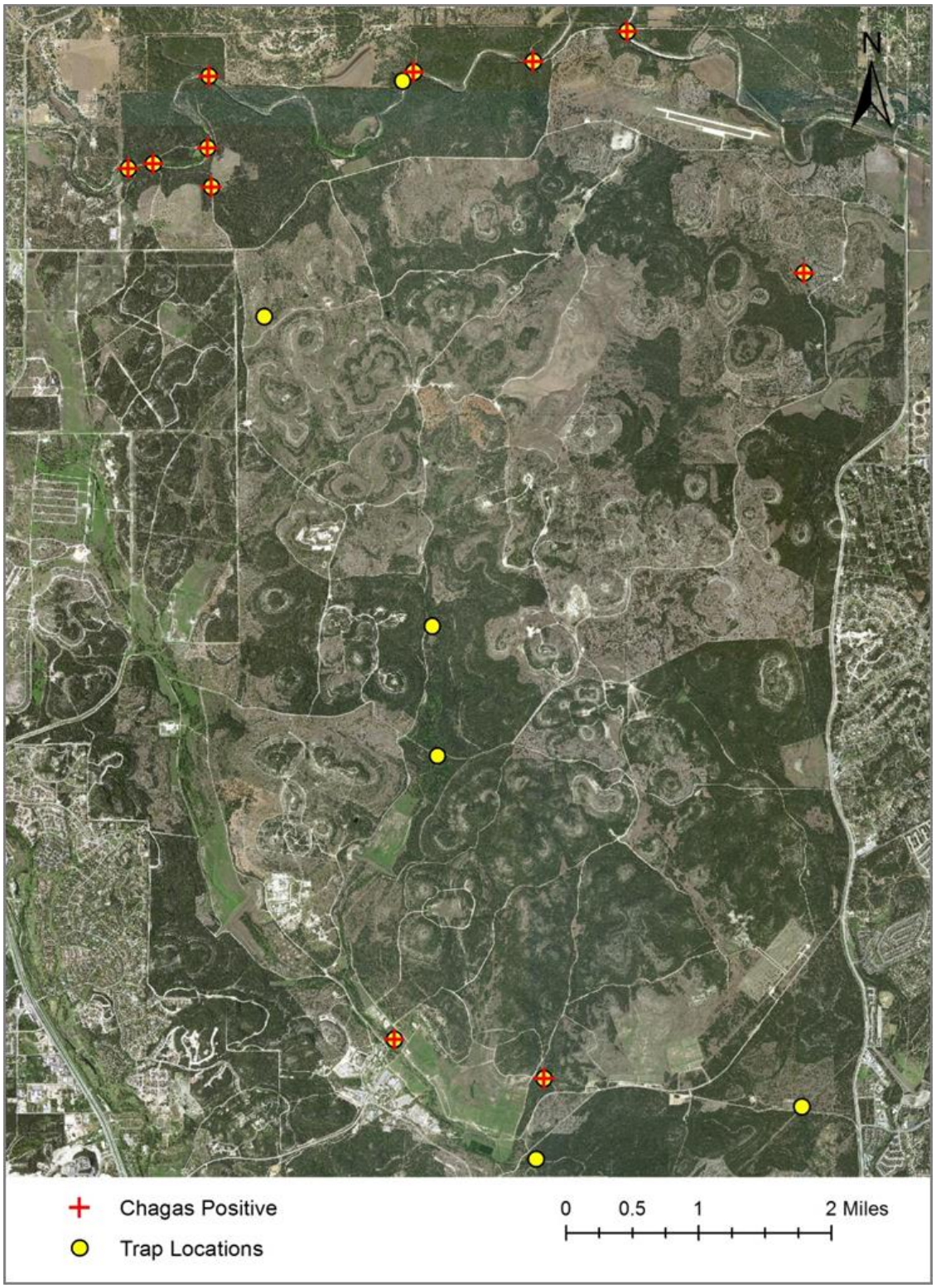

Figure 15. Chagas disease project small and meso-mammal capture locations with associated positive results, JBSA-Camp Bullis, San Antonio, Texas, 2016.

4.3 SPECIES RELATIVE ABUNDANCES AND POPULATION DENSITIES (JBSA-LACKLAND TRAINING ANNEX)

DITCs recorded 13 mammal species in a total of 2,302 photographs. We found that white-tailed deer (*n* = 683 photographs) and raccoons (*n* = 539 photographs) had higher relative abundances in photographs compared to other mammalian species (Fig. 1). However, selection of vegetative types and topographies differed among species (Fig. 2, Fig. 3). *T. cruzi*-positive species were found throughout the study area with raccoons

We pooled mark-recapture data into 12–16-week trap periods due to acceptable mark retention to support data collection. We determined densities by study season and year; however, we had insufficient data to calculate raccoon population densities in 2013. We had sufficient mark-recapture data to determine densities of the primary reservoirs of *T. cruzi* (raccoons, Virginia opossums, striped skunks). The study found that densities of species with confirmed *T. cruzi* infections were comparable among seasons, though striped skunks showed relatively higher population densities (Table 2). We had insufficient data to calculate densities for hispid cotton rats.

5.0 SUMMARY

Core areas for Chagas disease infection were primarily found within floodplain or riparian deciduous forests. The Chagas disease-positive species included raccoons, Virginia opossums, striped skunks, and hispid cotton rats, which are typically generalists with the ability to live in human-dominated areas but also naturally gravitate towards riparian zones and forested habitats. It is likely that animals tested were the primary hosts for the pathogen and the low numbers of test-positive animals in grasslands or semi-improved woodlands is reflective of habitat preferences for these species. This is supported by research that indicates triatomines are located in a variety of habitats including grasslands, woodlands, and human-dominated areas such as houses and often feed on mammals (Bern et al. 2011). Additionally, these species are at heightened risk of triatomine exposure as primary or occasional fossorial (burrow-dwelling)

mammals. Mammals in our study that rarely if ever utilize burrows (e.g., white-tailed deer and feral hogs) showed surprisingly no measurable level of infection. This project recognized meso-mammals as localized primary *T. cruzi* mammal reservoirs, which supports the hypothesis that these common mammals help maintain and transmit these parasites in association with vector triatomines. Furthermore, despite the different characteristics of the 2 types of woodlands we studied, they still had significantly higher rates of mammal infection than the upland grasslands; thus, lending credence that these species (woodland and riparian generalists) are important in pathogen persistence.

The lack of evidence of *T. cruzi* in all rodents except in hispid cotton rats is contrary to the findings of Pinto et al. (2010), and may be attributed to phylogenetic lineage differences between the protozoan pathogen and diversified mammal composition found within specific vegetation communities (Roellig et al. 2009). Similarly, no evidence was found that white-tailed deer and feral hogs are major contributors of the pathogen, and can probably be attributed to the lack of burrowing activity (where insects are commonly found) and different resting locations. This project largely supports much of the available evidence of mammal host roles in *T. cruzi* persistence. Beard et al. (2003) reported that *T. cruzi* infection of canine species in South Texas may be endemic. A recent study in Mexico identified the blood meal origins of 47 triatomines and found that raccoons and armadillos (*Dasypus* spp.) were the main blood meal hosts (Bosseno et al. 2009). Another study in the U.S. reported *T. cruzi* infection among 11 reservoir species from 6 southern states, and detected a higher incidence of the infection in Virginia opossums and raccoons (Brown et al. 2010). These studies suggest that *T. cruzi* prevalence varies between preferred host species and geographic regions.

The distribution of *T. cruzi* prevalence in mammal species has implications for managing wildlife populations to limit disease potential to humans. Raccoons, Virginia opossums, and striped skunks occur in relatively high population densities in our study area. Additionally, these species prefer deciduous forests located near bodies of water

and actively utilize burrows, hollow logs and other enclosed areas. Based on this project, no management action is recommended for the majority of rodent species and larger game species. Instead, management minimizing the risk of exposure to target host species should focus on meso-mammals, specifically raccoons, Virginia opossums, and skunks. Due to the high incidence of *T. cruzi* among these animals, however, their simple removal should be applied with caution because the kissing bugs typically associated with them could become displaced and search for alternative blood meals from pets and people (Barr 2009). Also, removal or large population reduction of meso-mammal species may lead to unpredictable trophic cascades or species adaptations (e.g., reduction of raccoons may allow Virginia opossum populations to increase). Modeling of meso-mammal population dynamics is proposed to evaluate various management actions *a priori* to include management of vegetative communities having relatively high prevalence of *T. cruzi* infection. Peridomestic meso-mammals are typically not constrained by natural predators, and are occupying South Texas microhabitat regimes that increase the human interactions threat of zoonotic diseases. Managing vector and reservoir movement requires an integrated control approach that includes, identifying harborage locations, select spatial insecticide applications, mechanical or chemical vegetation management to discourage vectors and reservoirs, and Environmental Protection Agency (EPA) approved mammal bait formulations impregnated with systemic insecticide (McPhatter et al. 2012) that produces hematophagous triatomine mortality without deleterious effects on vertebrate hosts. These management recommendations are detailed in the following section.

6.0 MANAGEMENT RECOMMENDATIONS

6.1 VEGETATION AND PEST MANAGEMENT

Perimeter maintenance will be important around human sleeping facilities and working dog kennels and holding areas. The keys are preventing triatomine access to vulnerable facilities.

1. Forested areas appear to be the primary vegetation type harboring triatomines and mammal hosts. We recommend maintaining a minimum 100-ft offset between these buildings and surrounding forested areas. This may require limited mechanical tree removal and herbicides approved by the EPA for application near living areas. We also recommend that bushes and rocks be removed when temperatures are below 55°F to limit triatomine cover and movement. This will limit, though not prevent, triatomine travel to the target buildings. Additionally, this will minimize, though not prevent, free-ranging mammal contact with the target building areas.

2. We also recommend consistent mowing of this perimeter area, particularly during the triatomine active period (April–July). Tall grass should be prevented to minimize cover for triatomines and burrowing mammals. Prescribed fire would also be an option in both grasslands and forested areas. This would need to be coordinated with licensed prescribed burn specialists and be integrated into the existing INRMP and an appropriate burn plan.

3. The perimeter should be monitored for mammal burrows. Burrows should be filled and mammals should be removed or relocated. Trash cans and other mammal attractants should be secured or removed to discourage meso-mammals.

4. Light Management (Attraction). Facility lighting and sanitation are undoubtedly the two primary contributing factors leading to an arthropod infestation and its continuation as a pest problem. Lights are used to provide facility security both inside and outside at night when the facility is not in operation, but also attract flying insects to and into the structure. These pests attract other pests. We recommend special lights that emit light frequencies that are less attractive to light-attracted insects. Light management is critical. When identified as a contributing factor creating the existing infestation or if uncorrected can result in

continued re-infestation, pest control personnel will not respond and implement a control until these issues have been corrected.

5. Appropriate pesticides should be applied around the perimeter of the buildings to discourage triatomine infiltration. Pesticides must conform to U.S. Environmental Protection Agency (EPA) regulations regarding application around areas of human and canine habitation. The CDC indicated that synthetic pyrethroid sprays have been used successfully in Latin America. Similar chemicals are available in the United States, though they have not been label approved for use against triatomines, but synthetic pyrethroid products can be used on the site. Only pest control licensed professionals may apply these products. This is in accordance with pesticide product label and JBSA Integrated Pest Management Plan (2016). Due to triatomines requiring a blood meal for growth, habitats should be surveyed at least weekly between March and September, or when temperatures are sufficient for blood feeding and development (usually above 65 degrees F for a minimum of 2 weeks).

    a. Pesticide Use
        i. Pesticides are poisonous. Always read pesticide label to determine active ingredients and signal words. Carefully follow all precautions and safety recommendations given on the container label.
        ii. Store all chemicals in the original labeled containers in a locked cabinet or shed away from food or feeds and out of the reach of children, unauthorized persons, pets and livestock.
        iii. Pesticides can move and contaminate creeks, lakes, and rivers. Confine chemicals to the property being treated and never allow them to get into drains or creeks. Avoid drift onto non-target properties.
    b. Pesticide and Container Disposal
        i. Do not dispose of containers with pesticides in the trash. Do not pour pesticides down sink, toilet or outside drains.

   ii. Use pesticides according to the label until the container is empty or properly dispose according to installation hazardous waste guidance.
   iii. Dispose of empty containers by following label directions.
   iv. Never reuse or burn the containers or dispose of them in such a manner that they may contaminate water supplies or natural waterways.
6. Mechanical control of insects is more difficult but can be used as a supplement to habitat and chemical control strategies. These methods include bottle and sticky traps that can provide both monitoring data and control benefits. These generally rely upon mechanical capture with an associate lure (e.g., CO_2). For example, bottle traps are generally made from 2-3 liter soda bottles.

6.2 HUMAN AND CANINE SAFETY

Maximizing human safety requires minimizing human contact with triatomines. Monitoring of canines at JBSA-Lackland MWD facilities revealed a *T. cruzi* antibody prevalence of 8% (DoD Military Working Dog Veterinary Service, unpublished data, 2011). We recommend a two-fold strategy to reduce the possibility of human and canine exposure to Chagas disease.

6.2.1 Facility Security

1. Facilities supporting sleeping personnel or working dogs should be carefully secured. Thoroughly check the exterior and interior of the facility for openings such as torn screens, vents, cracks, and poorly adjusted windows and doors. Cover vents with screen mesh. Seal cracks when detected. Keep window screens closed and in good condition. Close doors securely when entering or exiting the facility.

2. Frequently check all sleeping areas and kennels and holding areas when people or dogs leave or return to the facilities. Kissing bugs can hide in bedding materials or cracks and crevices in sleeping areas. Working dogs should sleep inside secured facilities.

3. Any accumulation of organic materials (manure, kennel wastes, etc.) should be eliminated if these accumulations become breeding sources for these pests or attractants for meso-mammals such as raccoons. Kennel personnel are responsible for managing the waste from MWD in such a manner to eliminate these materials from becoming pest breeding sites or meso-mammal attractants.

4. Dogs should be carefully monitored when they are outside. Dogs can infect themselves when ingesting kissing bugs. Conduct an external check of the dog if it has walked through a forested area. If a kissing bug is observed, remove any MWD from the area.

5. All internal and external areas of target buildings should weekly be checked carefully for triatomine presence. **Never handle kissing bugs with bare hands.** Chagas Disease is communicable to dogs and humans. If a kissing bug is detected, remove it using a plastic bag or disposable gloves. Wash hands thoroughly with soap and water.

6. Working dogs should be tested semi-annually for Chagas disease and to monitor prevention efficacy.

6.2.2 Human Field Safety

Triatomines are most active during the periods of April – July. They are known to actively prey upon sleeping humans and animals. Personnel that must maneuver or camp in areas known to support triatomines should take precautions. This is especially important for forested areas or other areas that support burrowing animals.

1. Personnel should carefully check clothing after excursions into forested areas.

2. Insect repellent should always be worn including Deet and Permethrin.

3. All personnel sleeping in the field should take care to wear loose clothing that stays away from the skin, face netting away from the face to prevent triatomine feeding through the mesh (Fig. 16), and application of recommended insect repellents. No bare skin should be approachable by triatomines. Preferably, personnel will use well-maintained tents without ingress points for triatomines.

4. Tents, bedding and sleeping areas should be carefully checked for triatomines prior to sleeping. The external areas of bedding materials can be treated with permethrin.

Figure 16. Example head netting that helps separate the net from the face.

Human and canine safety can be maximized through vigilance and common sense. It is recommended that installation safety personnel discuss these issues with relevant commanding officers. Brief informational meetings would help to highlight the ways to minimize the risk of exposure. Additionally, reminder flyers can be posted at critical facilities (see draft flyer at end of document).

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Chagas Disease

U. S. Department of Defense
Joint Base San Antonio

Be Alert. Be Thorough.

Chagas disease has been confirmed on **JBSA-Lackland Training Annex** and **JBSA-Camp Bullis**. Chagas disease is an incurable and chronic illness transmitted by triatomines (kissing bugs, see below) when they feed on animals (locally, humans and other mammals) or when the insect is ingested (such as when a dog eats one). Recent research efforts have found that kissing bugs and infected burrowing mammals largely reside in forested areas. However, Chagas disease has also been confirmed in working dogs trained at this installation. Kissing bugs can enter facilities through poorly sealed openings, stay hidden in small cavities, and emerge to feed on personnel or working dogs.

Photos courtesy of S. Kjos

Protect Yourself. Protect Your Colleagues. Protect Your Animals.

Secure all openings to the outside of the facility. Thoroughly check the exterior and interior of the facility for openings such as torn screens, vents, cracks, and poorly adjusted windows and doors. Cover vents with screen mesh. Seal cracks when detected. Keep window screens closed and in good condition. Close doors securely when entering or exiting the facility.

Check all kennels and holding areas when dogs are removed and returned to the facilities. Kissing bugs can hide in bedding materials or cracks and crevices in sleeping areas. It is advised that dogs stay in controlled facilities when not training or recreating.

Monitor dogs carefully when outside. Dogs can infect themselves when ingesting kissing bugs. Conduct an external check of the dog if it has walked through a forested area.

Observe internal and external areas carefully. Never handle kissing bugs with bare hands. If a kissing bug is detected, remove it using a plastic bag or disposable gloves. Wash hands thoroughly with soap and water.

For more information contact Dr. Matt Kramm (210-395-1749).