Geographic Distribution and Bionomics of *Triatoma dimidiata* (Reduviidae: Triatominae) in Northern Belize, Central America

by

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Angela T. Caranci

[May 20th, 2016]

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ABSTRACT

Geographic Distribution and Bionomics of *Triatoma dimidiata* (Reduviidae: Triatominae) in Northern Belize, Central America:

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The triatomine vectors (Hemiptera: Reduviidae) responsible for the transmission of Chagas disease are widely prevalent from the southern United States southward throughout much of Central and South America, ending in southern Argentina and Chile. Across this broad region, several important vector species serve as the main mode of transmission of *Trypanosoma cruzi*, the causative parasite of Chagas disease, to human hosts. Belize, a country in Central America, has reported the presence of a competent, infected vector species and limited instances of human disease. While several factors influencing vector presence have been described in the southern districts of Belize, the species distribution and associated attributes of household invasion in the north had not been investigated.

Here, we compare methods for surveillance of triatomine vectors within households of northern and central Belize. The only vector species recorded, *T. dimidiata*, was designated sylvatic in nature, having strong implications for further

surveillance and control strategies. Surveys targeting 20 ecological and social attributes associated with local households were modeled to determine association with *T. dimidiata* invasion. The final multivariate regression model developed from this data determined that *T. dimidiata* invasion was associated with the presence of peridomestic animals and proximity of community light sources.

Because the presence data of triatomine vectors in Belize are scarce, ecological niche models were developed with source data from neighboring countries of Central America. Presence data from the region were modeled with respect to altitude and climate data layers to develop predictive maps for *T. dimidiata* and another important Central American vector, *Rhodnius prolixus*. Altitude and temperature profiles were both associated with the predicted presence of these common vector species.

Initial assays for determining the effects of commonly used insecticides on colony-reared *T. dimidiata* are also described. There is some evidence that common control practices must be altered to achieve any effect on the target vector population. Collectively, the information gained from this research has direct bearing on surveillance and control of Chagas disease vectors in Belize, and may be used to strengthen ongoing efforts of local programs.

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CHAPTER 1: General Introduction

CHAGAS DISEASE: GENERAL EPIDEMIOLOGY

Varying levels of success have been attained in decreasing the incidence of Chagas disease throughout endemic areas of Central and South America, yet many aspects of the biology of transmission and control of the insect vectors must remain a focus of current research to maintain this decline (11). *Trypanosoma cruzi* is the causative agent of Chagas disease, and is vectored by true bugs of the order Hemiptera in the family Reduviidae, subfamily Triatominae (7). The parasitic protozoan undergoes cyclodevelopmental transmission and must develop and propagate through both vertebrate and invertebrate hosts to complete its lifecycle (42). When humans encounter infectious feces of invertebrate vectors, *T. cruzi* parasites can enter and infect human hosts resulting in a spectrum of disease symptoms referred to as Chagas disease. Human Chagas disease can range from asymptomatic infection to chronic disease affecting the tissue of major organs including the heart, esophagus, and rectum (42).

Trypanosomiases occur mostly in the tropics and subtropics throughout the world, causing high levels of morbidity and mortality in endemic areas (42). *T. cruzi* is known only from the New World, where the distributions of the pathogen and known vectors span from the southern United States south to areas of southern Argentina and Chile (7). Within this range, the disease is only second to malaria in the amount of area that is endemic for the parasite, causing an estimated 8 million current infections and threatening approximately 109 million people who live at risk (73). Due to increases in globalization and immigration, as many as 300,000 people infected with *T. cruzi* may be

currently living in the US, with thousands more documented in Europe, Australia and Japan (6; 24).

Chagas disease has been listed among the major neglected tropical diseases largely due to a lack of adequate diagnostic tools and treatment protocols (36). The success gained in decreasing the incidence of Chagas disease in the New World has varied due to differences in surveillance and control of the disease and associated insect vectors among afflicted countries (12). The programs that have shown success in controlling Chagas disease have largely centered on locating human houses with high rates of vector infestation and treating these areas with insecticides in order to diminish the risk of insect-human contact (12). While these efforts have reported success in some regions where transmission is predominantly due to domesticated vector populations, it is becoming apparent that control programs must be more integrated and targeted to local transmission ecology (12). In order to develop focused control programs that highlight regional aspects of disease transmission, it is essential to determine locally occurring vector species, assess vector population infection rates, model factors affecting vector behavior, compare efficient means of vector surveillance, and regularly evaluate the effects of common control methods on local populations.

Trypanosoma cruzi and Human Chagas Disease

Parasite Biology and Development

T. cruzi, a protozoan parasite, requires development in both vertebrate and invertebrate hosts (Figure 1) (90). As with other species in the order Trypanosomatida, the morphology of the parasite changes throughout the course of development (Figure 2) (90). Trypomastigotes are picked up in the vertebrate host blood meal by the vector insect

and enter the proventriculus where the parasites differentiate into sphaeromastigotes and epimastigotes. Later, in the midgut, the parasites exist as epimastgotes which multiply by binary fission. Epimastigotes migrate to the hindgut, wherein, depending on temperature, metacyclic trypomastigotes develop after 1-2 weeks. Here, metacyclics may attach to the inner cuticular layer of the rectum (22; 40). Competent vectors defecate during or immediately after feeding, leaving feces on the human host's skin surface (22). Metacyclic trypomastigotes in the feces can enter through the bite, through the penetration of mucus membranes on the face, through previously existing abrasions or orally through food contaminated with vector feces (38). Overall, the interaction between the vector gut and parasite is limited compared to related parasites such as *Trypanosoma brucei* in Glossinidae and *Leishmania* spp. in sand flies (22). As opposed to *T. brucei* and *Leishmania* spp., *T. cruzi* parasites develop and proliferate solely in the gut of invertebrate hosts and are not challenged with escaping gut barriers. Defenses in the insect gut are limited to the actions of proteases and lytic enzymes released in the midgut; however, *T. cruzi* has shown little weakness to vector defense mechanisms (22). The lack of efficient defense responses available to vectors contributes to *T. cruzi*'s ability to experimentally infect several different arthropods including bed bugs and ticks, while maintaining infection rates greater than 50% (82).

The full development of the parasite in the gut of the insect vector may take 6-15, days depending on ambient temperature, with transmission by insect vector via the posterior station, or stercorarian route. The parasites then enter the blood stream by mechanisms mentioned above and infect specific cells including smooth or striated muscle cells, fibroblasts, glial cells, and neurons, or are engulfed by phagocytes, most

readily macrophages (3). In the host cells, trypomastigotes transform into dividing amastigotes that undergo binary fission in pseudocysts. When the parasite reaches high numbers, the pseudocyst ruptures and spills parasites into the interstitial space. The amastigotes transform to promastigotes and again to epimastgotes before becoming trypomastigotes again and re-entering the bloodstream (3). In the bloodstream, trypomastigotes can infect additional host naïve cells or be taken up in a blood meal by the insect vector (22).

Parasites that require completion of life cycle in both vertebrate and invertebrate hosts commonly have a definitive host where genetic exchange takes place (42). Historically, *T. cruzi* was thought to only replicate by way of asexual, binary fission, but recent studies have shown that genetic exchange may occur in sylvatic transmission cycles (48). While authors provide some evidence for variability that infers the presence of genetic exchange in wild populations, little evidence is known to conclusively state that exchange occurs or if so, at what stage in development (48).

Chagas Disease Pathology

With approximately 8 million people currently infected with *T. cruzi*, and 109 million people living at risk across 21 nations, it is easy to highlight the importance of effective treatment and control of Chagas disease (73; 88). The disease progresses through three stages in the human host (Figure 3). While most infections remain asymptomatic, an initial acute phase can begin within days of inoculation and last for several weeks. Uncommonly and in severe cases, usually associated with inoculation in childhood, the acute phase can be fatal. The acute phase commonly occurs in children with associated symptoms including fever, swelling of lymph nodes, rash and some acute

cardiac conditions (3). This stage of the infection can go unnoticed particularly in endemic areas where the disease may not manifest in severe symptoms, or in areas where other infections associated with similar symptoms are endemic (38). The appearance of a spontaneously healing chagoma, which is an area of erythematous skin at the bite site, can serve as a diagnostic feature. Romaña's sign, which is swelling around the eye that causes reduced vision, may occur when the mode of entrance is through mucosa around the eye (7). These tissue changes are the result of the amastigote stages of the parasite in infected cells of the localized tissue and muscle (3).

As the acute phase wanes, the parasite continues to invade and proliferate within human tissue, but does not cause overtly symptomatic disease. Thus the intermediate phase is designated as the period where clinical disease is lacking, but *T. cruzi* antibodies are detectable in the human bloodstream (40). A few years to many decades later, the chronic phase of disease can manifest in 30-40% of infected people. Chronic disease symptoms can include enlargement of cardiac tissue and destruction of cardiac muscle leading to cardiac disease, and/or the enlargement of digestive organs particularly the esophagus and colon (3). Approximately 2/3 of patients with chronic Chagas disease have cardiac symptoms, which can cause heart rhythm abnormalities leading to death. Less than 1/3 of patients with chronic disease develop thinning of the muscle layer of digestive organs resulting in difficulties in swallowing and malnutrition (86). These massive dilations may disrupt digestive function, or predispose to acute tearing and sepsis.

Treatment of Disease

There is no current vaccine available to prevent infection with *T. cruzi* (42). Nifurtimox is widely used in the chemotherapeutic treatment of acute or early chronic cases, but cannot be used during pregnancy. The mode of action involves inhibition of intracellular development by the parasite (38). Benznidazole has been used regularly in Brazil, Argentina, and Chile (11; 14). The mode of action of benznidazole is thought to be through the development of free radicals that damage the parasite. Resistance of laboratory isolated strains of *T. cruzi* obtained from wild reservoirs has been reported for both nifurtimox and benznidazole (38). Another chemotherapeutic, allopurinol is enzymatically digested by the parasite to become adenine analogues that are toxic to the parasite itself. A study in Argentina focused on allopurinol and showed comparable efficacy to both nifurtimox and benznidazole, but with fewer side effects. These treatments have shown success in curing acute and early chronic disease, but the treatment of late chronic stage disease is limited to treatment of symptoms. Although less than 30% of infected individuals develop the manifestations of chronic disease, in those people it is likely that the individual will succumb to symptoms over time (38). Treatment issues are compounded by the expense of treatment over long courses. Because the chronic stage of Chagas disease can be prolonged, the cost per patient per year in endemic countries is estimated at $1,028 (US dollars), with average lifetime costs of $11,619 (US dollars) per patient (8).

Disease Transmission

Transmission to humans can occur in several ways. The primary mechanisms of transmission include classic vector-borne transmission, as well as through infected blood

transfusions, congenital transmission, and mucosal transmission by tainted vector or reservoir feces (7). Secondary transmission includes indirect methods such as lab accidents, organ transplants, sexual transmission, or spread through open wounds not classically associated with local deposits of insect feces (7). In endemic areas, 70% of cases are spread by vectors, but up to 20% can be spread through blood transfusions where the screening process is not stringent (38). Congenital transmission makes up the third highest percentage of transmission route and can be vary from 0.5% to 10% across countries such as Chile, Bolivia, and Paraguay (12).

Chagas disease is considered a zoonotic disease in many locations where domesticated animals live in close association with humans and serve as reservoirs to sustain both triatomine colonies and increased infection rates (42). The sylvatic, or enzootic, cycle has probably been maintained over millions of years in mammalian hosts, infrequently infecting humans that may have inhabited caves for short periods. Human cases in mummies can be dated back to only 4-9,000 years ago (12). The agricultural revolution and associated domestication of animals changed the lifestyle of humans, bringing reservoir animals in close contact with human dwelling areas. A leading risk factor associated with Chagas disease is rustic human housing construction styles which have remained largely unchanged in many remote, undeveloped areas of South and Central America (1). The domestication of animals for human use also led to domestication of some insect vectors species. Adaptations toward domestication include behavioral changes that increase vectorial capacity, such as shortened life span and passive mobility (48).

Migration of infected individuals leads to economic, medical, and political complications (54). As individuals move from Central and South America to more developed nations in North America, Europe and now Asia, this may lead to transfusion and congenital transmission events in countries with limited previous exposure to the pathogen and that may be unprepared to diagnose and treat new cases (50).

TRIATOMINAE: INSECT VECTORS

Vectors of Chagas disease are hemipteran insects of the family Reduviidae, subfamily Triatominae. Important vectors include: *Triatoma brasiliensis*, *Triatoma dimidiata*, *Triatoma infestans*, *Rhodnius prolixus*, and *Panstrongylus megistus* (12). *T. infestans* may be considered the most widely important vector because it has adapted to living in human housing throughout much of South America, while *R. prolixus* is an important, domesticated vector in Central America and Mexico (40). *P. megistus* is a major vector in coastal regions of Brazil, while *T. dimidiata* is a common vector found throughout Central America. Although these few species are considered of particular epidemiologic importance, up to 150 different species of Triatominae have been experimentally or found naturally infected with *T. cruzi*. Species of Triatominae are found throughout much of the New World as far north as the Great Lakes of the US. Other, related species are found in Asia, Africa, or Australia, yet *T. cruzi* has never been naturally reported from the outside of the New World (42).

The family Reduviidae is classified in the order Hemiptera that includes families that possess shared characteristics of piercing-sucking mouthparts and usually two pairs of wings, one set modified with hemelytra, which are half hardened elytra and half membranous. The piercing-sucking mouthparts allow this group to feed on a diverse

number of fluids, most commonly mammalian blood, plant fluids and invertebrate hemolymph. Species of concern to humans are generally limited to the obligate blood feeders, particularly those species in the subfamily Triatominae that are capable of disease transmission (40).

Characteristic morphology within the subfamily Triatominae includes adult sizes that range from 20-28mm, black or dark brown coloration, and a characteristic violin shape adaptive for hiding and maneuvering in crevices. This group exhibits hemimetabolous development, which involves the progression of immature nymph stages that appear similar to the adult stage but lack fully developed wings and reproductive capacity. In the case of triatomines, both sexes of all nymphal and adult stages are obligate blood-feeders (40). Progression over the five nymphal stages of these insects can take up to two years and adult longevity has been reported in laboratory colonies at around one year (12). Due to this extended growth period, and mortality across instar stages, when building colonies for use in controlled laboratory assays, immature stages have previously been used (59; 78). When hosts are available, triatomines may feed every 4-9 days, which increases the likelihood of encountering an infective host. The feeding behavior and longevity of these insects equates to each individual serving as a vector for up to three years (11; 59).

Triatomines display behaviors adaptive to feeding on mammalian hosts. These behaviors include feeding at night when most hosts are unaware of their presence, the tendency of species to adapt to living in or near human dwellings, and the reported behavior of hitchhiking on humans or human belongings (40). The vectors with the greatest capacity for spreading *T. cruzi* to human hosts are determined through three

characteristics: the vector's degree of susceptibility to the parasite, the time interval between feeding and defecation, and the degree of anthropophagy (12). Triatomines tend to spend daytime hours in sheltered, stable niches, often closely associated with their primary host. For sylvatic species, these areas may include nests and burrows or more natural areas such as caves, rock piles, tree holes, or bromeliads all of which are attractive to small hosts including mammals, birds, and reptiles (40). The peri-domestic species tend to inhabit housing of associated peri-domestic animals such as chicken coops, stables, or smaller houses for pigs and guinea pigs (40). Fully domestic species infest human housing in areas where there are available crevices that provide shelter during daylight hours (40). These areas may include breaks in the flooring or roofing, areas between thatched walls and crevices in furniture. Several domestic and peri-domestic species may continue to feed on wild animal reservoirs and migrate from sylvatic to domestic habitats (40). Triatomines have been known to make short, nocturnal flights for host seeking and some are attracted to lights; but the flight tendency and the flight range of many species have not been well described. Dispersal rates of 100 meters have been reported and this motility may assist sylvatic species to encounter human hosts (12). Passive transport of insect eggs on palms leaves or other organic materials used in housing construction may introduce vectors into a domestic habitat (1). Monitoring new cases of Chagas disease has shown that a larger percentage of cases occur in houses that are colonized with triatomine vectors; however, short flight ranges and host-seeking behavior may account for cases that occur in housing structures that do not show signs of vector colonization (69).

Generally, infestation is a term used to describe the transient presence of vectors within the household, while colonization describes permanent growth and reproduction of vector populations in a localized area (29). True colonization of a household occurs when vector populations are continually present in the house structure such that at least one full generation of insect development has completed. For the purposes of this study, colonization is characterized by finding several stages of the insect (such as eggs, immature insects, and adults) present in the same household location. In other instances, infestation will be defined as the fleeting presence of adult or possibly late instar vectors within a household. The terms infestation and invasion will be used interchangeably. Infestation is likely linked to the adult insect house-seeking behavior. In the instance of household invasion, eggs and young instars are not commonly found, but are likely developing in a sylvatic, or wild, setting. Re-infestation has been defined as the collection of any stage of triatomines within the household subsequent to clearance of previously documented infestation or colonization by way of chemical control methods (28).

Physical and chemical cues serve to initiate feeding behavior by triatomines, similar to other insect species that feed on animal hosts. Carbon dioxide causes increased activity in triatomines, while heat receptors on the insects' antennae have reportedly been stimulated to intiate probing behavior (40). Pheromones in the feces of blood-fed nymphs and adults act as an attractant to unfed nymphs. Several species defecate during or soon after feeding on a host, and this behavior may explain aggregation behavior or attraction associated with insect feces (40). Because these nymphs and adults may take in large amounts of blood, equating to nearly 3 times their unfed body weight, triatomines often need to defecate during or soon after feeding to release water and other less nutrient-rich

materials obtained from the meal. As mentioned, this interval is significant in determining the vectorial capacity of a given species, since a shortened time interval between feeding and diuresis will more likely occur in transmission to a vertebrate host as the defecated parasite comes in contact with the host or host surrounding (7).

Control Methods Targeting Triatomines

Historically in the 1940s and 1950s, the first methods of control involved use of residual insecticides such as gamexane and BCH to control vector populations. These insecticides applied as indoor sprays in and around domestic habitats greatly diminished insect populations (54). The next wave of insecticides included DDT, lindane and dieldrin, which were valued due to high efficiency at the onset of use, but intensive use led to increased resistance in several vector populations (12). Therefore newer classes of insecticides were investigated. Currently, organophosphate and pyrethroid insecticides have replaced DDT and lindane, but increased cost coupled with decreased efficacy has led to the encouragement of more integrated control methods (11; 27; 28).

For a control program to efficiently diminish the number of human *T. cruzi* infections, several factors must be in place. As with any vector control program, contact between vector and human must be interrupted. This can be achieved indirectly through environmental manipulation to improve housing structure, thus decreasing the areas for vectors to enter and/or infest domiciliary units (14). Sanitation can also reduce the presence of reservoir rodents which can lead to the reduction of the local vector population (14). Prompt diagnosis and treatment of new cases is necessary to halt further transmission from humans to vector populations (45). Application of residual insecticides would obviously decrease the vector population and therefore the likelihood of human-

vector interaction. Other control measures should include relocation of peri-domesticated animal enclosures away from human housing areas (12). Housing structures of domestic animals may also be modified to incorporate insecticide treated materials to discourage invasion of vector species (54).

Triatoma dimidiata

Triatoma dimidiata is a vector species that transmits Chagas disease and is found commonly throughout the Yucatan Peninsula (16; 17; 18; 70), with a range extending throughout most of Central America (10; 15; 93) and south to Ecuador and Peru (2). Throughout this large expanse, *T. dimidiata* populations are known to inhabit a variety of habitats, domestic or sylvatic in nature (2; 17).

T. dimidiata is reportedly common in central and southern Belize (70). In these regions, *T. dimidiata* collected by community participation were found to be more abundant from March through June within domiciliary units (70). The inhabitants of participating households throughout the study region collected *T. dimidiata* as they were found within the household and results suggested seasonal patterns in the infestations of domiciliary units by non-domesticated adult triatomines with very low numbers of nymphal stages collected. Under previously described definitions, the age profile of specimens collected negated the presence of true colonization (70). Continued studies of *T. dimidiata* in the Yucatan Peninsula of Mexico have demonstrated similar seasonal variation in the collection of specimens within domiciliary units and infection rates with *T. cruzi* have reached 48% during May to June (17; 18; 31).

Due to the transient nature with which *T. dimidiata* are found indoors in this region, it has been proposed that populations showing associations with domestic or peri-

domestic habitats originated from sylvan populations and that re-infestation is a common occurrence after treatment of houses with insecticide-spraying (28; 75). Because *T. dimidiata* displays a range of behavior with respect to house invasion or colonization throughout its large distribution area and its control programs often resort to the treatment of household interiors with insecticides, it is important to determine the behavior of the vector population throughout a given regions in order to develop more targeted control programs and to reduce operational costs.

RESEARCH GOALS

The general objective of this study is to assess and report on Chagas disease vectors in regions of Belize where such information remains unknown. The information gained should inform local health and vector control authorities on the risks associated with Chagas disease vectors as well as provide officials with preliminary methods for developing efficient surveillance and control programs. Primary objectives include: comparing the effectiveness of vector surveillance methodologies, developing a distribution map of vector species inhabiting northern Belize, assessing risk factors associated with household invasion, modeling environmental data layers, and evaluating the effectiveness of commonly used control methods.

Belize Geography, Climate, and Socio-economic Profile

The nation of Belize covers 22,700 km^2 of land on the Central American isthmus shared with the Yucatan region of Mexico. It lies between 15º45' and 18º30' N and 87º30' and 89º15' W. The national language is English and the country's capital is Belmopan in the Cayo District. Belize has a population density of 30.9 inhabitants per square mile. A 2000 census indicated that the total population was approximately

249,800 individuals, which grew to 351,700 individuals in 2014, signifying a 1.87% annual population growth rate. The national population is made of several ethnic populations including: Mestizos (52.9%), creoles (25.9%), Mayas (11.3%), Garifuna (6.1%), Mennonite (3.6%), and East Indians (3.9%) 60 (10). The remaining subset of the population represents Chinese, Arab, African and white Caucasians combined. Situated southeast of Mexico and north-northeast of Guatemala, Spanish is also a predominant language throughout the country. Belize has a simple structured economy supported mostly by agriculture and services (61). From 1976 through 2016, the exchange rate has averaged US$1.00 to BZ$2.00. Household poverty rates can reach 46.4% in lower income regions and per capita gross domestic product is equal to an estimated US$ 4,070 (62). Agricultural exports include sugar cane, citrus, bananas, and marine products. This average earned income does not represent the strong economic disparity among the people of Belize. While the data is not available to explore this disparity, it can be widely seen in the broad range of living conditions observed. In the northern districts, which also tend to bring in more income from the large sugar cane and agricultural opportunities, housing structure tend to be more finished, with tin roofing, concrete/cement walls, and fully or partially screened windows (Caranci personal observation, 2012). In the south, where economic opportunity is less stable, housing structures are similar to other Central and South American mud and thatch structures. In these regions, thatched or palm roofs cover walls made of wood and mud, with many open doors and windows (Caranci personal observation, 2012). It is important to note that this separation of housing structure from north to south is not all-encompassing, there are many regions in both the

north and south with varied housing structures, and the level of diversity in housing structures will be captured by the methods listed herein.

Belize is divided into six districts: Corozal, Orange Walk, Belize, Cayo, Stan Creek, and Toledo (listed from north to south as seen in Figure 4). The country's terrain is generally low and flat along the coast and in the northern region, while the central and southern regions have low mountainous areas that reach 1,123 meters (5). There are two seasons in Belize: a rainy season and a dry season. Historically, most of the rainfall throughout the year was concentrated in the June to November timespan (the rainy season) and the transition from rainy to dry season was classically abrupt in nature (as seen in Figure 5) (5). Mean annual rainfall ranged from 152 cm in the north to 406 cm in the south. In recent years, the classically defined wet and dry seasons have not been as regular as in the past, with dry conditions beginning in earlier in the year (5).

Ecology of *T. cruzi* and *T. dimidiata* in Belize

Lainson (41) was the first to report *T. cruzi* in Belize, which at that time was British Honduras. Later, Petana and Coura (65) reported finding *T. cruzi* in *T. dimidiata* collected from the Cayo District. The incidence of disease in humans in the Cayo District of Belize was the first human infection reported in the literature (64). The only evidence of cross sectional serologic studies reported in the literature focusing on populations in Belize attempted to compare immigrant populations to local, healthy military personnel (37). The subjects included in this study ranged from 15 to greater than 35 years of age. No positive samples were collected from military personnel but positive sera were obtained from both locally living, immigrant farming populations and from the screened patient population at Belize City Hospital (37). Authors suggested that autochthonous

transmission, which refers to locally occurring inoculation, is likely present in Belize, but a large proportion of infected people living in Belize have migrated from other endemic, Central American countries, particularly El Salvador and Guatemala (37). Jaramillo et al. (37) was the first and only group who reported the prevalence of sero-positive individuals in Belize and much more information is needed to determine the prevalence of disease in the human population and the proportion of autochthonous cases. Currently, blood-donors are screened for *T. cruzi* and patients are referred for medical treatment, but these positive cases are not reported in any accessible format. Nationally, Belize is provided with pharmaceutical treatments under PAHO guidelines, yet there are resource and knowledge gaps between the public understanding of treatment accessibility, the local public health offices in the districts of Belize, and the central public health offices in the capitol.

Animal reservoir populations from Belize have been found infected with *T. cruzi* since 1968, at which time *T. dimidiata* was also implicated as the most likely vector (63). The only comprehensive study focusing on the possible animal reservoir species implicated coati, opossums and rodents as likely reservoirs (63). A single recent study regarding *T. cruzi* transmission in Belize used community-based collections to report an infection rate of 28% in the *T. dimidiata* population (70). Community collections were performed by the people living in settlements throughout Cayo and Toledo Districts and specimens were collected from the community participants by investigators for subsequent PCR testing (70). This has been the only recent study to report broad collection of *T. dimidiata* in Belize. Overall, current published literature alarmingly lacks any reporting on the overall distribution of *T. cruzi* in northern Belize, presence of vector

populations throughout the country as a whole, and any detail regarding risk factors associated with disease particular to the region. Therefore, more work is needed to determine the distribution of the vector population in northern Belize as previous work largely centered in the central and southern districts of Belize.

Polonio et al. (70) reported increases in household invasion by vector insects throughout May and June (beginning of rainy season: Figure 5). The differences in vegetation, housing structure and precipitation patterns can be vast when comparing the northern and southern regions of Belize. As these factors can all affect vector behavior and the likelihood of vectors to enter households, it is essential to determine any seasonal trend in vector activity in the northern districts as well. Because of the existence of geographic barriers dividing the northern and southern districts of Belize and due to the reported existence of several clonal populations throughout Central America, it is possible that behaviors of *T. dimidiata* populations reported in the south are different from those exhibited by populations in northern Belize. As vector behavior is strongly linked to vector capacity, it is imperative to investigate and report seasonal trends in vector behavior throughout northern Belize as well. These few reports are the extent of what is known on any activity of Chagas disease transmission, human infection, and vector behavior in Belize. In terms of control measures, very little proactive surveillance is maintained nationwide. Citizens are encouraged to capture suspected triatomines observed in and around their houses, and submit samples to their local Ministry of Health officials for identification. If Ministry of Health officials decide that samples are suspected triatomines, chemical control methods in the form of pyrethroid sprays are performed in and around the house. Little follow up is maintained and no associated

disease detection is performed (Belize Ministry of Health, personal communication, March 2012).

With respect to the knowledge gaps surrounding triatomine vector distribution and behaviors, specifically in northern Belize, it is essential to define and begin to describe this important vector species in order to strengthen vector control efforts. In order to more completely describe Chagas disease transmission dynamics in the region, key characteristics regarding vector ecology must be investigated.

SPECIFIC AIMS AND HYPOTHESES

Hypothesis 1: Community-based collections are the most successful methodology for collecting *Triatoma dimidiata* in Belize and implementation of a broad program will reveal widespread vector distribution in northern Belize.

Objective 1: Compare the effectiveness of collection techniques

Objective 2: Map the geographic distribution of *T. dimidiata* in northern Belize

Objective 3: Assess characteristics of household structure associated with the invasion of households by *T. dimidiata* in northern Belize

Hypothesis 2: If triatomine vector presence records are modeled alongside environmental attributes, then output models will confirm suitable ecological settings within Belize that could support triatomine vector populations of medical importance including *T. dimidiata* and *R. prolixus*.

Objective 4: Model ecological aspects that may predict the distribution of key vector species in Belize and the surrounding Central American region

Hypothesis 3: The response of *T. dimidiata* to commonly used insecticides differs based on the type of housing material serving as the application surface.

Objective 5: Evaluate the efficacy of a commonly used insecticide on three types of commonly used, Belizean housing materials and document the mortality rate of colony-reared *T. dimidiata* when exposed to insecticides for various time frames.

Justification of Methods

The methods used to achieve study goals were often adapted from available literature pertaining to triatomine surveillance and control methodologies. Methods for collecting triatomines include active searching and collecting in suspected resting areas in and around human housing (11; 17; 18; 29). Community–based collections, which involve the assistance of community members in the real-time collection of vectors within the household, may be used to assess what species are locally important or if new species are emerging as human feeders. Knockdown insecticides have been used to some extent to collect triatomines in enclosed spaces (30), but this requires true colonization of housing structures. Sensor boxes have been used to passively determine local infestation within the household (29). It is likely that several collection methods are required to fully describe the level of vector infestation and the species locally present (28).

Studies report that developing a targeted cross-sectional sampling method is no more successful in locating large vector colonies than randomized cross-sectional surveys. The authors remark that random cross-sectional surveys may be equally effective and less expensive to perform (39). Authors that discuss the importance of surveillance suggest that community-based surveillance systems would be more efficient at locating vector specimens due to the irregular nature with which some vector

populations tend to invade households (19). Some success has been shown by educating school children on the importance of the vectors and how to report finding local populations (28). Additional methods include the collection of vector specimens at man-made light sources during nocturnal hours (75) and the use of avian-baited trap boxes to attract vectors in a peri-domestic setting.

Rebollar-Tellez et al. (75) were the first to perform collections solely based on insect attraction to artificial light at night. In fact, more recent research has shown that light may play an important role in the attraction of sylvatic *T. dimidiata* to housing structures. Pacheco-Tucuch et al. (60) published controlled chamber tests supporting the hypothesis that *T. dimidiata* are attracted to incandescent light during nocturnal hours, as well as field data which showed that houses closer to street lights were more likely to be infested. As mentioned, *T. dimidiata* is the most common vector described in Belize and surrounding countries, and has a tendency to only be found indoors on a seasonal basis (17; 18; 31; 70). This aspect of vector behavior has led to the incorporation of less traditional collection methods. However, because very little is known of the vector populations in northern Belize, the ideal trapping methodology has yet to be investigated. It is therefore essential to assess the success of several commonly used collection methods as proposed here.

GIS and the mapping of T. dimidiata

Geographic information systems (GIS) have been used to map the distribution of vector species including mosquito vectors of malaria, sand fly vectors of leishmaniases, and triatomine vectors of trypanosomiasis (13). In the case of mapping *T. dimidiata*, GIS outputs have been used to visualize the distribution and density of the species,

particularly in the Yucatan region of Mexico (17; 31). Much of the mapping in these instances has focused on determining patterns of infestation on the community or household level in order to determine risk factors associated with the overall position of houses within villages (17; 31). Authors have reported that the likelihood of house invasion increases with decreased distance to surrounding vegetation, but that some variability can occur based on the infectious status of the vector (71). Interestingly, vectors infected with *T. cruzi* have been shown to develop larger wings than non-infected individuals; this modification in wing morphology may lead to variability in dispersal distance (58).

Advanced analyses involving spatial modeling such as ecological niche modeling have also been applied to the distribution of *T. dimidiata* in order to determine associations with temperature, humidity, precipitation, and vegetation type (16). Results indicated that warmer, drier regions were more likely to overlap with *T. dimidiata* distribution and that the presence of the vector species was highly associated with disturbed vegetation types such as pasture and agriculture (16). The authors remark that this seemingly seasonally dependent distribution pattern is different from that reported in other regions; thus, more work is needed to determine climactic correlates in different regions. This type of mapping has centered on regions of the Yucatan peninsula of Mexico just north of Belize, yet no modeling has been performed in Belize to date.

Molecular Methods of Vector Ecology

When describing the biology and ecology of an insect vector of disease, it is vital to ensure the correct identification of specimens collected from field studies. This can be achieved using morphological keys such as the one used here, describing the distribution

and morphology of triatomine vectors presented by Lent and Wygodzinsky (43). Morphological identification is supported by genetic evidence of species identification using species-specific primers often targeting regions of the internal transcribed spacer (ITS) regions of rDNA (21; 44).

Similarly, when investigating vector-borne diseases, methods for determining the infection status of vector specimens have been developed. The complex development of *T. cruzi* in the triatomine gut has implications for determining the infectious nature of a vector specimen. As discussed previously, parasites must develop and travel to the hindgut for excretion with feces in order to contact and infect human hosts. Microscopy has been used to visually confirm the presence of *T. cruzi* parasites in feces expelled from insect specimens. However, when field collection methods rely on interval collections performed by community members, the resulting specimens are often dead and desiccated. Therefore, molecular methods for the detection of parasite DNA are needed to confirm the infection status of field samples. Such methods have been developed and standardized for isolating *T. cruzi* DNA from triatomine specimens (76; 86).

The methods described in the literature provide a strong basis to explore the research goals stated previously. The basic science developed to study the vector ecology and infectious status associated with triatomine vectors can be modified to investigate similar themes in northern Belize, where prior knowledge is lacking. The resulting information is essential to the strengthening of ongoing vector control efforts in the region.

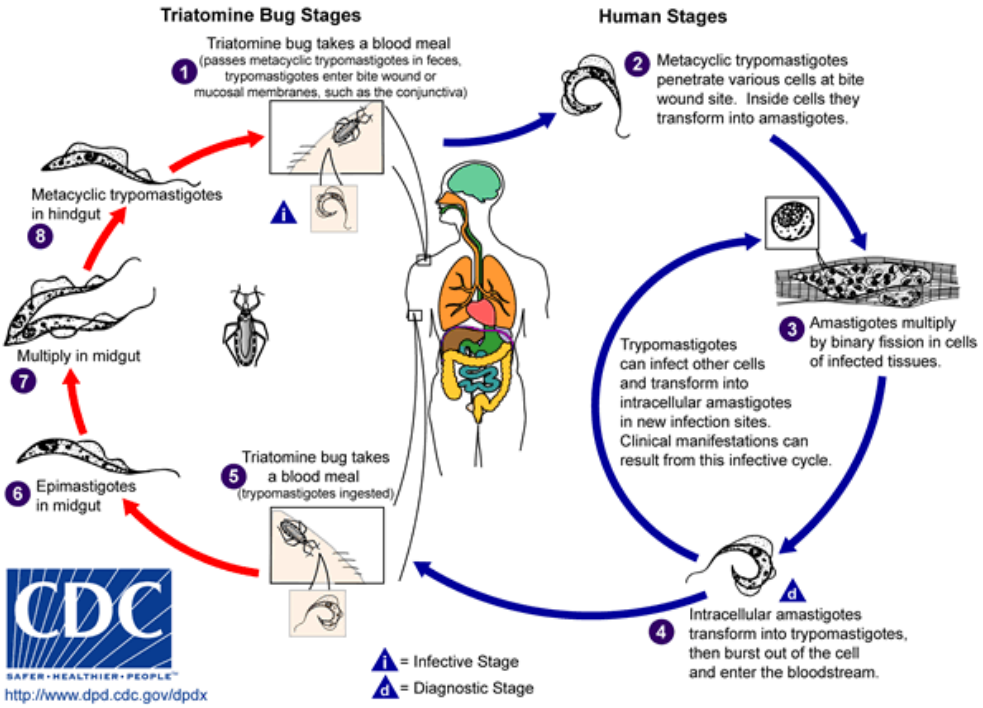

Figure 1. Life cycle of *Trypanosoma cruzi* as the parasite develops and reproduces through both vertebrate and invertebrate hosts (9).

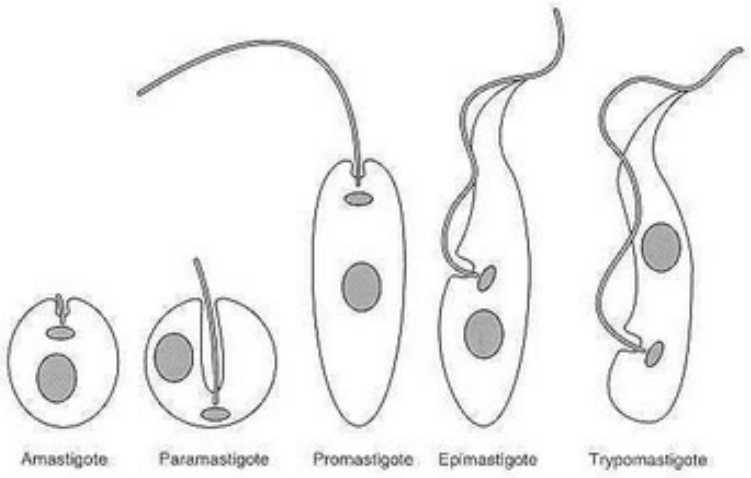

Figure 2. Morphology of life stages as seen in the development of species in the genus *Trypanosoma* (42).

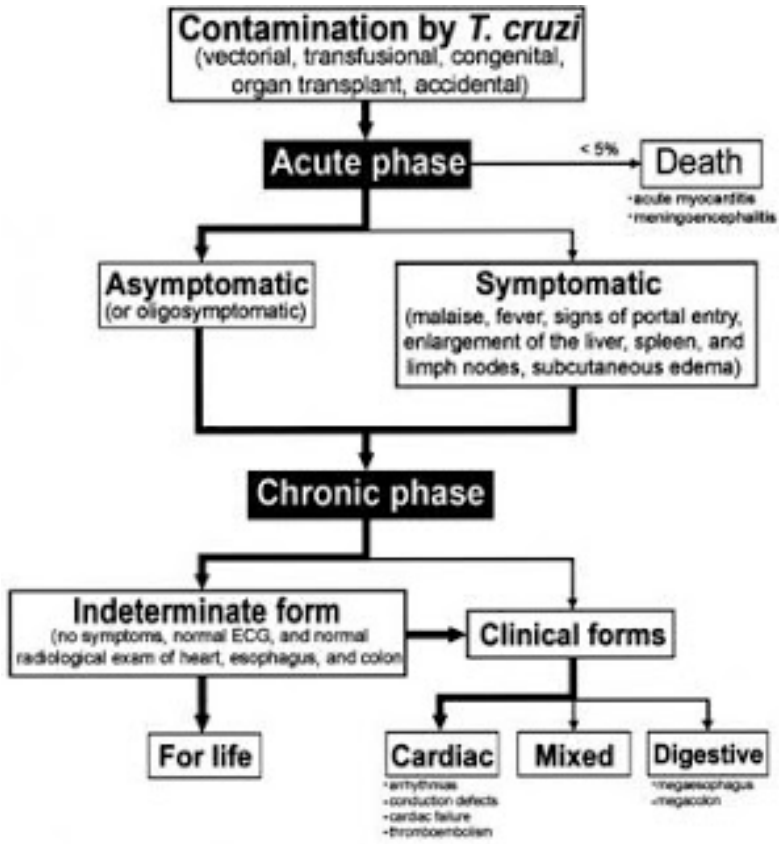

Figure 3. Schematic that maps the possible paths of disease progression after human infection of *T. cruzi* (74).

Figure 4. The nation of Belize, colored areas designate the six separate districts.

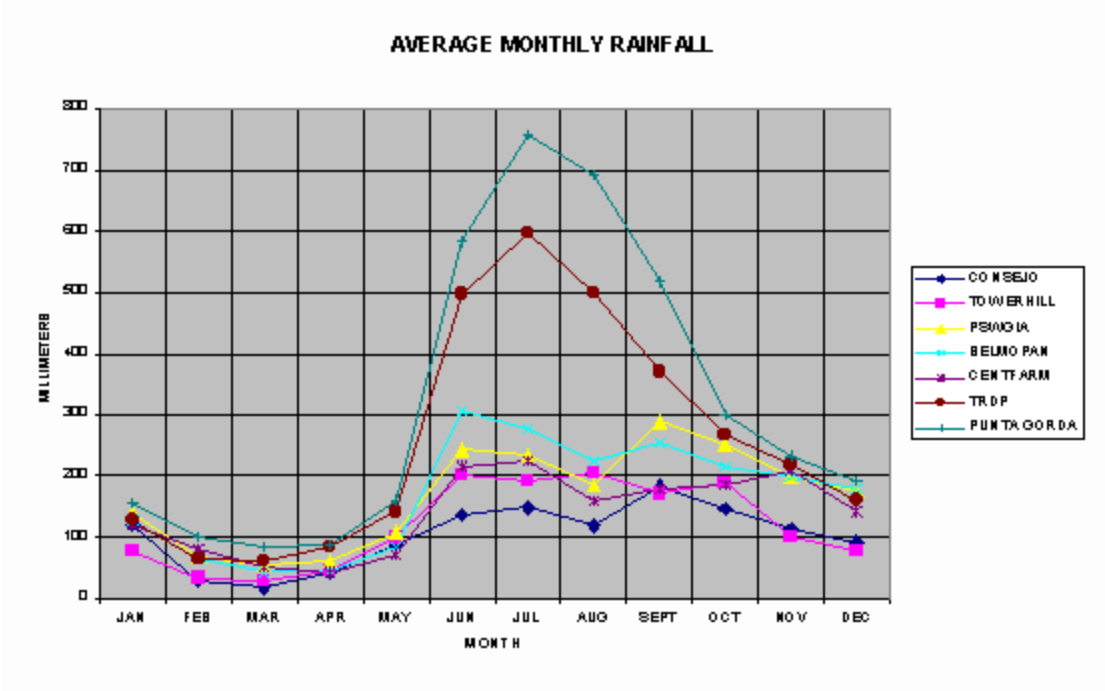

Figure 5. Graph representing the average monthly rainfall throughout regions of Belize. Rainy season begins in early May in the south (Punta Gorda and Toledo Research and Development Program - TRDP) and begins later in May on the central (Belmopan and Central Farm) and northern regions (Consejo, Tower Hill) (5).

CHAPTER 2: Distribution of *Triatoma dimidiata* (Reduviidae: Triatominae) and Risk Factors Associated with Household Invasion of in Northern Belize, Central America*

ABSTRACT

To date, *Triatoma dimidiata* (Reduviidae: Triatominae (Latreille, 1811) remains the sole vector species associated with Chagas disease transmission reported from Belize. Available human infection data are limited for Belize and the disease transmission dynamics have not been thoroughly investigated, yet the likelihood of autochthonous transmission is supported by the widespread collection of infected vectors from within local households. Here, we report updated infection rates of the vector population and infestation rates for villages in north and central Belize. Overall, 275 households were originally enrolled in an ongoing vector surveillance program. Of the 41 insects collected, 25 were PCR positive for *T. cruzi*, indicating an infection rate as high as 60%. To further characterize the epidemiological risk of human-vector contact, determinants of household invasion were modeled. Local households were surveyed and characterized with respect to 20 key factors that may be associated with household infestation by *T. dimidiata*. The final multivariate regression model developed from this data included household risk factors that were significantly associated with the presence of *T. dimidiata,* including the presence of peridomestic animals and the proximity of community light sources.

*Adapted from manuscript prepared for submission to the Journal of Medical Entomology: **Distribution of *Triatoma dimidiata* (Reduviidae: Triatominae) and Risk Factors Associated with Household Invasion of in Northern Belize, Central America.** Angela T. Caranci, Nicole L. Achee, David F. Hoel, Kim Bautista, Ireneo Briceno, Russell King, V. Ann Stewart, Jittawadee Murphy, Penny Masuoka, Cara H. Olsen, and John P. Grieco

Unlike other reports, the presence of peridomestic chickens appears to be protective. In northern Belize, *T. dimidiata* is confirmed to be sylvatic in nature as opposed to the classical paradigm of domiciliated vector populations. This designation has strong implications for vector surveillance and control; therefore, the risk factors reported here may guide integrated control efforts to reduce invasion and limit human-vector contact.

INTRODUCTION

Chagas disease continues to be one of the most widespread, neglected tropical diseases in Latin America despite several regions reporting decreased incidence of human infections throughout endemic areas of Central and South America (57). Although varying levels of success have been attained in controlling Chagas disease over the last 40 years, the biology of transmission and control of the insect vectors must remain a focus of current research to maintain this decline (12). *Trypanosoma cruzi* is the causative agent of Chagas disease and is vectored by true bugs of the order Hemiptera in the family Reduviidae, subfamily Triatominae (36). Following contact with feces of infectious invertebrate vectors, *T. cruzi* parasites can enter and infect human hosts resulting in a spectrum of disease symptoms referred to as Chagas disease (3). The spectrum of disease can range from asymptomatic infection to chronic pathology affecting the tissue of major organs including the heart, esophagus, and rectum, resulting in an estimated annual loss of 430,000 Disability-Adjusted Life Years (DALYs) (57).

Chagas disease has been listed among the major neglected tropical diseases largely due to a lack of adequate diagnostic tools and treatment protocols (36). In Latin

America, the disease is second to malaria in the amount of area that is endemic for the parasite, causing an estimated 8 million current infections and threatening approximately 109 million people who live at risk (86). Because no vaccine is available, many nations have employed blood screening and case detection to prevent spread of the parasite by congenital transmission or blood transfusion (12). The success gained in decreasing the incidence of Chagas disease in the New World has varied due to differences in the efficacy of surveillance and control methods focused on insect vectors across varied ecological settings throughout the large endemic region (12). Control efforts have reported success where transmission is predominantly due to domesticated vector populations; however, widespread sylvatic populations require the need for control programs that are integrated and targeted to local transmission ecology (17; 19).

Belize, on the eastern coast of Central America (Figure 6) is one of several nations with limited surveillance of Chagas disease transmission. In this region, information related to human cases of Chagas disease as well as the infection and transmission dynamics of the insect vectors is largely lacking. Lainson (41) was the first to report *T. cruzi* in Belize, known as British Honduras at that time. Later, Petana and Coura (65) reported finding *T. cruzi* in *T. dimidiata* collected from the central region of the country. The first human infection reported in the literature was from the same central area in Cayo District of Belize (63). To date, the only cross sectional serologic study focusing on human disease incidence in Belize compared immigrant populations to local, healthy military personnel via the analysis of blood donations (37). The authors reported only one serologically positive sample from a Belizean citizen, stressing the prominence of imported cases from bordering regions of Central America. Animal reservoir

populations from Belize have been found infected with *T. cruzi* since 1968, at which time *T. dimidiata* was also implicated as the most likely vector (63). The authors provided the only comprehensive study focusing on the possible animal reservoir species implicating coati, opossums and rodents as likely reservoirs (63). A single recent study regarding *T. cruzi* transmission in Belize, limited to the southern and central districts, used community-based collections to report an infection rate of 28% in the *T. dimidiata* population (70). Together these studies reveal the presence of pathogen, vector, and evidence of human infection from this nation, necessitating additional study of local vector ecology.

The general objective of this study was to assess and report on Chagas disease vectors in regions of northern Belize where such information was largely unknown. Efficient methods of capturing the distribution of local triatomines were assessed. Collected specimens positively identified as vectors were analyzed for *T. cruzi* infection status. Household risk factors were modeled to determine association with the presence of invading *T. dimidiata*. The information gained should inform local health and vector control offices on the risks associated with Chagas disease vectors as well as provide officials with preliminary methods for strengthening surveillance and control programs.

MATERIALS AND METHODS

Study Area and Collection Period

Specimen collection occurred within the two most northern administrative districts of Belize, Corozal District and Orange Walk District (Figure 6), from November 2012 to September 2014. The more centrally located Cayo District (Figure 6) was added in June of 2013 with collection continuing through September 2014. In all, the collection

region spanned between longitudes 88.4º and 89.1º west and latitudes 17.1º and 18.4º north (Figure 7). All collections across the sampling region were performed within the same four week period and repeated at three-month intervals. Any household that was not able to be sampled at any two recurring visits was discontinued as loss to follow up.

Vector Collection Methods

Initially, 210 houses were randomly selected within 23 villages throughout Corozal and Orange Walk Districts in order to evaluate collection methodologies. Villages were included based on input from local Vector Control District offices, but also with the aim of surveilling a wide extent of the target region. Subsequent addition of Cayo District added an additional 65 households across 8 villages to the total number of collection sites (total 275 enrolled). Common collection methods were adapted from the literature and employed at various locations in each village. Regardless of the collection method performed at a given location, an adult member of each household was presented with an educational pamphlet regarding the transmission of Chagas disease and the associated disease vectors reported from surrounding regions (color pictures provided for reference). After informed consent was granted (Appendix C), we stressed the importance of avoiding direct contact when handling any possible vectors. The first method, referred to hereafter as 'community collection' involved community-assisted surveillance (19; 70). Each household was provided with collecting materials and again coached on the importance of minimizing contact when collecting any insects that resemble the vectors of interest. Cooperating household members were asked to collect any insects that resembled the reference images provided in the educational pamphlet and to record the date and location of collection on the specimen container. New pamphlets

and collection materials were offered at each return visit throughout the study period. The second collection method employed across all villages was referred to as active searching. Active searching has been a common means of locating and collecting vectors associated with household domiciliation (12). In the interest of standardizing this collection method to account for the different size and types of households present within and across local communities, the time allotted for searching each house included 20 minutes per common use household area and an additional 5 minutes per bedroom. During this time, 2 trained entomologists simultaneously searched the indoor area. Additionally, another trained entomologist concurrently searched the peridomestic area for an allotted 25 minute period. This process was performed during each visit for one year. The third collection method involved the placement of sensor boxes (29). This means of surveillance is meant to offer shelter to domiciliated vectors and allow for the passive detection of vectors within the household. Plywood boxes (14 x 25 x 9 cm) lined with accordion-folded construction paper were placed at each location at one of three possible locations: behind headboard of bed, behind other large furniture, or in roof rafters (this was often guided by discretion of household inhabitants to ensure limited tampering) and inspected upon subsequent visits for one year. The final collection method involved nocturnal lighting as described in Rebollar-Tellez et al. (75). Ultra-violet (15 W) tube lights (BioQuip Products Inc., Rancho Dominguez, CA) were hung against white canvass sheets (3 x 3 m) and monitored every 30 minutes for the 12 hour period from dusk until dawn. Due to lack of vectors collected by means of active searching, sensor box surveillance, and nocturnal lighting, these methods were discontinued as primary collection methods after the first year of the study. Community

collection was then employed at all sites for the duration of the study. All specimens were identified using a morphological key (43) and confirmed by PCR of the ITS-2 region as previously described by Richards et al. (77).

Determination of *T. cruzi* Infection Status of Vector Specimens

Because of the lag period occurring between collection periods, most specimens were too desiccated to attempt determination of infection status by microscopic examination of fecal matter. Therefore, for each specimen, the terminal segment of the abdomen was removed using sterile dissection tools. Individual samples were then pulverized in phosphate-buffered saline (PBS) using a hand operated, cordless pellet pestle with sterilized tissue grinders (Fisher Scientific, Pittsburgh, PA). The slurry was then processed by PCR to determine *T. cruzi* presence according to methods reported in Virreira et al. (86). DNA was extracted from these samples using QiaAmp DNA Blood Mini Kit (QIAGEN, Valencia, CA). Separate PCR reactions were run to amplify sequences targeting both genomic DNA (TCZ) and kDNA minicircle segments (S35/36). PCR products were run on 1.5% agarose gels stained with 0.5 μg/ml ethidium bromide. Samples from which both target sequences were successfully amplified were considered positive for *T. cruzi*.

Collection of Household Attribute Data

At the time of enrollment and after informed consent was obtained, adult inhabitants were given a brief survey that inquired on the individual's previous encounters with *T. dimidiata* as well as additional behavioral information regarding household use of insecticides. Additional information regarding the visual household structure and presence of domesticated animals was recorded. The attributes recorded

included: GIS location, number of rooms, number of bedrooms, presence of screened doors, presence of screened windows, presence and type(s) of animals in domestic area, presence and type(s) of animals in peridomestic area, external light sources, community light sources, distance to community light sources, status of surrounding vegetation (primary, secondary, agricultural), wall material, roof material, floor material, number of wall gaps larger than 2 cm, and opening between roof eaves and wall structure. Later, the distance from each house location to the village periphery was measured using Google Earth.

Data Analysis

Data of vector presence of absence were mapped at the village level using ArcGIS 10.1 software (ESRI, Redlands, CA). Village infestation rates were calculated as the percentage of households positive for vector presence. Dispersion was calculated as the percentage of villages positive for vector presence. Chi square analysis tested trends in vector presence for sex differentiation and seasonality. Univariate analysis of each attribute was performed to determine correlation of each characteristic with the presence of *T. dimidiata* within the household (SPSS 22, Armonk, CA). Characteristics appearing to have significant associations were included in the final binomial logistic regression.

RESULTS

T. dimidiata Distribution

A total of 41 *T. dimidiata* specimens were collected across three administrative districts throughout the collection period. On occasion, possible vector specimens were occasionally submitted to local district-level Belize Ministry of Health: Vector Control offices. Although these submissions were not part of our initial collection protocol, these

submissions were tracked to the associated village, georeferenced, and included in the distribution maps reported in order to be as inclusive as possible. All of the specimens collected were adults; no eggs or nymphs were reported throughout this collection period. Approximately 54% of the adults collected were male with the remaining 46% female. Of the total specimens collected, the distribution among collection methods was as follows: community collection n = 39; active searching n = 2; sensor box surveillance n = 0; and nocturnal lighting n = 1. Village infestation rates (Table 1) and district dispersal rates are reported (Table 2). There is no visually apparent trend in the distribution of *T. dimidiata* positive villages throughout the northern region of Belize (Figure 7), other than to say the distribution appears to be dispersed throughout the region. Throughout the collection period, 13 specimens were collected from February – May 2013-14, 17 specimens from June-September 2012-14, and 11 specimens from October- January 2012-14. Commonly, the cool dry season in Belize stretches from November to early February, with the strong heat and rains occurring from May through September (5). Collection timing was designed with the aim of capturing seasonal trends; however, there was no significant trend in the seasonal distribution of reported vector presence.

T. cruzi Infection Rates of Vector Populations

The overall infection rate of the target vector population was 61%. Infection rates by district ranged from approximately 58% to 64% and are reported in Table 2. Due to the low number of individual vectors collected, we were not able to report any trends associated with district level distribution or seasonal presence and *T. cruzi*-positive *T. dimidiata*.

Risk Factors Associated with Household Invasion

Of the 41 specimens collected during the study, 38 were associated with households enrolled into the survey portion. Due to the fleeting nature of the vector's presence in households, it is important to report on trends associated with household characteristics that are local to northern and central Belize. The survey data reported that 80% (n = 203) of households lacked screened doors, with 66% (n = 166) of houses having no or only partially screened windows. Only 6% (n = 15) of households reported housing an animal within the home overnight, while 83% (n = 209) of households reported ownership of animals kept in the surrounding peridomestic area. The most common animal associated with the peridomestic setting was dogs (78%, n = 196) and chickens were the second most common (37%, n = 93). All but one village was equipped with community lighting in the form of street lights that were kept on throughout the night. Household structure materials are also commonly associated with vector presence. In this study region, the most common wall material was cement (56%, n = 141) followed by treated wood (41%, n = 103). Roof material was most commonly made of sheets of corrugated zinc (83%, n = 209). Locally acquired thatch and vegetation material were rarely used as the main household structure material (5%, n = 13). Additionally of note, interview data showed that 87% (n = 219) of household adults surveyed had no prior knowledge or experience with *T. dimidiata* as pictured in the educational pamphlet. The use of some form of commercially available insecticide occurred in 88% (n = 221) of surveyed households.

Of the total number of houses initially enrolled (n = 275), we lost 23 of them (8%) due to loss of follow up and only 26 household locations were positive for *T. dimidiata*

presence. Univariate analysis explored the relationship between each household risk factor surveyed and the presence of *T. dimidiata* reported from within the household as a binary state. Of the attributes, only three were significantly associated ($P<0.05$) with the presence of *T. dimidiata*: peri-domestic dog, peri-domestic bird, and community light source within 20 meters. These variables were included in the final regression analysis, with the odds ratios reported in Table 3.

DISCUSSION

The design of a successful vector control program principally depends on initial investigations into the distribution and ecology of the target vector species. This initial reporting sought to not only provide some basic data to support a growing vector control program, but also to formally report early properties of the transmission dynamics of Chagas disease in northern Belize. Widespread surveillance of villages in the northern and central region of Belize resulted in the collection of *T. dimidiata,* which remains the sole vector reported from this Central American nation. At this early point in our understanding of *T. cruzi* transmission in this region, a few key observations should be noted. Throughout two collection seasons, only 41 specimens were collected despite the use of four different surveillance methodologies in an attempt to capture this vector species. Community collection efforts were by far the most efficient means of collecting *T. dimidiata* in the region. The village and district level infestation rates were low compared to neighboring regions in the Yucatan peninsula (17). This low level of capture may be due to naturally small populations of endemic *T. dimidiata*. All of the 41 specimens collected were adults; therefore, there is little evidence of household domiciliation of this vector population in the northern districts and it is likely that the

presence of vectors within human habitations is transient or intrusive as defined by Waleckz et al. (87). Because the local vector population does not seem to display behavior associated with regular household infestation, it may also be possible that Belizeans living within this region are not as familiar with the vector itself. Of the people surveyed in this study, 87% reported never having previously seen *T. dimidiata* prior to enrollment in this study. Not only is this a likely reflection of the small size of local vector populations, but also a low level of local familiarity with this neglected disease and associated vectors. Continued surveillance and educational interventions at the village level are needed to ensure full capture of *T. dimidiata* distribution throughout this region, particularly when relying on community collection. Similarly, ecological trends associated with seasonality of household invasion may emerge as the reporting of local vector populations strengthens.

Despite the seemingly scant local vector population reported here, the infection rate in specimens collected within households was 60%. This rate is higher than previously reported (70), but supported by literature reporting vector infection rates greater than 50% (82). Similar infection rates were also reported when modeling the relationship between vector abundance and population infection rate in the bordering Yucatan region of Mexico (17). Authors reported a regionally specific inverse relationship between the density of local vector populations and *T. cruzi* infection rates of vectors. It is important to note that currently the reported incidence of human-vector contact is low, as local *T. dimidiata* populations are highly sylvatic. However, with the high infection rates of the local populations, fluctuations in the sylvatic setting that impact vector behavior may lead to increased human contact with infectious vectors.

Sylvatic *T. dimidiata* populations have been reported from elsewhere in Central America, where adult vectors invade the household area from surrounding secondary brush vegetation and agricultural plots (51). This complex relationship requires additional and ongoing surveillance to fully determine any risk factors associated with vegetative patterns in Belize and elucidate possible shifts that may impact human risk.

The fleeting, non-domiciliated nature of the vector population in northern Belize seems similar to that reported in neighboring areas of Yucatan, Mexico where climate and ecology are comparable (18). Many of the household risk factors investigated in this study were chosen based on previously reported associations with *T. dimidiata* infestation. As reported, the northern region of Belize does not currently sustain fully domiciliated populations of *T. dimidiata*. This may be due to nature of household structures that are common throughout the region. Currently, most homes in Belize are composed of cement, treated wood, and zinc materials, already a substantial improvement of traditional housing materials in control domestication of triatomine vectors. The thatch roofing and locally sourced logs that have been reported to be associated with *T. dimidiata* infestation were present in less than 5% of households included in this study. Of the thirty household characteristics recorded, only three were significantly associated with household infestation (Table 3). As reported in other studies, the results presented here support the association between the presence of dogs in the peridomestic setting, as well as the relative location of community lighting, and the collection of *T. dimidiata* within the household (18). The relationship between peridomestic animals leading to increased risk for vector invasion relies on the premise that these transient, blood-meal seeking vectors may be attracted to the additional hosts. However, here we report the

seemingly protective presence of chickens in the peridomestic setting. Because chickens are not commonly kept localized in coop structures throughout this region (personal observation, November 2012), the free-range nature of these birds may allow them to serve as predators of host-seeking *T. dimidiata* in the peridomestic setting. Additional research is necessary to elucidate this possibly protective attribute. The third factor associated with *T. dimidiata* presence was the household distance to community light sources. Pacheco-Tucuch et al. (60) published controlled chamber tests supporting the hypothesis that *T. dimidiata* are attracted to incandescent light during nocturnal hours, as well as field data showing that houses closer to street lights were more likely to be infested. The trend associating community light sources with the presence of *T. dimidiata* was further supported by Dumonteil et al. (18). This may have implications for village-level control interventions targeting *T. dimidiata* if additional research can determine possible preferential behavior associated with specific wavelengths of light.

In conclusion, we report the widespread distribution of *T. dimidiata* throughout northern and central Belize. Despite low levels of household invasion, the infection rates of local vector populations averaged 60%. Therefore, the likelihood of human-vector contact remains low, but the risk for human infection remains. Factors such as the presence of dogs in the peridomestic setting and community light sources within 20 meters of the house are predictors of household invasion by *T. dimidiata*, while the practice of keeping free-range chickens in the peridomestic setting was protective. This study was an initial investigation into the ecology of *T. dimidiata* in this setting and should be used to strengthen vector surveillance programs. Additional human case data are crucial in calculating human risk. We suggest that further, more inclusive research be

performed in order to determine the interaction of additional ecological factors including land use and local reservoir animal populations.

Figure 6. Belize and administrative districts. (A) The left image displays the location of Belize in the context of Central America. (B) The administrative district of Belize, this study investigated households in the northern districts of Corozal and Orange Walk, and centrally, Cayo.

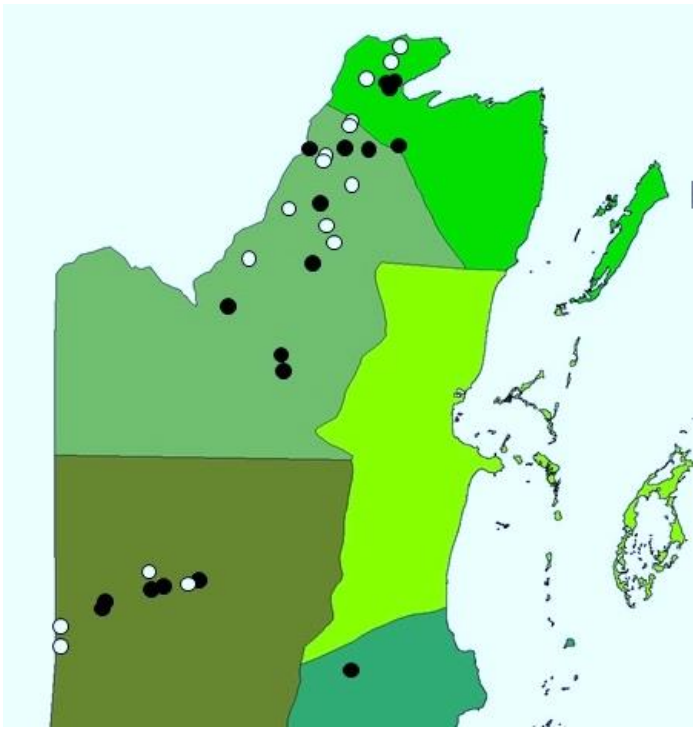

Figure 7. Surveyed villages and reported distribution of *T. dimidiata*. All circles represent villages included in the surveillance portion of this study; darkened circles represent villages reporting the presence of *T. dimidiata* in the household.

Table 1. Village level distribution of *T. dimidiata*. Villages reporting vector presence, number of *T. dimidiata* (n) and infestation index, defined as the proportion of houses reporting vector presence. *Collected in Village in Stan Creek District but reported to Cay District Vector Control Office

Village Level Distribution of *T. dimidiata* in Belize

Corozal District			Orange Walk District			Cayo District		
Village	**n**	**Infestation Index**	**Village**	**n**	**Infestation Index**	**Village**	**n**	**Infestation Index**
Ranchito	4	30%	Indian Church	8	40%	Santa Elena	3	20%
Buena Vista	2	20%	San Carlos	5	20%	Blackman Eddy	2	10%
Xaibe	2	10%	Douglas	2	20%	Esperanza	2	13%
San Andres	2	10%	Trial Farm	1	10%	Unitedville	2	NA
Caledonia	1	10%	Guinea Grass	1	10%	Camelote	1	13%
Progresso	1	10%	San Felipe	1	10%	Middlesex	1	NA

Table 2. Vector ecology of *T. dimidiata* at the district level. Infestation index was defined as the proportion of households reporting vector presence, dispersal was defined as the proportion of villages reporting vector presence, and infection rate is the percentage of vectors positive for *T. cruzi*.

District Level Attributes of *T. dimidiata* Vector Ecology			
District	**Avg infestation index**	**Dispersal rate**	**Infection rate**
Corozal District	15%	60%	58%
Orange Walk District	18%	46%	61%
Cayo District	14%	50%	64%

Table 3. Modeling of household risk factors. Logistic regression coefficients, Wald test, and significance for the variables included in the final regression model for predicting the infestation of *T. dimidiata* in northern and central Belize.

Logistic Regression Predicting Presence of *T. dimidiata* in Households				
Variable	**B**	**Wald**	**Sig.**	**Odds Ratio**
Peridomestic dogs	1.33	3.739	<0.05	3.78
Peridomestic chickens	-0.99	3.846	<0.05	0.37
Community lighting	1.85	4.644	<0.05	6.36

CHAPTER 3: Ecological Niche Modeling of Common Chagas Disease Vectors in Central America, with a Focus on Belize*

ABSTRACT

Chagas disease is considered one of the most widespread and problematic neglected diseases in Central America. The causative parasite, *Trypanosoma cruzi*, can infect humans through a variety of methods including blood transfusions, congenital infection, and by means of contact with insect vectors of the subfamily Triatominae (Hemiptera: Reduviidae). In endemic regions of Central and South America, Chagas disease incidence has been diminished by efficient and sustained vector control programs. In order to strengthen vector surveillance and control programs targeting triatomine vectors, vector distribution maps are often developed to target resources in high risk areas. The Central American nation of Belize has reported the presence and distribution of a sole vector species, *Triatoma dimidiata*, which is common throughout surrounding regions of Mexico and Guatemala. Here, we include distribution data recently reported from northern Belize, in the development of an ecological niche model. *Rhodnius prolixus* (Stal, 1859), another triatomine vector, has been reported from countries surrounding Belize in the past and has also served as an important vector of Chagas disease in Central America; however, this vector has not been found during vector surveillance programs in Belize.

*Adapted from manuscript prepared for submission to PLoS Neglected Tropical Diseases: **Ecological Niche Modeling of Common Chagas Disease Vectors in Central America, with a focus on Belize.** Angela T. Caranci, Penny Masuoka, John P. Grieco, Nicole L. Achee, David F. Hoel

We develop an ecological niche model using occurrence data reported from neighboring countries to explore the areas within Belize that could possibly provide a suitable habitat, based on basic ecological requirements. Temperature variables were both vital to the development of the final models; therefore, temperature may be a controlling factor in the distribution of both species. Together, these models may offer guidance to local efforts in sustained and efficient vector surveillance.

INTRODUCTION

In Central America, Chagas disease is a widespread and problematic parasitic disease with a high disease burden, yet is listed among the neglected tropical diseases in terms of the level of funding and focused research programs (12). With an endemic area stretching from Mexico to Chile and southern Argentina, Chagas disease is thought to cause an estimated annual loss of 430,000 Disability-Adjusted Life Years (DALYs) (11; 57). The etiological agent of Chagas disease, the protozoan parasite *Trypanosoma cruzi*, can be transmitted to human hosts by means of contaminated food or beverages, congenital passage from mother to fetus, or via blood or tissue donation (7; 11). However, the predominant means of human infection in regions with autochthonous transmission occurs when naïve human hosts encounter the feces of infectious insects of the subfamily Triatominae (Hemiptera: Reduviidae). Due to the lack of a vaccine and limited success of drug treatment, vector control remains vital to the reduction of disease burden in endemic regions (32). In Central and South America, targeted and sustained vector control programs have made great strides in reducing domiciliated vector

populations and disease incidence (32), yet additional research regarding vector distribution and local transmission ecology are required to sustain such efforts.

Within some regions of Mexico and Central America, the sole reported vector of Chagas disease is *Triatoma dimidiata* (Latreille, 1811) (18; 70). Local vector behavior and ecology have been extensively studied, while recent information regarding niche differentiation among cryptic species within the *T. dimidiata* complex lends another layer of complexity to assessing vectorial capacity throughout the endemic region (4; 17). As the Chagas disease vector surveillance program in Belize strengthens, new distribution data are being reported with the aim at broadening our understanding of *T. dimidiata* population distribution in this region. Vectorial capacity and level of domicilation of *T. dimidiata* vary throughout the expansive region that is considered endemic for this vector species (4; 17; 70). Therefore, it is vital to investigate and report novel incidence data so that surveillance programs may guide their efforts appropriately.

Rhodnius prolixus (Stal, 1859) has also been an important vector of Chagas disease in Central America. First reports of *R. prolixus* outside of South America were from El Salvador in the early 1900s, which led to an expansive, multi-country control effort to eliminate the vector from the region (33). The successful spread of *R. prolixus* through Central America from what is thought to be a single introductory location has been attributed to the exclusively domestic behavior in Central America, tendency to passively spread, and comparatively short reproduction cycle (92). Only two countries in Central America did not report the presence of *R. prolixus*: Belize and Panama. While the domestic nature of this vector has largely allowed for the success of targeted control

strategies, surveillance programs in the region should be prepared for re-emergence so that control strategies can be efficiently implemented.

The publication data regarding Chagas disease vectors in Belize have been limited to a single publication in the last 30 years (70). The authors described the broad distribution of *T. dimidiata* in central and southern Belize; however, surveillance records for the northern region bordering the Yucatan region of Mexico had not been previously reported. Here, additional presence data are presented for use in developing tools for strengthening vector surveillance.

Ecological niche models (ENM) have been used as a tool in integrated control programs targeting Chagas disease vectors (26; 66; 67). Presence data of a target species are used to extract associated environmental and/or land use data in order to build a model that predicts the presence likelihood over a broader region. Here, our aim was to develop ENMs for two important Chagas disease vectors and use these as tools to determine the risk of vector presence throughout Belize.

MATERIALS AND METHODS

Study area

The regions included for development of the ecological niche models included areas of Mexico, Guatemala, Belize, and Honduras. The corner coordinates delineating the area for the models were -103.58ºE, -86.94ºE, 13.28ºN, and 22.38º N.

Species Distribution Records

Studies reporting the distribution of Chagas disease vectors from Belize were previously limited to the central and southern regions of the nation. Trained entomologists conducted a surveillance campaign across 31 villages, incorporating 275

households, in order to track Chagas disease vectors throughout Corozal, Orange Walk, and Cayo Districts of Belize. The program was run from November 2012 to September 2014 and incorporated passive and active collection methods.

In order to strengthen the species presence database we sought out additional presence records from neighboring areas of Central America. Records from published scientific literature from 1970 through 2015 were reviewed. The literature searches centered on the two species of interest: *T. dimidiata* and *R. prolixus* individually, but some publications may have contained presence data for both. Records were used if they met the inclusion criteria of: 1) being collected in a region within the study area, and 2) being a novel location such that village level locations were not included more than once. Publications were excluded if collection data were not reported with associated village-level location information. Any records that noted the village or city name but no geographic coordinates were geocoded using GoogleEarth (Google Inc., Mountain View, CA). For each species, the species location data were compiled into a database totaling 110 location records for *T. dimidiata* (Appendix D) (4; 15; 17; 26; 34; 52; 70; 84) and 37 records for *Rhodnius prolixus* (Appendix E) (25; 32; 33; 46; 47; 53; 56; 62; 72; 80; 83; 91).

Model Development

Altitude and bioclimatic data layers (Table 4) from WorldClim (35) database version 1.4 were obtained for use in building species distribution models. The 1km resolution ESRI format data used were derived from weather station data spanning the years 1950-2000. These layers were imported to ArcMap 10.1 (ESRI, Redlands, CA) and the data associated with the study area previously described were extracted. The species

distribution data and environmental data layers were then input into niche modeling software, MaxEnt version 3.2.1 (68). This program uses principles of maximum entropy to build models of species distribution. The individual models built for *R. prolixus* and *T. dimidiata* used 80% of the presence data as training points and 20% were randomly selected by the program for testing the model's validity. Jackknife tests were performed to determine which variables contributed substantially in model development.

In order to explore differences in altitude and environmental data associated with species presence records, altitude and temperature layers were then displayed in ArcMap along with the village level location data for each species. Using the Spatial Analyst tools in ArcMap, values for each data layer at those points were extracted and analyzed by student's t-test to determine significant differences in mean values.

RESULTS

Vector Surveillance: Northern and Central Belize

Here, we report the results of a surveillance campaign conducted in central and northern districts of the country (Table 5). The majority of specimens (95%) of the 41 specimens were collected by means of community cooperation. The only vector species reported from this surveillance effort was *T. dimidiata*, which was found in 18 of the 31 villages studied.

Prediction model for *T. dimidiata*

The overall distribution records compiled for *T. dimidiata* are mapped in Figure 8. The MaxEnt output model of predicted distribution is shown in Figure 9. The area under the curve (AUC) as calculated for the training points used in the model was 0.956 and the AUC for test points was 0.868. With the reported threshold, the model predicted presence

significantly better than by random selection ($p<0.01$). Results of the jackknife analysis of variable importance are presented in Figure 10. The highest gain was achieved when variable bio07 (temperature – annual range, Table 4) was used in isolation.

Prediction model for *R. prolixus*

The distribution records associated with *R. prolixus* in Central America are shown in Figure 8. The MaxEnt output model of predicted distribution is shown in Figure 11. The AUC for the training points used in the model was 0.925 and the AUC for test points was 0.947. With the reported threshold, the model predicted presence significantly better than by random selection ($p<0.01$). Results of the jackknife analysis of variable importance are available in Figure 12. The highest gain was achieved when variables bio03 (isothermality, Table 4) and bio04 (temperature seasonality, Table 4) were used in isolation.

Environmental Values: Species Comparison

In exploring the environmental input layer data with respect to the presence data set, mean values of altitude and temperature information are reported in Table 6. The mean altitude of *T. dimidiata* presence points was 381.2 meters. The mean of the maximum temperatures associated with presence data was 32.0° C and the mean of the coldest temperatures was 15.8° C. The mean temperature associated with presence data points ranged by 16.1° C. The mean altitude of *R. prolixus* presence points was 673.6 meters (Table 6). The mean of the maximum temperatures associated with presence data was 32.5° C and the mean of the coldest temperatures was 15.1° C. The mean temperature associated with presence data points ranged by 17.4° C. According to the results of

student's t-tests, mean altitude, isothermality, and seasonality were significantly different when comparing species location data.

DISCUSSION

Despite the relatively low level of new reports pertaining to the presence of *T. dimidiata* in northern and central Belize documented here, the model supports the widespread presence of this important vector species throughout the region. This surveillance data provides additional baseline information to track future trends in vector distribution and supports the need for the northern and eastern districts to strengthen surveillance and control programs. Alongside additional investigations into the genetics of these new samples, this new distribution data can be integrated into ongoing discussions related to niche differentiation among sympatric sibling clades of *T. dimidiata* throughout Central America (26). Determining ecological and behavioral aspects associated with sibling clades may reflect differences in vectorial capacity, a vital designation of determining disease risk to local human populations. Because the development of a strong model depended on the number of presence input records, the current study aimed at developing a model for the greater Central American region. Additional vector surveillance and reporting of *T. dimidiata* presence within Belize would allow for the development a more specific model predicting the presence probability within country, which may be useful for guidance of control efforts.

Because *R. prolixus* has never been reported from Belize, the presence data set was limited to countries at the regional level that have published records. Overall, this dataset was not complete, in that much of the vector surveillance referred to in previous publications was performed by government sponsored programs and these data may not

have been available in the general scientific literature. However, the model does include areas of central and southern Belize in the predicted distribution of *R. prolixus*. Based on this evidence, this portion of the country displays environmental attributes similar to locations from which *R. prolixus* has been reported. This supports the potential for *R. prolixus* to take hold within this geographic region if it were to be introduced. This information is of some value to district level vector control programs in training and preparing their personnel for vector surveillance.

While altitude does not appear to strongly impact gain in each model (Figures 10 and 12), the predictive distributions of each species as seen in Figures 9 and 11 seem to be linked to the altitude profile of the region. Also, the comparison of mean altitude associated with the presence data for each species was statistically significant such that *R. prolixus* was associated with higher altitudes than *T. dimidiata*. Despite previous reports associating the presence of *R. prolixus* with areas lower in altitude (33), the model developed here predicts the presence of this vector in regions of Belize with the highest altitude. It is important to note that the highest elevation found in Belize is approximately 1,100 meters which is markedly lower than mountains associated with previous distribution records, as some of the higher elevations of the Sierra Madre de Chiapas mountain range of Mexico exceed 4,000 meters. In order to rationalize this apparent disagreement between the previous reports and the models developed here, it may be necessary to further define the cutoff for what is considered high and low altitude. For instance, what is considered high altitude within Belize is likely still within the lower altitude range when considering the greater Central American region. Differentiating the impact of altitude and temperature on the distribution of a species can be difficult, since

the two factors are inherently linked. It is interesting that both predictive models associate the presence of each vector with temperature variables; yet overall the distribution predictions do not overlap. This may be attributed to metabolically favorable temperature profiles intrinsic to the successful growth of each vector species. Here we report significant differences in seasonal and isothermic temperature profiles which may have a greater impact on species distribution than the more straightforward minimum and maximum mean temperature values. Additional environmental variables must be modeled to determine if the temperature associations here are not simply a correlative relationship with ties to outside factors such as animal reservoir distribution, human housing characteristics, land use, or vegetation. In the study region, *R. prolixus* was highly domesticated and it is possible that the introduction of this invasive species was limited to areas with specific temperature profiles. Such a high level of domesticity may lessen the impact that climatic variable have on species distribution, stressing the need for a more extensive surveillance program.

Lastly, it is important to note that the presence data used to develop these models were from studies focusing on the indoor capture of Chagas disease vectors. These presence records are limited and do not capture species distribution in sylvatic environments which is of particular importance when considering non-domesticated populations of *T. dimidiata*. However, human-vector contact is the predominant means for disease transmission and this contact is most likely to occur in the home. Epidemiologically, instances of household invasion may be the more valuable predictor for the risk of human-vector contact, and thus serve as a tool for guiding control methods such as indoor residual spraying (IRS) of insecticides that operate at the household level.

As novel control methods targeting non-domesticated vector populations develop, additional presence data may be useful in guiding the implementation of such efforts.

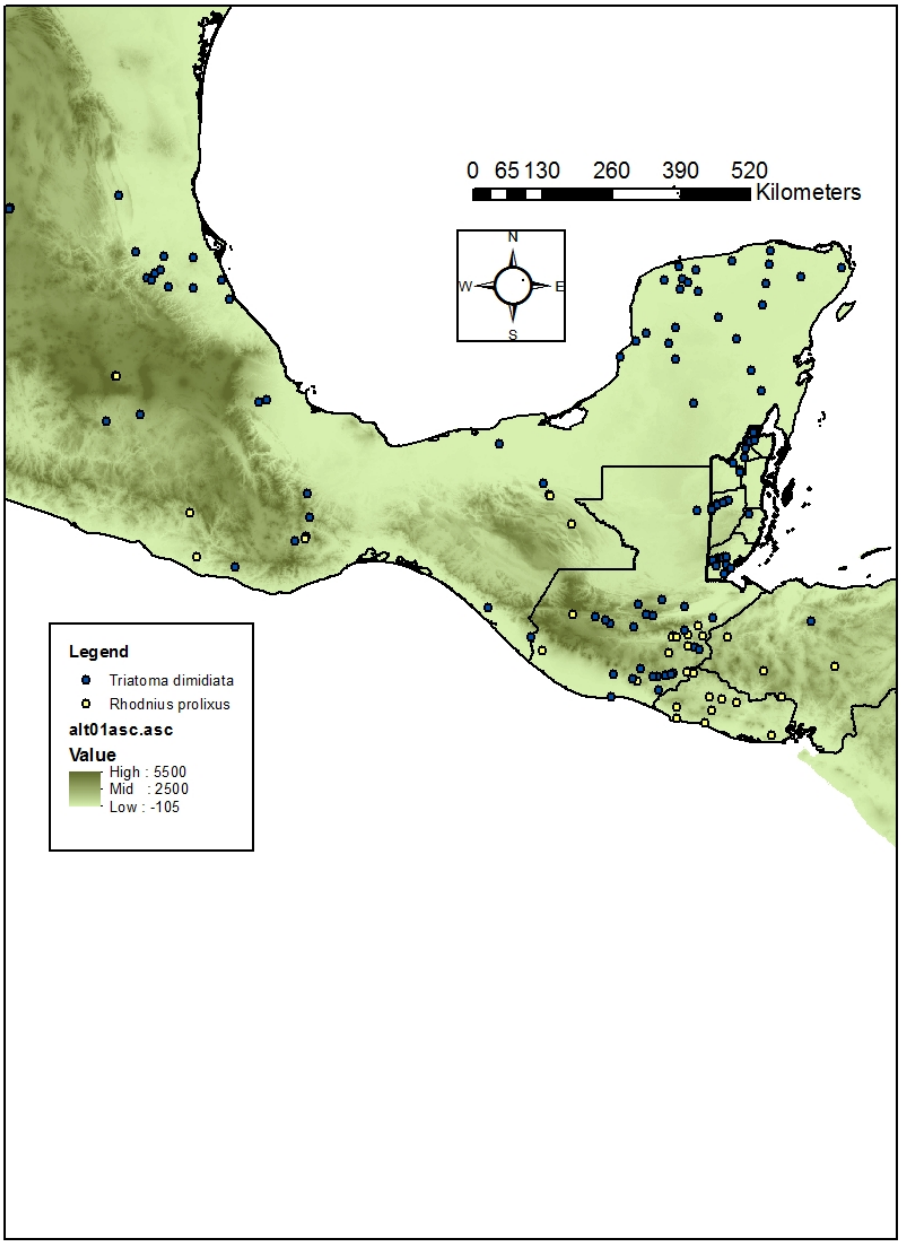

Figure 8. Distribution map of presence data for *T. dimidiata* and *R. prolixus*. Yellow circles represent presence data location of *T. dimidiata*; blue circles represent presence data location of *R. prolixus*. Base-map shading is altitude.

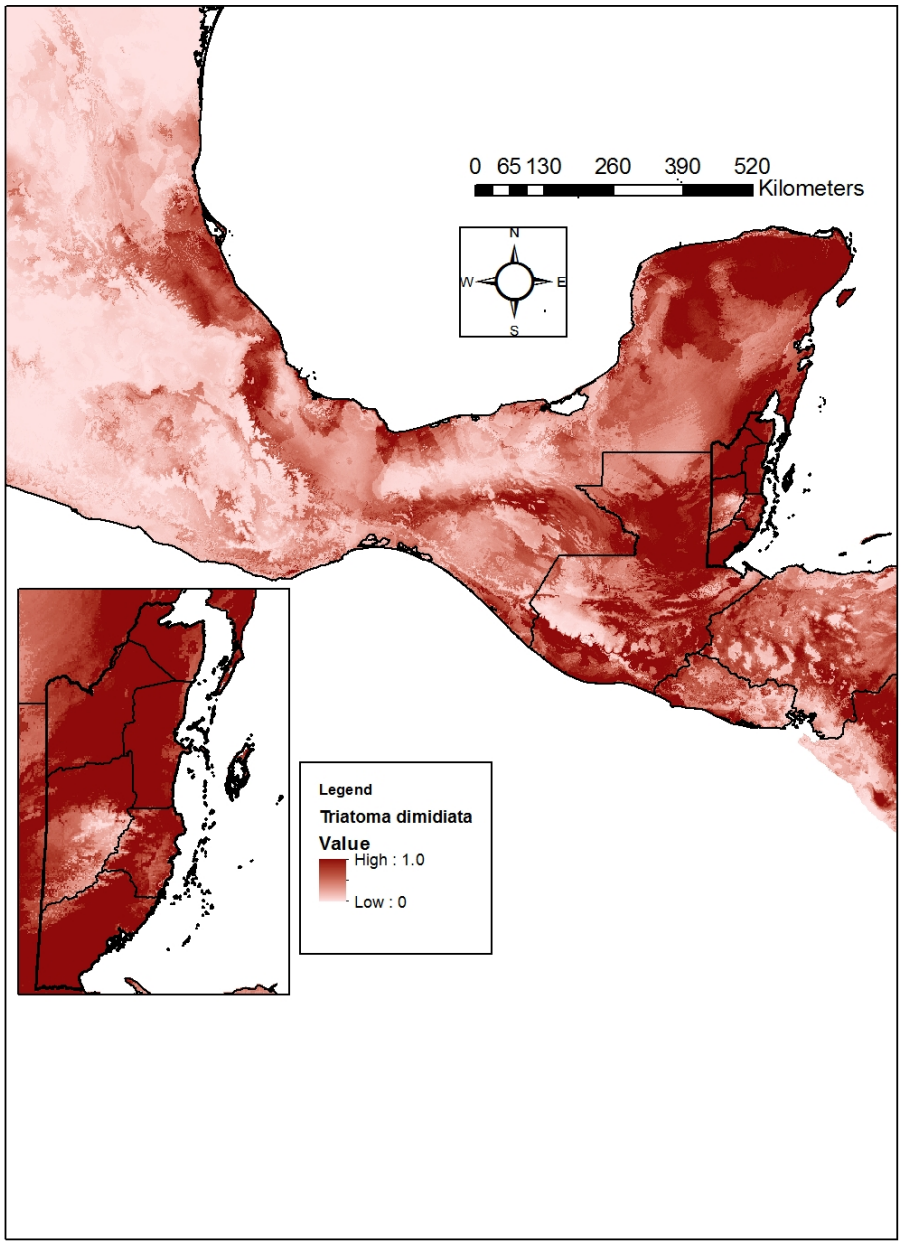

Figure 9. Predicted distribution of *T. dimidiata* developed in MaxEnt. The red regions represent areas that have a high probability for the presence of *T. dimidiata*. The insert in the bottom left provides a closer view of Belize.

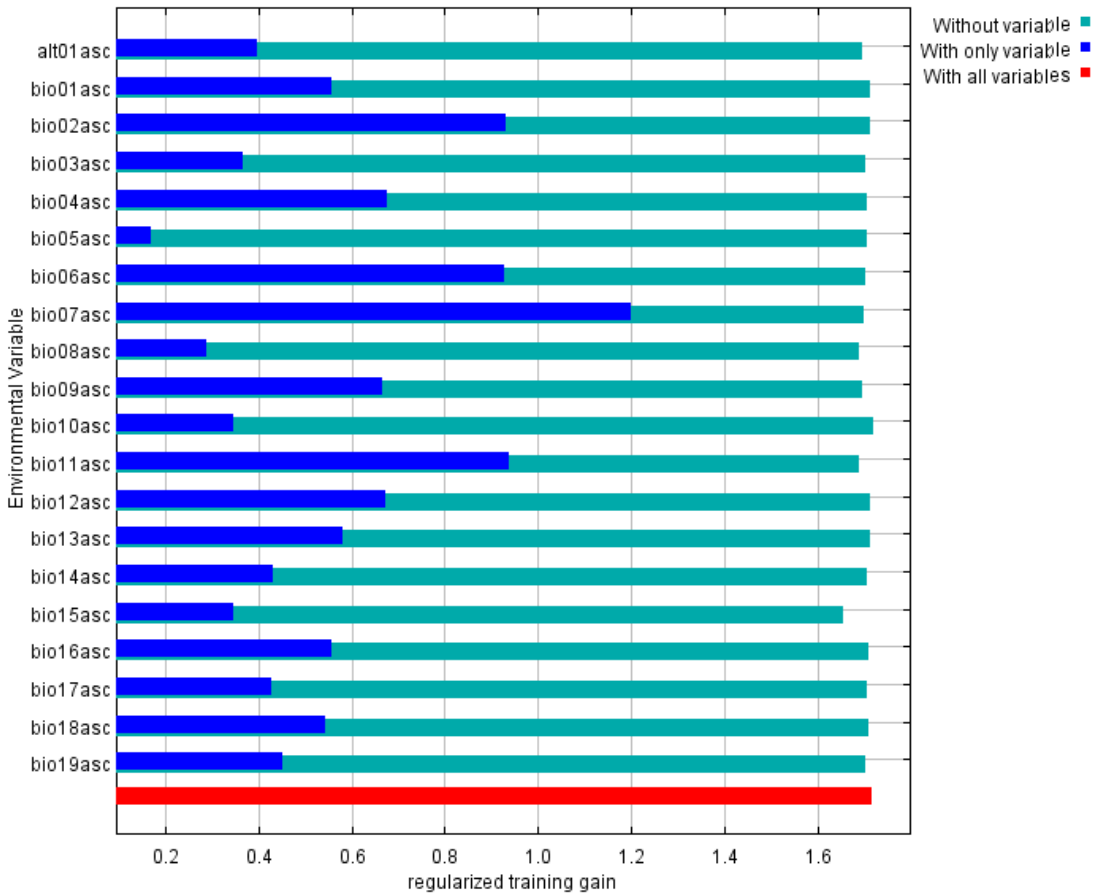

Figure 10. Jackknife test of variable importance for model predicting *T. dimidiata* presence

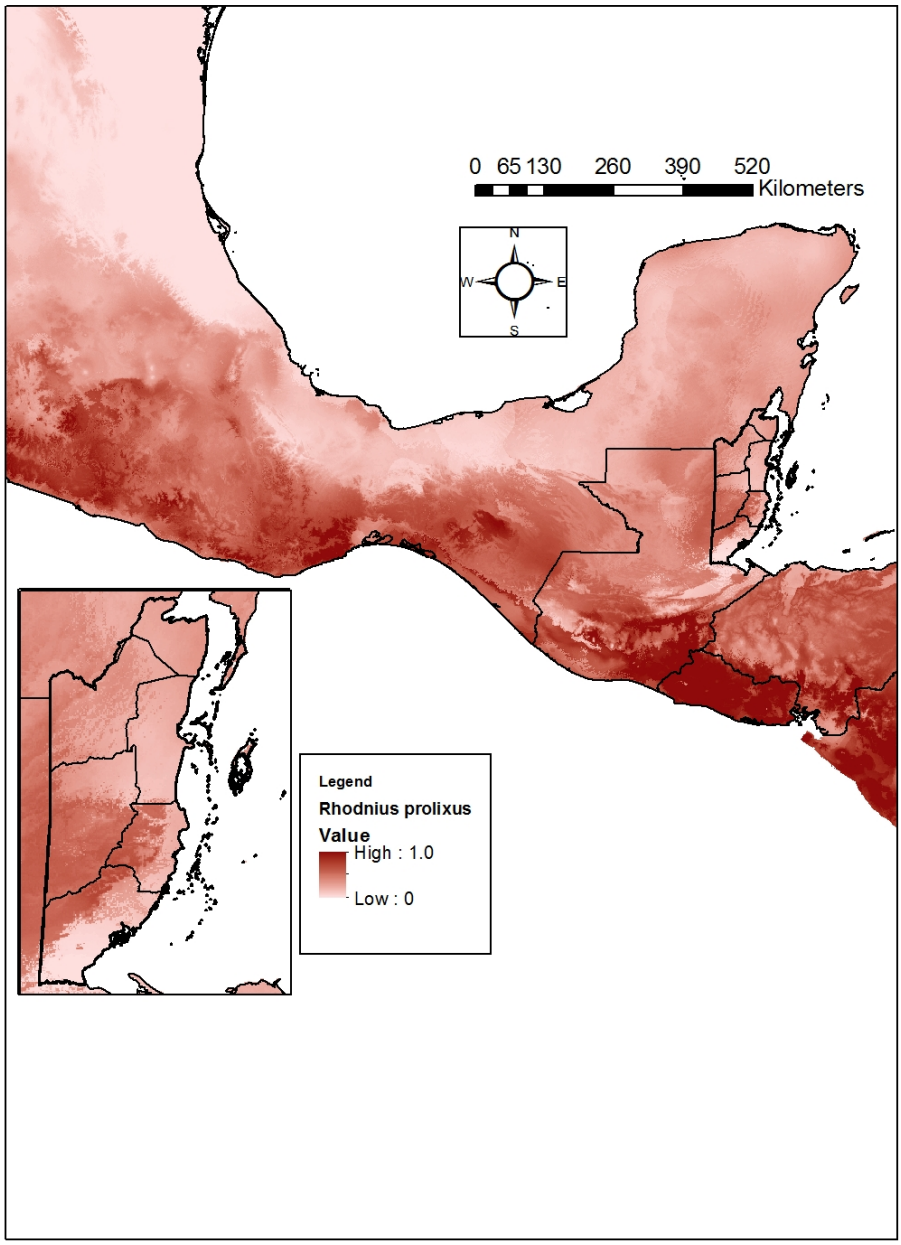

Figure 11. Predicted distribution of *R. prolixus* developed in MaxEnt. The red regions represent areas that have a high probability of presence of *R. prolixus*. The insert in the bottom left provides a closer view of Belize.

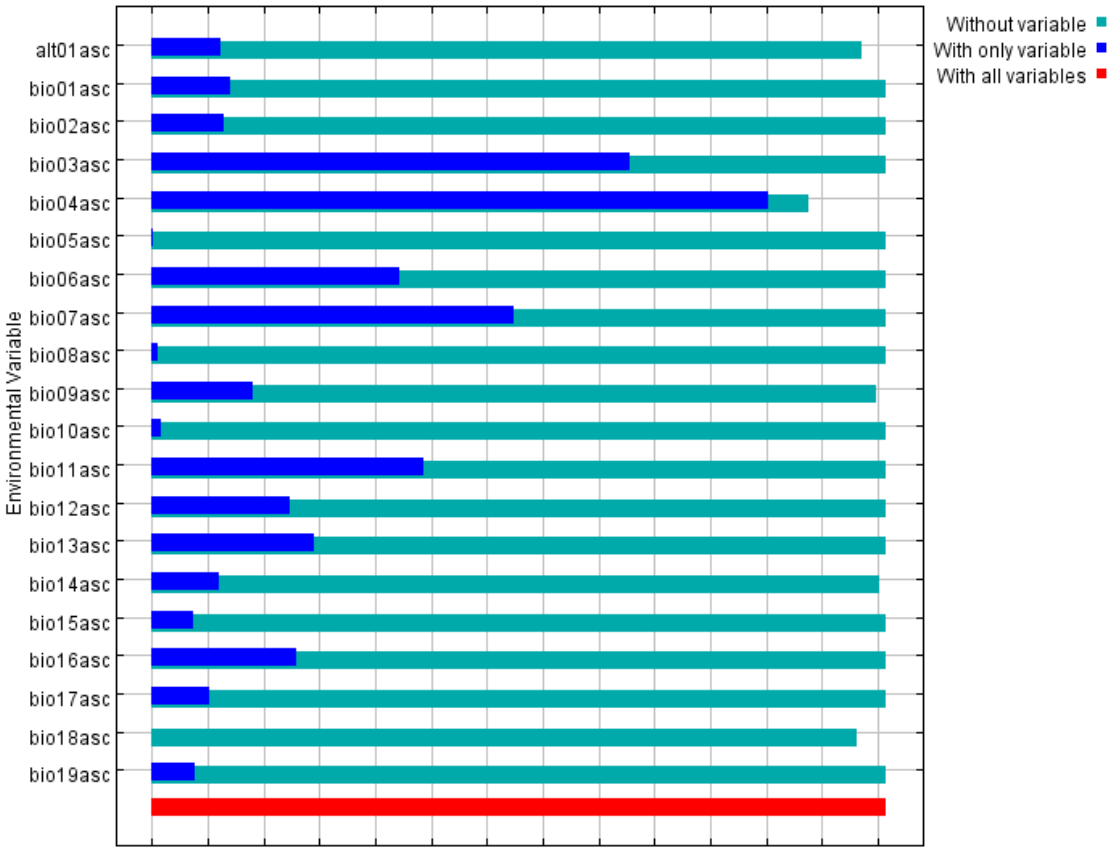

Figure 12. Jackknife test of variable importance for model predicting *R. prolixus* presence

Table 4. Descriptions of altitude and environmental variables used in ecological niche modeling

Variables Defined	
alt	Altitude
bio1	Annual Mean Temperature
bio2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
bio3	Isothermality (BIO2/BIO7) (* 100)
bio4	Temperature Seasonality (standard deviation *100)
bio5	Max Temperature of Warmest Month
bio6	Min Temperature of Coldest Month
bio7	Temperature Annual Range (BIO5-BIO6)
bio8	Mean Temperature of Wettest Quarter
bio9	Mean Temperature of Driest Quarter
bio10	Mean Temperature of Warmest Quarter
bio11	Mean Temperature of Coldest Quarter
bio12	Annual Precipitation
bio13	Precipitation of Wettest Month
bio14	Precipitation of Driest Month
bio15	Precipitation Seasonality (Coefficient of Variation)
bio16	Precipitation of Wettest Quarter
bio17	Precipitation of Driest Quarter
bio18	Precipitation of Warmest Quarter
bio19	Precipitation of Coldest Quarter

Table 5. Novel presence records for north and central Belize

District/Department	Longitude	Latitude	Village
Cayo	-89.04365	17.16642	Santa Elena
Cayo	-89.03856	17.18172	Esperanza
Cayo	-88.93860	17.21100	Unitedville
Cayo	-88.91422	17.22103	Blackman Eddy
Cayo	-88.83715	17.23312	Camelote
Cayo	-88.50950	17.02430	Middlesex
Corozal	-88.40407	18.23652	Progresso
Corozal	-88.43007	18.38790	Xaibe
Corozal	-88.41719	18.37561	Ranchito
Corozal	-88.52200	18.23580	Buena Vista
Corozal	-88.47786	18.23111	Caledonia
Corozal	-88.40550	18.39264	San Andres
Orange Walk	-88.77357	17.86910	San Felipe
Orange Walk	-88.65935	17.75550	Indian Church
Orange Walk	-88.65387	17.71817	San Carlos
Orange Walk	-88.59084	17.96799	Guinea Grass
Orange Walk	-88.57293	18.10550	Trial Farm
Orange Walk	-88.59084	17.96799	Douglas

Table 6. Environmental variable values associated with presence distribution points; *designates significant difference in mean values at *P<0.05*

		T. dimidiata		***R. prolixus***		**t-test**		
		Mean	St. dev.	Mean	St. dev.	T	SE	p value
alt*	mean altitude (meters)	381.2	385.37	673.6	569.37	3.51	83.1	0.0006
bio3*	Isothermality	65.6	5.80	71.2	4.39	5.33	1.05	0.0001
bio4*	Seasonality	173.2	67.40	124.7	42.30	4.07	11.9	0.0001
bio5	Warmest temperature (Celsius)	32.0	2.47	32.5	2.77	1.03	0.50	0.3050
bio6	Coldest temperature (Celsius)	15.8	3.13	15.1	4.08	1.09	0.65	0.2795
bio7	Temperature range (Celsius)	16.1	3.44	17.3	2.86	1.90	0.63	0.0598

CHAPTER 4: Evaluation of IRS control methods targeting *Triatoma dimidiata* (Reduviidae: Triatominae): susceptibility to alphacypermethrin

ABSTRACT

Presently, application of pyrethroid insecticides in households at high risk for triatomine colonization or invasion is a common means of vector control. Specifically in Belize, reports of vector presence within households are followed by the application of pyrethroid insecticides by means of indoor residual spraying (IRS) or thermal fogging. Here, we explore the effects of alphacypermethrin on the sole vector species reported from Belize, *Triatoma dimidiata*. In a controlled setting, colony-reared, 3rd instar nymphs were exposed to control and insecticide treated surfaces for three exposure periods: 5 minutes, 30 minutes, and 3 hours. Insects were then housed to record 72 hour post-exposure mortality. Three types of treated surfaces (wood, tile, and cement) were used as testing surfaces with the intention of mimicking common household wall materials used in home construction throughout Belize. The insecticide application rate (5%) and methods (IRS) also emulated current vector control practices commonly performed in response to reports of the presence of *T. dimidiata*. No initial knockdown was observed among all treatment materials and across all exposure periods. When comparing 72 hour post-exposure mortality, a single treatment group significantly differed from the control group. *T. dimidiata* exposed to 5% alphacypermethrin applied to tile for a 3 hour exposure period suffered a significantly higher rate of mortality than all other treatment groups.

In order to further evaluate the susceptibility of *T. dimidiata* to alphacypermethrin, we performed a topical assay on 3rd instar nymphs and developed a response curve in order to calculate lethal dose 50% and 90% (LD_{50} and LD_{90}) values. The resulting values indicate that the commonly used application rate is likely much lower than the LD_{90} calculated by means of the topical assay. Further field evaluation is required to determine what, if any, effect current control methods have on local sylvatic vector populations.

INTRODUCTION

Reducing incidence of Chagas disease by means of vector transmission is often listed as a high priority action of Latin American governments (1; 11; 33). The Southern Cone Initiative, organized by WHO in conjunction with several nations in South America, achieved some success in the elimination of *Triatoma infestans*, an important Chagas disease vector. This commitment to vector control was reflected in the reduction of disease incidence (49; 88). Similarly, in Central America, a multi-nation, government-guided control effort targeting *Rhodnius prolixus* succeeding in eliminating this invasive species from many countries previously reporting vector presence (33). These broad vector control campaigns implement methods, such as IRS and thermal fogging, at the household level, principally impacting domesticated triatomine vector populations.

Belize, on the eastern coast of Central America, is one of several nations with underdeveloped surveillance and control programs concerning incidence of human Chagas disease, infection rates of insect vector populations, and success rates of control programs targeting triatomines. Presently, the sole Chagas disease vector reported from the nation of Belize, *T. dimidiata*, is widespread throughout the region (70). Often

sylvatic in nature, this vector species intermittently invades houses in search of a blood meal, but does not mate and reproduce in the domestic settings (70). While this behavior limits human-vector contact and thus reduces vectorial capacity, insecticide control methods applied within the household do not impact sylvatic vectors to the same degree as domestic vector populations (12).

Currently, the control methods targeting *T. dimidiata* in Belize are limited to the responsive application of pyrethroid insecticides to households reporting vector presence. A common pyrethroid used for indoor applications against triatomine insects is alphacypermethrin (59; 79). Pyrethroid insecticides inhibit the closing of sodium channels along nerve axons of susceptible vector insect. Alphacypermethrin is included within this class of insecticides that affect the nervous system and are used in pest control at low doses. Formulations with high residual activity, such as wettable powder and paint based product, of alphacypermethrin and other pyrethroid insecticides have been favored in the control of domesticated triatomine vector populations when considering cost effectiveness (88).

In this study, the main objective was to evaluate the impact of a commonly used, pyrethroid insecticide, alphacypermethrin, on colony-reared *T. dimidiata*. The insecticide was applied to several different housing materials by means of IRS application, emulating the real-world insecticide control methods as they are applied within homes in Belize. Three exposure times were utilized to explore the irregular exposure length experienced by sylvatic vectors: 5 minutes, 30 minutes, and 3 hours. Lastly, topical applications of various concentrations of alphacypermethrin were performed in order to

develop a response curve and calculate associated LD_{50} and LD_{90} values. These results should have implications for the effectiveness of current vector control campaigns.

MATERIALS AND METHODS

Maintenance of *T. dimidiata* colony

T. dimidiata vector colonies were maintained according to the protocol described in Durvasula et al. (20). Insects were housed in a Percival I-36VL incubator (Percival Scientific, Perry, IA) which was maintained at 23ºC and 70% RH. Plastic containers were stuffed with accordion-folded strips of heavy-weight rosin paper (Triamco, Morrisville, NC) and served as colony-rearing containers. The containers were fastened with 1 mm cross-section netting (Bioquip, Rancho Dominguez, CA) covers to seal the top, offering an opening for air circulation and a surface for feeding. Every two weeks (with the exception of fasting periods prior to insecticide assays) colony containers were offered blood via an artificial feeding apparatus. Heated water circulated through open ended glass bells, which maintained blood at or near 36ºC. Approximately 15 ml type O negative whole blood (Interstate Blood Bank Inc., Memphis, TN, USA) was then pipetted into the glass bell after incubating in a 36º C water bath for 20 minutes. The feeding contact surface provided to the colony was made up of stretched Parafilm® (Bemis NA, Neenah, WI). Each container was covered with black plastic sheet and allowed to feed for 45 minutes. The external surfaces of the feeding apparatus and associated tools were cleaned with bleach between uses. This vector species developed through 5 instar stages before becoming a functionally reproductive adult. Because the growth period between these stages can exceed 6 weeks and the colony suffered high mortality at these developmental transitions, 3rd instar nymphs were used in the assays described below.

IRS application of alphacypermethrin

Preparation and application of alphacypermethrin by means of IRS were performed using WHO insecticide application guidelines (89). A three-gallon stainless steel Hudson® sprayer (H.D. Hudson Manufactoring Co., Chicago, IL) was calibrated with laboratory-grade distilled water according to WHO guidelines (89). This preparation was then used to apply water as a control onto a set a set of three surfaces: tiles, wood, and cement. The sprayer was then refilled with a sachet of Fendona 5WP®, a commonly used commercially produced formulation of alphacypermethrin, and mixed with distilled water according to package instructions. This was used for insecticide application to treatment surfaces. Spray swaths measured approximately 75 cm, no overlap spray was necessary to achieve full coverage of the testing surfaces. The spray tip was kept 45 cm from the spray surface which produced a 650 ml/min (+/- 10 ml/min) rate of application. According to package labels, this process results in a 5% alphacypermethrin solution, with coverage rate of 0.03 g/m^2. All control and treatment surfaces were prepared in the same setting within 4 hours of each other and stored in separate areas to avoid contamination.

Cone Assay

In order to evaluate the impact of insecticide treated surfaces on vector mortality, 3rd instar *T. dimidiata* nymphs were placed in plastic cone holding containers attached to surfaces within the spray swath described in the treatment methods above (Figure 13). Three replicates, for a total of 15 insects, were tested at three different exposure periods: 5 minutes, 30 minutes, and 3 hours. This process was repeated against the three types of treatment surfaces: wood, tile, and cement. Insects were then removed from underneath

the plastic cones and placed in clean, labeled, plastic holding containers for observation. Initial knockdown was observed and recorded. Mortality at 24, 48, and 72 hours post-exposure was logged. Student's t-test analysis was performed to determine significant difference in 72 hours post-exposure mortality of treatment groups compared to controls.

Droplet Assay

Laboratory grade, 99.9% alphacypermethrin (Supelco, Bellefonte, PA) was diluted in acetone to 5 concentrations: 130 mg/ml, 65 mg/ml, 0.65 mg/ml, $6.5x10^{-3}$ mg/ml, and $6.5x10^{-5}$ mg/ml. *T. dimidiata* 3rd instar nymphs, reared at the conditions described above, which were bloodfed and then fasted for two weeks were used in groups of ten for control and treatment groups. For control cohorts, 1 μl of acetone was applied by micropipette to the dorsal abdomen. Treatment cohorts received 1 μl of insecticide solution. Groups of ten treatment and control cohorts were treated each time, with each treatment repeated on at least one separate occasion. After topical application, insects were kept in clean, plastic holding containers for observation. Initial knockdown was observed, as well as mortality rates at 24, 48, and 72 hours post exposure. Initial knockdown was defined as the loss of directed, coordinated locomotion. Mortality was assessed as the lack of response to external stimuli. For each treatment group, percent mortality at 72 hours post-exposure was calculated and probit analysis performed to extrapolate LD_{50} and LD_{90} values.

RESULTS

Cone Assay

As seen in Table 7, challenging *T. dimidiata* against field application rates of alphacypermethrin applied to common household surfaces results in no knockdown

activity across all exposure times. Only one treatment group recorded a mortality level significantly different from control, the cohort exposed for 3 hours against treated tile exhibited a loss of 7/15 individuals 72 hours post-exposure.

Droplet Assay Response Curve

After insecticide solution was applied directly to the dorsal abdomen, 72 hour post-exposure mortality was measured (Table 8). In the application of acetone control, 1 of the 15 insects was dead 72 hours post-exposure. At the lowest concentration used, $6.5x10^{-5}$ mg/ml, 5 insects died. Nine and 13 specimens were dead at 72 hours post-exposure for concentrations $6.5x10^{-3}$ mg/ ml and 0.65 mg/ ml, respectively. The remaining concentrations resulted in 100% mortality at 72 hours post-exposure. From this data, 3 probit values were calculated as plot points for the final regression model (Table 8): 4.42, derived from the mortality rate at $6.5x10^{-5}$ mg/ml exposure, 5.18, derived from the mortality rate at $6.5x10^{-3}$ mg/ml exposure, and 6.04, derived from the mortality rate at 0.65 mg/ml exposure. These probit values were modeled against the respective Log10 concentrations to achieve the resulting regression equation: $y = 0.405x + 6.0991$ (Figure 14). This equation was then used to calculate the LD_{50} and LD_{99} values of $6.8x10^{-3}$ mg/ml and 2.75 mg/ml, respectively.

DISCUSSION

Vector surveillance and control programs are implemented in a public health context to reduce human-vector contact with the final aim of decreasing vector-borne disease incidence (88). These programs should be subject to regular inspection to determine if the ongoing methods used to achieve these goals are impacting disease transmission. Unfortunately, vector control methods that have proven successful in one

system are then applied to a new region without follow up studies regarding efficacy or cost effectiveness with regards to controlling a target vector species. While the application of pyrethroid insecticides by means of IRS have appeared efficacious when targeting domesticated *T. infestans* and *R. prolixus* vectors (33; 88), this study is the first evaluation of such control methods against *T. dimidiata* populations native to Belize.

It should be noted that successful control campaigns reported on in the past have taken steps to evaluate the appropriate insecticide concentrations for use in the field when targeting local triatomine vectors. Here, we present data that strongly support a need for such evaluation in the insecticide control protocol currently in place for the control of *T. dimidiata* in Belize. The field application rate of alphacypermethrin failed to achieve notable mortality on 3rd instar *T. dimidiata* nymphs. This is particularly concerning when considering additional vector behavior information regarding local *T. dimidiata* populations. For instance, it has been reported that *T. dimidiata* populations within Belize are largely sylvatic in nature and the vectors intermittently enter household in search of a blood meal (70). This behavior limits the time spent within the household and may reduce any impact of insecticide control measures implemented within the home. Because these local populations tend to be sylvatic in nature, only adult vectors are commonly found within households. It is possible that the current rate of field application of insecticides would have even less of an impact on larger adult insects compared to the 3rd instar nymphs used in this study.

When assessing the effect of surface material on mortality of insects exposed to insecticide-treated surfaces, it is interesting to note that tile surfaces were the only treated surface with an observable impact on *T. dimidiata* mortality in this study. It is possible

that the non-porous tile surface allowed more of the active ingredient to remain on the surface of the material. Porous wood and cement materials may absorb more of the insecticide solution, leaving less active ingredient at the surface-insect interface. However, the porous nature of these materials may also allow for longer residual maintenance of insecticide presence. The interplay between surface material, application rate, and residual efficacy must be further investigated with respect to control methods targeting adult *T. dimidiata*.

The results of the topical assay are revealing with respect to the concentration administered for testing in cone assay. From a cursory comparison, the LD_{99} calculated from the results of the topical assay far exceed the field application rate used in the treating surfaces for the cones assay. The true level of insecticide exposure an insect receives when contacting a treated surface is difficult to assess. Most triatomines are more likely to walk across a given surface than to fly and intermittently land, measuring the length, duration, and surface area an individual vector contacts is a difficult task, and not particularly feasible in the performance of ongoing insecticide resistance practices. Therefore, this study provides some evidence for the need to thoroughly investigate a working relationship between the suggested concentrations calculated by means of topical assays, with the efficacy data from the field, such that evidence based changes in control methods can include the information gained from controlled insecticide assays.

Lastly, it is imperative for local vector control personnel to regularly evaluate control results by exploring a number of output measures, including vector presence and human disease incidence. Additional work must be performed, particularly concerning

disease transmission in Belize, in order to report on the current impact of vector control campaigns and guide future campaigns against local vector populations.

Figure 13. Cone assays performed on three different surface materials for three exposure periods.

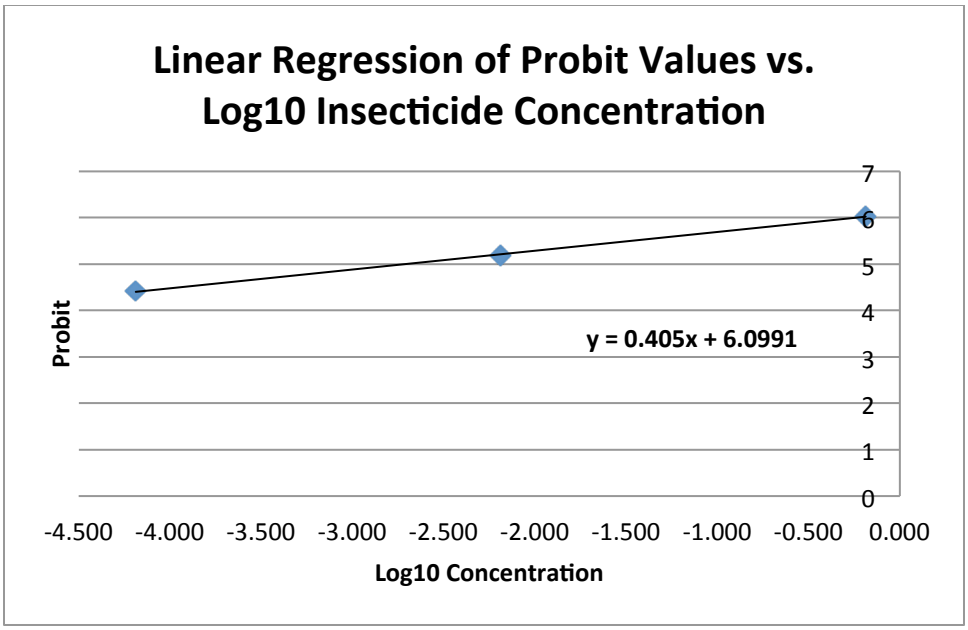

Figure 14. Linear regression of probit values vs. Log10 of insecticide concentrations.

Table 7. Resulting mortality of cone assay. Insects were exposed to insecticide treated surfaces for specific exposure periods. *designated significant difference from control where $P<0.05$.

Observed mortality of *T. dimidiata* against insecticide treated surfaces						
		Knockdown	**24 hr mortality**	**48 hr mortality**	**72 hr mortality**	**Total Mortality**
Control	**wood**	0/15	1/15	0/15	0/15	1/15
	tile	0/15	0/15	0/15	0/15	0/15
	cement	0/15	0/15	0/15	0/15	0/15
5 min	**wood**	0/15	0/15	0/15	0/15	0/15
	tile	0/15	0/15	1/15	0/15	1/15
	cement	0/15	0/15	0/15	0/15	0/15
30 min	**wood**	0/15	0/15	0/15	0/15	0/15
	tile	0/15	0/15	2/15	0/15	2/15
	cement	0/15	0/15	0/15	0/15	0/15
3 hr	**wood**	0/15	1/15	2/15	0/15	3/15
	tile	0/15	2/15	3/15	2/15	7/15*
	cement	0/15	0/15	0/15	0/15	0/15

Table 8. Mortality and associated probit analysis of *T. dimidiata* exposed to topical application of alphacypermethrin

Insecticide concentration mg/ml	Log10 of Conc.	Total N	n dead	% mortality	corrected % mortality	probit
Control		15	1	6.67		
6.50E-05	-4.187	15	5	33.33	28.57	4.42
6.50E-03	-2.187	15	9	60.00	57.14	5.18
0.65	-0.187	15	13	86.67	85.71	6.04
65.00	1.813	15	15	100.00	100.00	
130.00	2.114	15	15	100.00	100.00	

CHAPTER 5: Summary and General Conclusions

DISSERTATION SUMMARY

Belize, on the eastern coast of Central America, is one of several nations with underdeveloped surveillance of both Chagas infection rates and insect vector populations. Basic ecological aspects of the vector population went unreported, particularly in the northern regions of the country. The geographic distribution of Chagas disease vector populations within this region had not been available in the literature. Despite the stress placed on the link between vector infestation and aspects of human housing structures in several regions throughout Central and South America, regions of northern Belize had not been investigated in this manner (17; 31). We sought to perform some of these baseline surveillance studies in order to provide local vector control authorities with a working knowledge of the current status of both the distribution and bionomics of key Chagas disease vectors in northern and central Belize.

Based on the one previous study focusing on surveillance of triatomine vectors in Belize, community-based collections became a cornerstone of our work from the onset (70). Although we attempted to integrate additional methods for vector collection, the number of triatomines collected did not offset the highly invasive nature of alternate methods. Active searching and nocturnal lighting put undue burden on homeowners as these collection methods can largely disturb daily life. These methods also provide minimal output considering the comparatively high input of equipment and man-hours. Our means of methods comparison was helpful in providing evidence of a lack of domesticity in the local vector populations, but continued surveillance should focus on community collections to maximize efficiency. Community-based collecting has become

a popular means of surveillance in similar settings throughout central and southern Belize and regions of Mexico (19; 28; 54; 70). In these regions, authors have reported on the importance of integrating the local community in collection efforts to bolster awareness and strengthen effective coverage of vector surveillance. Education-based outreach focusing on school-age children has assisted in improving surveillance programs in Brazil (23). Better integration by means of community educational outreach focused at school-age children and/or community health workers could be readily applied at the village level in northern Belize. Community health workers are already in place to assist in epidemiological surveillance targeting malaria (Caranci personal observation 2013). It may prove useful to resource-limited vector control offices working alongside community health workers to coordinate efforts against other emerging infectious diseases. This would allow the Belize Ministry of Health to readily address knowledge gaps with respect not only to Chagas disease, but also to dengue, chikungunya, zika virus, and other emerging threats.

Here we report the broad distribution of what continues to be the sole vector species from Belize, *T. dimidiata*. This species appears to be locally sylvatic in nature, with only adult insects found intermittently within the household. While vector presence within households appears low, difficulties in reporting the presence of sylvatic vector species may lead to an underestimation of true levels of household invasion. Despite apparently low level of human-vector contact, local populations of *T. dimidiata* display high rates of infection with *T. cruzi*, the causative parasite of human Chagas disease. In previous studies that focused on triatomine surveillance in central and southern Belize, 87% of insects collected in households were adults (70). Here, we report 100% of

invading *T. dimidiata* to be adults. When viewed collectively, we can conclude that *T. dimidiata* populations in this region are largely sylvatic, but occasionally invade household spaces late instar nymphs or adults. The data presented here focusing on northern Belize was limited to cover only 10 houses per village on average. Because this was an initial effort aiming to describe the regional distribution of *T. dimidiata*, the number of household collection points was limited in order to increase the number of villages included for coverage. This limitation is one likely reason for the few specimens collected, but it serves to illustrate the need for increased incorporation of community health workers to strengthen surveillance programs.

In the development of molecular assays for the identification of *T. cruzi* parasites in human and vector hosts, several different primer sets have been developed and optimized. Each set had previously been optimized to detect the parasite in a specific type of tissue sample. In this study, we followed protocols used in the detection of *T. cruzi* from field collected triatomines by entomology laboratories operating at the Centers for Disease Control and Prevention (Ellen Dotson personal communication 2013). This protocol was adapted from a study that had comparatively assessed the validity and reliability of primer sets in similar investigations (86). Because the PCR primers used in our study reliably detects all strain *T.cruzi* species, information regarding the circulating strains of *T. cruzi* in Belize remains unknown.

We found risk factors associated with household invasion appear to include the presence of peridomestic dogs, the absence of peridomestic chickens, and distance of a household from community light sources. All of the attributes included in the survey portion of this study were chosen based on similar studies in the literature that have

investigated household risk factors associated with the indoor presence of triatomines (18; 31; 60; 71). The presence of peri-domestic mammals, particularly dogs, is a common risk factor that has been noted in previous studies (18; 69; 71). It is possible that particularly with sylvatic vectors, dogs that freely roam the village periphery and surrounding vegetated areas may be more likely to interact with triatomines. This has two key implications for human disease epidemiology. Dogs that feed on infected triatomines far from the household setting can keep the pathogen in circulation in and around the village setting, increasing the likelihood that vector populations near human populations may carry the *T. cruzi* pathogen. Free-roaming dogs may also serve to passively transport triatomines that are living in vegetation at the outskirts of villages. This may serve to bring these vectors closer to human households and to increase the likelihood of human-vector contact. These ecological and epidemiological trends need further research to determine the underlying relationship between peri-domestic dogs and household risk of vector invasion. The presence of chickens in the peri-domestic setting can also impact vector ecology in several ways. While birds are not reservoir hosts for *T. cruzi* because the avian immune response prevents sustained infection, birds can serve as a blood meal source for local vector populations (40). When in place, chicken coops can provide ideal shelter for triatomines which would increase the likelihood of human-vector contact. However, the likelihood that such populations are infected with *T. cruzi* would require further investigation. In Belize, chicken coops are often an added expense that many households do not allocate resources toward (Caranci personal observation 2013), as they often require the input of supplies and maintenance to ensure their protective purpose. In this setting, chickens are often free to range around the houses and do not necessarily

offer a large nesting site that would be as suitable for triatomines. These free-ranging chickens may also be more likely to feed on insects in the peri-domestic setting, including triatomines. Lastly, the presence of nearby street lights has also been associated with household invasion by triatomines (60). Only presence, absence, and distance from community light sources with respect to surveyed households were determined in this survey. Additional work to determine impact of different types of lighting on vector behavior may lead to the development of applicable interventions. These household attributes may be used to guide surveillance methods of vector control personnel and may prove valuable in the ongoing community educational programs pertaining to the behavior of local vector species.

One observation in the literature describes behavioral and morphological changes of vectors infected with *T. cruzi* (58). Authors reported increased wing-length in infected triatomine adults, which could impact flight-range. This study was largely limited by the number of vector specimens collected. A stronger community outreach program involving a health education component could strengthen this type of vector surveillance. If and when such a program is strengthened, household attributes and vector infection status could further be correlated.

When modeling the regional probability of distribution of *T. dimidiata* in Central America, temperature and altitude seem to be key determining variables. This is in line with previous work that has concluded that temperature, above precipitation, is closely associated with vector distribution (26; 33). A study in Belize linked *T. cruzi*-infected *T. dimidiata* with higher altitudes, but the small number of insects collected in this study limited our ability to develop models based on infection status (70). The differential in

temperature profiles seen in the modeling developed here also correlate to reported optimal rearing temperature profiles for *T. dimidiata* and *R. prolixus* (20). This supports the notion that regional temperature profiles may impact vector species with respect to species-specific minimum and maximum temperature thresholds which support vector metabolism and development. While *Rhodnius prolixus* remains unreported from Belize, a niche model developed for Central America appears to include regions of central Belize within the potential distribution of this species. These models can be used to guide current surveillance training and implementation in Belize, and the growing species presence dataset should be improved to develop ecological niche models specific to Belize.

Our research supports the notion that current insecticide-based control methods employed to limit the contact between humans and triatomine vectors are insufficient, and may not be cost effective in targeting locally sylvatic populations of *T. dimidiata*. Because *T. dimidiata* can require 1-2 years to complete a full generation, this study was limited to the use of 3rd instar nymphs for insecticide assays (20). As the current colony source continues to develop, it may be useful to repeat controlled assays on all developmental stages in order to assess differential mortality across instar stages. It is also imperative to note that the colony was sources by 10-12 females all collected from the same cave populations. Populations of *T. dimidiata* have been shown to be isolated with little genetic exchange among focal populations, so the results of our assay were limited to what could be a genetically clonal population due to sampling (44; 48). Because the source population was found outside of a populated area, it is unlikely that the population would have any pre-exposure to insecticides or be genetically resistant to

commonly used insecticides. The triatomines found in peri-domestic settings may be genetically different and are more likely to have been pre-exposed to insecticides.

When exposing colony-reared *T. dimidiata* to surface materials sprayed with the field application rate of alphacypermethrin, minimal mortality was observed. Controlled experiments evaluating the impact of topically-applied alphacypermethrin against the same colony-reared population revealed that high rates of mortality were only achieved by higher concentrations of insecticide solutions. Similar topical assays have been performed on 5th instar nymphs of other triatomine species (59). The LD_{50} values extrapolated in this study are in the same range as those reported for *Triatoma infestans*, but higher than those reported for *R. prolixus*. The application rate of pyrethroid insecticides, include alphacypermethrin, has been shown to be highly species specific, thus increasing the importance of an integrated surveillance and control program that ensures adequate control measures targeted to specified vector populations. This has direct consequences for the current vector control methods employed in Belize, which appear to be ineffectual. As with any other vector control program involving the use of insecticides, regular assessment of the impact of insecticides on the mortality of target populations is vital to ensuring the ongoing performance of such programs. The use of sub-lethal levels of insecticides has been shown to drive increased insecticide resistance in target vector populations (27). It is unclear at this point whether the ongoing vector control activities in Belize are impacting local triatomine populations in such a manner. The transient nature with which vectors are present in the home may not allow for enough of an exposure to cause selection pressure on triatomine populations; however, the application of insecticides as described may also be negatively impacting non-target

mosquito populations. This study was not able to assess impact of vector control programs in the field setting, but such studies are warranted with respect to the results of the controlled assays reported here.

Future Research Needs and Knowledge Gaps

"When we try to pick out anything by itself, we find that it is hitched to everything else in the Universe." **John Muir**, 1911(55)

As with most scientific endeavors, the information here only leads us to focus on the additional research needed to elucidate the complex nature of vector-borne disease transmission and to frame recommendations for improving vector surveillance and control programs. Currently, the use of community collection is vital to determining the distribution and household invasion behavior of *T. dimidiata* populations in Belize. In order for this process to strengthen and capture the full distribution of this vector, the relationship between vector control personnel, researchers, and the local communities must take priority. Opportunities to increase the level of community education and awareness regarding Chagas disease transmission, vector biology, vector recognition, and the individual's role in vector control operations must be sought out to strengthen control campaigns. With the assistance of the public, vector control personnel may also be able to begin to address factors of human behavior and housing structure that may decrease human-vector contact.

While we were able to model basic household level and environmental attributes associated with the presence and invasion behavior of *T. dimidiata*, the need to continue these efforts in exploring additional factors is unmistakable. Research regarding the

effects of land-use and reservoir host biology on the distribution and behavior of *T. dimidiata* may offer additional guidance for vector control.

Due to our focus on the insect vectors of Chagas disease, the human infection rates in the Belizean population remains to be investigated. This piece of the transmission ecology is vital to our understanding of the key drivers of disease transmission and regular monitoring of human population should be an important metric by which control interventions are measured. In Belize, specifically, additional epidemiological work is needed to determine the circulating strains of *T. cruzi* present in the human populations and associated clinical manifestations of disease. Of particular importance should be the patients that are co-infected with HIV. Research has shown that chronic Chagas disease can be more likely to occur in HIV patients, and may manifest with complications such as meningoencephalitis (81). With reports that Belize has the highest HIV prevalence in Central America, the likelihood of coinfection and associated disease complications requires additional focus (85).

Lastly, while it is apparent that current control strategies must be adjusted to target local *T. dimidiata* populations, the real advancement in vector control for this transmission system lies in the development of novel control methods targeting sylvatic triatomine vector populations. Reduction in the population as a whole would likely reduce human-vector contact. Novel approaches to applying these control methods in the sylvatic setting, as opposed to the domestic setting, must be further explored.

APPENDIX A: Ministry of Health, Belize: Institutional Review Board Approval

MINISTRY OF HEALTH
Institutional Review Board
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May 7th, 2013

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Re: Research Protocol "Geographic Distribution and Bionomics of *Triatoma dimidiata* (Reduviidae: Triatominae) in Northern Belize, Central America"

IRB Tracking 05/13(4)

Dear Mr. Caranci:

The Institutional Review Board (IRB) of the Ministry of Health, Belize hereby approves the research protocol entitled ***Geographic Distribution and Bionomics of Triatoma dimidiata (Reduviidae: Triatominae) in Northern Belize, Central America*** to be executed as described in the submitted protocol and according to the conditions set out in the attached addendum.

[redacted]

Signature/Name, Director of Health Services

Signature/Name, Board Member
7/5/2013

[redacted]

Marjorie E. Parks
Signature/Name, Board Member

Angela Caranci
Signature/Name, Lead Investigator

APPENDIX B: Generalized script for addressing household members, obtaining and documenting verbal consent.

Household Participation Script and Record of Verbal Consent

HH #: ____ ____ Date of interview: __/__/_____

Greeting

Are you an adult and an inhabitant of this household?

We are members of a research group working in collaboration with the Belize Ministry of Health and the Uniformed Services University from the United States. With your help, we are hoping to understand more about the distribution of triatomines insects, which are responsible for the transmission of Chagas disease (show pamphlet). We are currently searching for households that would be interested in participating in a collection survey, which would allow us to better determine the distribution of these insects throughout the local area.

We would like to describe in detail what participation in this process would include, would you be willing to participate in this project if you are comfortable with the details after our discussion today? Keep in mind that this is voluntary and you are free to leave the project at any time.

Answer: Y or N

Describe and present specimens for inspection

These are examples of the insect we are studying. They have been collected here in Belize and with you assistance we are hoping to learn more about where they can be found and what factors lead human encounters with the insects. This information will be provided to Belize Ministry of Health officials so that their knowledge of the insect distribution can guide surveillance and control efforts here in _____ District.

If you decide that you would be willing to serve as a representative household of _____ District, we would ask that: (describe the collection type being used at this location)

a. should you see the presented insect in or around your household, you collect the triatome using the plastic bags provided and mark the bag with the date. Deliver bags to research team or Ministry of Health.

b. you allow two trained members of our team to actively search in and around your home approximately three times a year to collect any insect specimens of interest.

c. you give permission for the placement of box traps around your household and allow members of our team to return at least three times a year to monitor the boxes for any insect activity.

d. you consent to the use of your peri-domestic area for overnight light trapping which will be conducted by two of our team members at least three times a year. This will entail the use of moderately bright lights in association with white sheets to attract insects.

All participants will also be asked several questions about their house (allow interviewee to view survey questions and describe content). We would like to assure you that your name and specific house location will not be presented in any capacity or linked specifically with insect presence in any publications or presentations open to public viewing. We believe the information gained from this study will equip the local Ministry

of Health officials with tools to limit human encounters with potentially infected vectors. Please be aware that the handling of insects with bare hands may pose a health risk, but this risk does not exceed that of naturally encountering the insects in and around your home. Together we can assess the threat of infected vectors in your area and present this information to the Ministry of Health along with suggestions to prevent house infestations in the future.

(Lastly, after all instruction and descriptions are given, firmly obtain participant's consent)

Do you think you would participate in this study?

(If yes, interviewer's signed confirmation of verbal consent)

Interviewer: _____ Date: _____

(If no) Thank you, we appreciate you taking the time to listen to our request. Have a nice day.

APPENDIX C: Household Attribute Data Collection Sheet

Household Checklist

Administer informed consent and proceed only if subject agrees to participate

HH #: ____ ____ Date: __/__/_____

Visit # : _____

Location description: _____

Pic #: _____ Coll. Method: _____

GPS coordinates: Tracking number: _____

N: _____

W: _____

Household:

rooms: _____ # bedrooms: _____

Check if present:

Screened door ☐ Screened windows ☐

domestic animals (visibly housed indoors) ☐ animal types:

_____

peri-domestic animals ☐ animal types:

_____ ☐

External light sources on house External community lighting (CL) ☐

Distance to CL: (circle one) 5m 10m 20m ≥30m

Surrounding vegetation ☐

Direction (distance): N _____m S _____m E _____m W _____m

Types of surrounding vegetation:

Agriculture ☐ Secondary growth ☐ Primary growth ☐

Description of household materials:

Walls: mud/thatch ☐ tiled ☐ cement ☐ wood ☐

Other/description:

_____

Roof: mud/thatch ☐ tiled ☐ cement ☐ wood ☐
Other/description:
_____

Flooring: mud/thatch ☐ tiled ☐ cement ☐ wood ☐
Other/description:
_____

APPENDIX C: Household survey

Household Survey Determining Risk Factors for Triatomine Infestation

Administer informed consent and proceed only if subject agrees to participate

HH #: ____ ____ Date of interview: __/__/_____

Visit # : _____

Community name: _____

Description of house location in community:

_____

_____

Number of rooms in household: _____

Number of bedrooms in household: _____

Questions Pertaining to Household Characteristics:

1) How often do animals sleep inside the household?

a. Never b. 1-2 times a month c. 1-2 times a week

d. Every Night e. Seasonally Months: _____

2) Have you ever seen a triatomine in your household? (show examples to ensure clarity) Yes No (if no, proceed to question 4)

3) When is the last time you saw a triatomine in your household?

a. within the last week b. within the last month c. within the last year

d. greater than one year ago

4) Have you ever applied insecticides or pesticides to the interior surfaces of your household? Yes No (if no, proceed to question 6)

5) When is the last time you applied insecticides or pesticides inside the house?

a. within the last week b. within the last month c. within the last year

d. greater than one year ago

6) Have you ever applied insecticides or pesticides to the interior surfaces of your household? Yes No (if no, proceed to question 8)

7) When is the last time you applied insecticides or pesticides outside or around the house?

a. within the last week b. within the last month c. within the last year

d. greater than one year ago

8) Are their crop areas adjacent to your household that are regularly harvested? Yes No (if no, end survey)

9) When is the last time the surrounding crops were harvested?

a. within the last week b. within the last month c. within the last year

d. greater than one year ago

APPENDIX E: *Triatoma dimidiata* Presence Data

Country	District/Department	Longitude	Latitude	Village	Source
Belize	Cayo	-89.14	17.07	Benque Viejo	Polonio et al. 2009
Belize	Cayo	-89.13	17.12	Calla Creek	Dorn et al., 2009
Belize	Cayo	-89.13	17.08	Calla Creek	Original reporting
Belize	Cayo	-89.11	16.23	Santa Elena	Original reporting
Belize	Cayo	-89.07	17.15	San Ignacio	Polonio et al. 2009
Belize	Cayo	-89.04	17.18	Esperanza	Original reporting
Belize	Cayo	-89.04	17.17	Santa Elena	Original reporting
Belize	Cayo	-88.94	17.21	Unitedville	Original reporting
Belize	Cayo	-88.91	17.22	Blackman Eddy	Original reporting
Belize	Cayo	-88.84	17.23	Camelote	Original reporting
Belize	Cayo	-88.51	17.02	Middlesex	Original reporting
Belize	Corozal	-88.48	18.23	Progresso	Original reporting
Belize	Corozal	-88.43	18.39	Xaibe	Original reporting
Belize	Corozal	-88.42	18.38	Ranchito	Original reporting
Belize	Corozal	-88.41	18.39	San Andres	Original reporting
Belize	Orange Walk	-88.77	17.87	San Felipe	Original reporting
Belize	Orange Walk	-88.66	17.76	Indian Church	Original reporting
Belize	Orange Walk	-88.65	17.72	San Carlos	Original reporting
Belize	Orange Walk	-88.59	17.97	Guinea Grass	Original reporting

Belize	Orange Walk	-88.57	18.11	Trial Farm	Original reporting
Belize	Orange Walk	-88.52	18.24	Douglas	Original reporting
Belize	Toledo	-89.14	16.21	Pueblo Viejo	Polonio et al. 2009
Belize	Toledo	-89.08	16.23	Santa Cruz	Polonio et al. 2009
Belize	Toledo	-89.05	16.20	Blue Creek	Polonio et al. 2009
Belize	Toledo	-89.05	16.13	Santa Teresa	Dorn et al., 2009
Belize	Toledo	-89.02	16.27	Crique Jute	Polonio et al. 2009
Belize	Toledo	-88.95	16.27	San Pedro Columbia	Monteiro et al., 2013
Belize	Toledo	-88.93	16.29	San Miguel	Polonio et al. 2009
Belize	Toledo	-88.92	16.00	Barranco	Polonio et al. 2009
Belize	Toledo	-88.89	16.26	Big Falls	Polonio et al. 2009
Belize	Toledo	-88.89	16.28	Silver Creek	Polonio et al. 2009
Belize	Toledo	-88.88	16.15	Jacintoville	Polonio et al. 2009
Belize	Toledo	-88.80	16.10	Punta Gorda	Polonio et al. 2009
Guatemala	Alta Verapaz	-90.37	15.48	Cobán	Bargues et al., 2008
Guatemala	Alta Verapaz	-90.24	15.31	Tamahú	Monteiro et al., 2013
Guatemala	Alta Verapaz	-90.12	15.29	Tucurú	Monteiro et al., 2013
Guatemala	Alta Verapaz	-89.97	15.57	Lanquin	Bargues et al., 2008
Guatemala	Alta Verapaz	-89.58	15.45	Cahabón	Monteiro et al., 2013
Guatemala	Baja Verapaz	-90.45	15.11	Rabinal	Dorn et al., 2009
Guatemala	Chiquimula	-89.43	14.77	San Juan Ermita	Monteiro et al., 2013
Guatemala	Chiquimula	-89.35	14.72	Tuticopote	Monteiro et al.,

					2013
Guatemala	Escuintla	-90.82	13.93	Puerto de San José	Gomez-Palacio et al. 2016
Guatemala	Escuintla	-90.79	14.31	Escuintla	Bargues et al., 2008
Guatemala	Izabal	-89.11	15.27	Los Amates	Dorn et al., 2009
Guatemala	Jutiapa	-90.13	14.27	San José Acatempa	Monteiro et al., 2013
Guatemala	Jutiapa	-90.13	14.27	Valle Abajo	Dorn et al., 2009
Guatemala	Jutiapa	-90.04	14.27	La Brea	Dorn et al., 2009
Guatemala	Jutiapa	-90.04	14.28	Quezada	Monteiro et al., 2013
Guatemala	Jutiapa	-90.03	14.05	Conguaco	Monteiro et al., 2013
Guatemala	Jutiapa	-89.91	14.28	Juliapa	Bargues et al., 2008
Guatemala	Jutiapa	-89.81	14.30	Piedra Pintada	Bargues et al., 2008
Guatemala	Jutiapa	-89.78	14.33	Agua Zarca	Bargues et al., 2008
Guatemala	Peten	-89.38	17.07	Yaxha	Bargues et al., 2008
Guatemala	Quiche	-91.09	15.29	Chaoj	Dorn et al., 2009
Guatemala	Quiche	-90.92	15.22	San Andrés Sajcabaja	Bargues et al., 2008
Guatemala	Quiche	-90.85	15.17	Canilla	Monteiro et al., 2013
Guatemala	Quiche	-90.47	14.23	Pueblo Nuevo Viñas	Bargues et al., 2008
Guatemala	Santa Rosa	-90.33	14.40	Amberes	Monteiro et al., 2013
Guatemala	Zacapa	-89.58	15.05	Rio Hondo	Monteiro et al., 2013
Mexico	Campeche	-90.67	19.65	Seybaplaya	Tamay-segovia et al., 2008
Mexico	Campeche	-90.41	19.93	Hampolol	Tamay-segovia et al., 2008
Mexico	Campeche	-90.23	20.05	Tenabo	Herrera-Aguilar et al., 2009

Mexico	Campeche	-89.85	19.88	Hopelchen	Dumonteil et al. 2002
Mexico	Campeche	-89.75	20.15	Bolonchen	Dumonteil et al. 2002
Mexico	Campeche	-89.74	19.62	Dzilbalchen	Dumonteil et al. 2002
Mexico	Campeche	-89.43	18.88	Zoh-Laguna	Dumonteil et al. 2002
Mexico	Chiapas	-92.91	15.43	Mapastepec	Bargues et al., 2008
Mexico	Chiapas	-92.17	14.94	Tuxtla Chico	Monteiro et al., 2013
Mexico	Chiapas	-91.97	17.52	Palenque	Bargues et al., 2008
Mexico	Colima	-103.58	19.35	Alcaraces	Bargues et al., 2008
Mexico	Colima	-98.28	20.83	Xochiatipan. Acomul	Bargues et al., 2008
Mexico	Hidalgo	-98.66	20.99	Tlanchinol	Bargues et al., 2008
Mexico	Hidalgo	-98.57	20.95	Canalí	Bargues et al., 2008
Mexico	Hidalgo	-98.51	21.07	Huehuetla	Bargues et al., 2008
Mexico	Hidalgo	-98.42	21.13	Huejutla	Bargues et al., 2008
Mexico	Hidalgo	-98.37	21.35	Atlapexco	Bargues et al., 2008
Mexico	Morelos	-99.34	18.58	Amacuzac	Bargues et al., 2008
Mexico	Morelos	-98.77	18.68	Chalcatzingo	Bargues et al., 2008
Mexico	Oaxaca	-97.16	16.11	Nopala	Bargues et al., 2008
Mexico	Oaxaca	-96.16	16.55	Hierba Santa	Bargues et al., 2008
Mexico	Oaxaca	-95.95	17.35	San Juan Comaltepec	Monteiro et al., 2013
Mexico	Oaxaca	-95.91	16.95	San Juan Juquila Mixes	Monteiro et al., 2013
Mexico	Quintana Roo	-88.71	19.97	Presumida	Dumonteil et al. 2002
Mexico	Quintana Roo	-88.47	19.42	Valle Hermosa	Dumonteil et

					al. 2002
Mexico	Quintana Roo	-88.30	19.08	Andres	Dumonteil et al. 2002
Mexico	Quintana Roo	-86.94	21.17	Tres Reyes	Dumonteil et al. 2002
Mexico	San Luis Potosí	-100.98	22.15	San Antonio	Monteiro et al., 2013
Mexico	San Luis Potosí	-100.96	22.16	San Luis Potosí	Monteiro et al., 2013
Mexico	San Luis Potosí	-99.13	22.38	Tanchahuil	Monteiro et al., 2013
Mexico	San Luis Potosí	-98.85	21.42	Temalacaco, Axtla de Terrazas	Monteiro et al., 2013
Mexico	Tabasco	-92.71	18.20	El Rosario	Bargues et al., 2008
Mexico	Veracruz	-97.88	21.34	Citlaltépetl. La Mesa de Tlanchinol	Monteiro et al., 2013
Mexico	Veracruz	-97.87	20.83	Úrsulo Galván	Bargues et al., 2008
Mexico	Veracruz	-97.40	20.95	Túxpan	Dorn et al., 2009
Mexico	Veracruz	-97.27	20.63	Emiliano Zapata	Bargues et al., 2008
Mexico	Veracruz	-96.77	18.90	Atoyac	Bargues et al., 2008
Mexico	Veracruz	-96.63	18.93	La Luz	Bargues et al., 2008
Mexico	Yucatan	-89.93	20.96	Tetiz	Dumonteil et al. 2002
Mexico	Yucatan	-89.69	21.18	Dzidzilche	Dumonteil et al. 2002
Mexico	Yucatan	-89.66	20.79	Abala	Dumonteil et al. 2002
Mexico	Yucatan	-89.62	20.97	Merida	Dorn et al., 2009
Mexico	Yucatan	-89.53	20.92	Teya	Gomez-Palacio et al. 2016
Mexico	Yucatan	-89.37	20.76	Eknakan	Gomez-Palacio et al. 2016
Mexico	Yucatan	-89.01	20.17	Chacsinkín-Tres Reyes	Gomez-Palacio et al. 2016
Mexico	Yucatan	-88.79	21.28	Buctzotz	Dumonteil et al. 2002

Mexico	Yucatan	-88.27	20.54	Tixcacalcupul	Gomez-Palacio et al. 2016
Mexico	Yucatan	-88.21	20.89	Temozon	Dumonteil et al. 2002
Mexico	Yucatan	-88.17	21.21	Kikil	Dumonteil et al. 2002
Mexico	Yucatan	-88.15	21.44	Loche	Dumonteil et al. 2002
Mexico	Yucatan	-87.63	21.01	San Manuel	Dumonteil et al. 2002

APPENDIX F: *Rhodnius prolixus* Presence Data

Country	Longitude	Latitude	Location	Source
El Salvador	-89.72	13.75	Armenia	PAHO 2010
El Salvador	-89.56	14.34	Cabanas	PAHO 2010
El Salvador	-88.13	13.28	Capulin	PAHO 2010
El Salvador	-88.96	13.89	Ciquera	PAHO 2010
El Salvador	-89.18	13.93	Guazapa	PAHO 2010
El Salvador	-89.44	14.33	Metapan	PAHO 2010
El Salvador	-89.25	13.48	San Diego	PAHO 2010
El Salvador	-88.72	13.83	San Isidro	PAHO 2010
El Salvador	-89.13	13.69	San Salvador	PAHO 2010
El Salvador	-89.72	13.57	Sonsonate	PAHO 2010
Guatemala	-89.80	14.93	Cabanas	Nakagawa et al., 2003
Guatemala	-89.54	14.79	Chiquimala	Tabaru et al., 1999
Guatemala	-89.36	15.12	Gualan	Nakagawa et al., 2003
Guatemala	-91.47	15.31	Huehuetenango	Hashimoto et al., 2012
Guatemala	-89.72	14.93	Huite	Nakagawa et al., 2003
Guatemala	-89.89	14.28	Jutiapa	Hashimoto et al., 2012
Guatemala	-89.29	14.96	La Union	Nakagawa et al., 2003
Guatemala	-91.99	14.70	Las Palmas	Monteiro et al., 2000
Guatemala	-89.85	14.66	San Pedro Pinula	Yoshioka 2013
Guatemala	-90.38	14.20	Santa Rosa	Hashimoto et al., 2012
Guatemala	-89.78	14.95	Usumatlan	Nakagawa et al., 2003
Guatemala	-89.54	14.97	Zacapa	Nakagawa et al., 2003
Honduras	-88.86	14.94	Copan	Hashimoto and Schofield 2012
Honduras	-88.26	14.37	Intibuca	Hashimoto and Schofield 2012
Honduras	-87.95	13.92	La Paz	Hashimoto and Schofield 2012
Honduras	-87.06	14.45	Morozon	Monteiro et al., 2000
Mexico	-91.88	17.34	Arimatea	Mazanejo-Arana et al. 2002
Mexico	-99.18	19.34	Cerro del Aire	Goldsmith et al., 1986
Mexico	-99.16	19.34	Cotozocan	Salazar et al, 1987
Mexico	-97.82	16.28	Jamiltepec	Salazar et al, 1987
Mexico	-91.50	16.83	Lanandon Jungle	Mazariego-Anara et al., 2001
Mexico	-95.98	16.60	Nejapa de	Salud Publica Mexico

			Madero	2000
Mexico	-91.85	17.32	Nueva Galilea	Mazanejo-Arana et al. 2002
Mexico	-95.98	16.61	Oaxaca	Ramsey et al., 2000
Mexico	-95.97	16.63	Oxaca	Ramsey et al., 2000
Mexico	-97.93	17.03	Putla	Salazar et al, 1987

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