COMPARATIVE FEEDING AND DEFECATION BEHAVIORS OF TRIATOMINES AND THEIR RELATION TO THE RISK OF *Trypanosoma cruzi* IN THE UNITED STATES

A Thesis

by

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Submitted to the Graduate and Professional School of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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December 2021

Major Subject: Veterinary Public Health—Epidemiology

ABSTRACT

Triatomines are vectors of *Trypanosoma cruzi*, the etiological agent of Chagas disease. They exhibit stercorarian transmission, in which *T. cruzi* is transmitted via fecal contamination to the host. Differential feeding and defecation behavior may contribute to the lower burden of human Chagas disease in the U.S. compared to Latin America, but behaviors of species in the U.S. have been infrequently studied. We hypothesized that U.S. triatomines less commonly defecate during or shortly after blood-feeding when compared to a South American triatomine species. We reared *T. cruzi*-infected (TcI and TcIV) and uninfected *Triatoma gerstaeckeri* and *Triatoma sanguisuga* (both of the southern U.S.) and *Rhodnius prolixus*—a South American triatomine sourced from a colony maintained by the U.S. Centers for Disease Control and Prevention. Single nymphs were allowed to interact with a restrained guinea pig for one-hour during which insect feeding and defecation events were measured. In 148 trials across all three species, 40.0% of insects fed at least once, of which 71.2% defecated during the observation period. Compared to *R. prolixus*, *T. gerstaeckeri* were more likely to feed and had more feeding events, and *T. sanguisuga* fed longer. The average interval between feeding to the first defecation was 4.5 min for *R. prolixus*, 9.8 min for *T. gerstaeckeri* and 20.7 min for *T. sanguisuga*, and there were observations of simultaneous feeding and defecation in all three species. The defecation index reported a similar pattern with *R. prolixus* having the highest infection capacity, followed by *T. gerstaeckeri* and then *T. sanguisuga*. Triatomines that were infected with *T. cruzi* DTU

TcI were less likely to feed than uninfected controls, while those infected with TcIV had no significant differences. Finally, *T. cruzi*-infected insects had shorter post-feeding defecation intervals when considering multiple defecation events. These data suggest that while the feeding and defecation behaviors of *T. gerstaeckeri* and *T. sanguisuga* result in them being less efficient vectors compared to *R. prolixus*, they are still capable of stercorarian transmission. These observations suggest that other extrinsic and intrinsic factors contribute to the reduced autochthonous transmission of *T. cruzi* in the U.S. compared to elsewhere in the Americas.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Sarah Hamer, and my committee members, Dr. Gabriel Hamer and Dr. Keri Norman. I personally would like to thank Dr. Sarah Hamer for allowing me to join the team as an undergraduate researcher and then transition into my role as Research Assistant. I want to thank her for her support and guidance throughout my graduate studies. I have learned so much about disease ecology and epidemiology, specifically Chagas disease and kissing bugs. I want to thank Dr. Gabriel Hamer for his support and knowledge in handling kissing bugs and learning the ins and outs of arthropod containment which gave us the resources for our projects. I thank Dr. Keri Norman for her expertise in epidemiological methods and guiding me through my courses.

I want to thank my colleagues and friends of both Hamer labs for their guidance and support. I specifically want to thank Dr. Italo Zecca for allowing me to shadow under him when I first joined the lab. He has been a great mentor and friend—his advice has been helpful whether it was research, work, or personal related. I want to thank all of the friends I have made throughout my years at Texas A&M University for their fellowship and support.

Lastly, I am truly blessed and grateful for my mother and sister, who have supported and cherished me from the beginning, and for their love and patience whenever I get passionate in talking about my research. I am grateful for all of these people because I would have been lost if I did not have their support.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This research work was supervised by a thesis committee consisting of Drs. Sarah Hamer (advisor) and Keri Norman of the Department of Veterinary Integrative Biosciences at Texas A&M University, and Dr. Gabriel Hamer of the Department of Entomology at Texas A&M University. I would like to thank Dr. Jillian Wormington for her contribution in the research project with writing the proposal; Dr. Italo Zecca for his help and support with conducting the trials; and Dr. Luis Fernando Chaves for the statistical analyses of the data.

Funding Sources

This work was made possible in part by the National Institutes of Health under Grant No. 1-R03-AI144711-01. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of National Institutes of Health.

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CHAPTER I

INTRODUCTION

1.1. Chagas Disease and *Trypanosoma cruzi*

Chagas disease, also known as American trypanosomiasis, is a zoonotic disease caused by a protozoan parasite, *Trypanosoma cruzi*. The disease was discovered by Carlos Chagas in 1909, when he identified the parasites in the hind gut of triatomines, the vectors of Chagas disease [1]. Since its discovery, Chagas disease has become an increasing public health concern. It affects about 8 million people worldwide, with the majority of infected individuals living in endemic areas of the Americas. Some cases have been reported in non-endemic areas, such as Europe and Asia, but these are mostly due to global migration [2]. Chagas disease also accounts for an annual loss of 806,170 disability-adjusted life years (DALYs) globally [3], burdening those infected economically and socially [4].

The primary transmission route of Chagas disease is via the feces of an infected triatomine, where the feces can enter the host through an open wound or mucous membrane. Other ways of transmission routes are oral, congenital, organ transplantation, blood transfusion, or laboratory accidents [5]. Chagas disease has three stages: acute, indeterminate, and chronic. The acute phase occurs when *T. cruzi* is circulating in the host's blood, and symptoms may include nausea, fever, chills, or even the infamous Romana's eye. The indeterminate stage occurs when infected individuals do not show any clinical symptoms—and even may not realize they are infected [6]. This may lead

into the chronic stage which can result in severe cardiomyopathy or gastrointestinal issues in 20-30% of patients. Currently, there are no vaccines available and treatments for humans are limited.

Trypanosoma cruzi can affect a wide range of hosts, many of which serve as reservoirs in the transmission cycle—domestic canines, opossums, raccoons, and many other mammals [7]. *T. cruzi* has been identified to have seven discrete typing units (DTUs)—TcI to TcVI and TcBat. In North America, TcI and TcIV are the predominant DTUs. TcI is the predominant strain implicated in human Chagas diseases in the United States (U.S.) [8], and is also found in most of the U.S. vector species [9] and in domestic and wildlife animals [7]. TcIV has been mostly shown in wildlife reservoirs, which is important because it has been shown that there is spillover from wildlife to domestic animals [7].

1.2. Triatomines

Triatomines, or kissing bugs, are hematophagous insects that can transmit *T. cruzi*. These insects share the same family Reduviidae as other assassin bugs and over 130 triatomine species are capable of transmitting the parasite [10]. The parasite is found in the gut and feces of triatomines, which is one of the primary modes of transmission of Chagas disease. In order for triatomines to pass on the parasite to a host, they must rely on feeding and defecating near them, where *T. cruzi* must somehow enter the host's body through an open wound or membrane. This usually means a higher chance of parasite transmission. Because there are no vaccines available and limited treatments for

Chagas disease, efforts in public health interventions have been focused on controlling triatomine populations. Many countries in Central and South America have taken initiatives in reducing triatomine populations, which has greatly decreased the number of infected individuals compared to that of a couple decades ago [11, 12]. An example is the successful story of eliminating populations of *Rhodnius prolixus*, one of the most epidemiologically important vectors of Chagas disease, in Central America [13]. In the United States, reducing triatomine populations is difficult because the species found here are known to be sylvatic and do not colonize homes like those in Central and South America [6].

As we know, there are well-established triatomine populations distributed across a wide geographical range, expanding from the southern U.S. to near the southern tip of South America [10]. In Latin America, roughly 6-7 million people are infected with Chagas disease since the disease is endemic in these areas, and the majority of these infections were locally-acquired—humans are getting *T. cruzi* infections from the kissing bugs themselves [14]. However, in the U.S., we can estimate about 300,000 people infected with Chagas disease [15], but a low number of those infected persons acquired the infections from kissing bugs. In a recent review paper, 76 confirmed and/or suspected autochthonous cases were reported from the years 2000 to 2018 in the U.S. [16]. We are seeing a disconnect between well-established vector populations in the U.S. and not many locally-acquired cases. Possible explanations could be: 1) differences in domestic versus sylvatic triatomine species; 2) more robust housing available in the southern U.S. compared to some areas in Latin America; 3) differences in disease

surveillance and reporting; and 4) vector behavioral differences between the U.S. and Latin America.

1.2.1. Triatomine Feeding and Defecation Behaviors

Vector behaviors, such as feeding and defecation patterns, may play a role in the differences of disease burden between the U.S. and Latin America. It is essential to understand the biology and behaviors of these vectors because public health intervention is limited to controlling for triatomine populations. Studies conducted over feeding and defecation behaviors of triatomines have been done for decades since there has been an interest in how the defecation behaviors of triatomines relate to the risk of *T. cruzi* transmission. Many of these studies have looked at epidemiologically important species in Central and South Americas, such as *R. prolixus, T. infestans*, and *T. dimidiata*. It has been shown that these species, as well as others, are more efficient vectors of Chagas disease because they are more likely to defecate and have shorter post-feeding defecation intervals (PFDI) [17-20]. Zeledon (1977) conducted feeding and defecation behavioral studies on *R. prolixus*, *T. infestans*, and *T. dimidiata*, and found that *R. prolixus* were better defecators than the latter two species; however, all three species have been shown to be competent vectors for *T. cruzi* [17]. He also proposed the "defecation index", a standardized measurement for infection capacity of triatomines which accounts for how often triatomines will defecate within 10 minutes post feeding. This use of measurement has been used in many studies since then [20-22].

On the other hand, species that can be found in the U.S. may have longer PFDIs or might not defecate as often, which may lower the chance of parasite transmission.

This reasoning could account for the low disease burden seen in the U.S. However, only a few studies have extensively observed the feeding and defecation behaviors of North American species. Sherwin Wood's early studies looked at the contaminative effects of *T. protracta* and *T. rubida* and observed that these species had delayed PFDIs [23]. A more recent study found similar results in the same species and concluded that *T. protracta* and *T. rubida* would be inefficient vectors for Chagas in the southwestern parts of the U.S. [21]. Another study focused on the feeding and defecation patterns of *T. gerstaeckeri* and *T. sanguisuga*, two species that can be found in Texas, and compared them to that of *R. prolixus*. The author found that the U.S. species did not defecate as often, so they were less likely to contaminate their hosts [19]. However, many studies have not considered the effect *T. cruzi* may play on the feeding and defecation behaviors of species found in the U.S.—*T. cruzi* manipulation has been shown in some Latin American species [20, 24-26]. Learning the feeding and defecation behaviors of triatomines, especially with and without *T. cruzi* infection, will help us understand the disease risk of Chagas disease in the U.S. and determine that there is indeed a low probability of *T. cruzi* transmission in U.S. species.

CHAPTER II

COMPARATIVE FEEDING AND DEFECATION BEHAVIORS OF *TRYPANOSOMA CRUZI*-INFECTED AND UNINFECTED TRIATOMINES FROM THE AMERICAS

2.1. Introduction

Triatomines (Hemiptera: Reduviidae), or kissing bugs, are the vectors of *Trypanosoma cruzi*, the etiological agent of Chagas disease. They are arthropods that require blood meals in order to develop throughout their hemimetabolous life cycles. Over 130 triatomine species distributed from the southern United States to northern Argentina and Chile are capable of transmitting *T. cruzi* [10]. Triatomines exhibit the stercorarian form of biological transmission, in which they transmit the infectious state of *T. cruzi* via fecal contamination to the host. If an infected triatomine feeds on a host and defecates at the same time or shortly after, there is a higher risk for the parasite to enter the host via the bite wound or other orifices. Accordingly, there is a long-standing interest in triatomine defecation behavior as it relates to the risk of *T. cruzi* transmission [17, 21-23, 25, 27-30].

Studies observing the feeding and defecation behaviors of triatomines have been conducted for decades. It is commonly cited that South American triatomines are more efficient vectors of *T. cruzi* because they generally have shorter post-feeding defecation intervals (PFDIs) [17-20, 31], as compared to North American species that do not defecate while feeding nor immediately after [17, 19, 21-23]. Thus, the feeding and defecation behaviors of North American species may dampen transmission of *T. cruzi* in

the U.S. This has been a factor stated to contribute to why the human health burden of Chagas disease in the U.S. is relatively low compared to that in Latin America [21-23, 32-34], despite established vector populations in both locations. Despite this perspective, few studies have comparative feeding and defecation behavior data of species found in the U.S. and elsewhere in the Americas.

Based on public submissions in a community science program, both *Triatoma gerstaeckeri* and *Triatoma sanguisuga* are considered as the two most epidemiologically important vector species in Texas, and they both have relatively high infection prevalence of *T. cruzi* [9], with about 45-70% infection prevalence for *T. gerstaeckeri* [9, 19, 35, 36], and about 25-67% infection prevalence for *T. sanguisuga* [19, 35-37]. *Triatoma gerstaeckeri* is more likely to carry discrete typing unit (DTU) TcI, and *T. sanguisuga* is more likely to carry DTU TcIV [9]. The distribution of *T. gerstaeckeri* expands to all but the northern parts of Texas extending into parts of New Mexico [5, 35], while *T. sanguisuga* is broadly distributed from Texas to the east coast of the U.S. [5]. Although the distribution of *T. gerstaeckeri* is limited to two states, it is one of the most commonly collected species in the U.S. [5] with the majority of specimens found in Texas.

Rhodnius prolixus is a species native to South America and is one of the most competent vectors for *T. cruzi.* It has been thoroughly studied for decades as it can be easily colonized in laboratory settings [38, 39]. *Rhodnius prolixus* has been shown to have higher vectorial capacity given short PFDIs compared to triatomine species found in the U.S. [19], as well has other South American species [18]. Accordingly, this

species would be a good model to compare feeding and defecation behaviors with the U.S. species.

The objective for this study was to examine feeding and defecation behaviors of *T. cruzi*-infected and uninfected *T. gerstaeckeri* and *T. sanguisuga* in comparison with *R. prolixus* under laboratory conditions. Comparison of feeding and defecation behaviors between the two North American triatomines and *R. prolixus,* as well as between individuals infected with the *T. cruzi* (TcI and TcIV) and uninfected controls, will afford key information for parameterizing models of vectorial capacity as it relates to differences in human disease risk across the Americas.

2.2. Methods

2.2.1. Insects

All insects in this study were lab reared in our triatomine colony located in a USDA APHIS-PPQ, arthropod containment level 2 (ACL2) facility. The temperature in the room ranged from 24-27°C, and relative humidity levels ranged from 28-57%, although the microclimate experienced by the insects had higher humidity because they were housed in Nalgene primary containers (Avantor, Radnor, PA, USA) that were placed in a plastic secondary container filled with water to maintain humidity levels [40]. The secondary containers sit in tubs coated with fluon (BioQuip Products, Rancho Dominguez, CA, USA) to prevent the insects from escaping. Three species of triatomines were used in the experiments. *Rhodnius prolixus* were acquired from the Centers of Disease Control and Prevention whose original source was collected in

Colombia (BEI Resources, Manassas, VA, USA), and *T. gerstaeckeri* and *T. sanguisuga* were offspring of individuals caught locally in the wild between 2017 and 2019 [41]. All nymphs came from known *T. cruzi*-negative colonies which were separated from adults and subsampled to confirm infection status following testing protocols described below. Triatomines were maintained using defibrinated rabbit blood (Hemostat Laboratories, Dixon, CA, USA) fed weekly through artificial membrane feeders (Hemotek Ltd, Blackburn, UK).

2.2.2. Guinea Pigs

Because triatomines are able to take an average of 0.3 mL of blood per blood meal with the upper limits approximately at 0.75 mL (Wormington et al., unpublished data), the safe limits of blood loss could be exceeded in laboratory mice and rats when used repeatedly. Thus, guinea pigs (*Cavia porcellus*) were selected as the model to allow live animal feeding because their body size is larger than that of laboratory mice and rats, thereby allowing for triatomine blood feeding at two- and three-week intervals without concern for blood loss.

Fourteen adult, female guinea pigs were used in the trials (IACUC 2018-0484). They were uniquely marked with fur pigment markers (Stoelting, Wood Dale, IL, USA) and group-housed in animal BSL-1 containment. Although *T. cruzi* infection was not an expected outcome in animals in this study, each of the guinea pigs had their blood drawn at three different time points: pre-study, mid-study, and post-study. We extracted DNA from 100 µL of whole blood or 50 µL of blood clot with the E.Z.N.A.® Blood DNA Kit (Omega Bio-tek, USA). Extracted DNA was amplified for detection of *T. cruzi* using

previously published methods [42]. Guinea pigs were adopted at the end of the study after confirming they did not have evidence of *T. cruzi* DNA in their blood.

2.2.3. Parasite Cultures

We obtained *T. cruzi* metacyclic trypomastigotes by gently compressing the abdomen of a wild-caught *T. gerstaeckeri* nymph from Moore, TX from which the feces had previously tested positive for *T. cruzi* discrete typing unit (DTU) TcI. We obtained *T. cruzi* epimastigotes of *T. cruzi* DTU TcIV by hemoculture of a naturally infected non-human primate from a central Texas biomedical research facility [43].

These trypanosomes were cultured in liver-infusion tryptose (LIT) media (Difco, BD, Franklin Lakes, NJ, USA) supplemented with fetal bovine serum, penicillin-streptomycin, and nystatin (Sigma-Aldrich, Darmstadt, Germany) [44-46]. Culture flasks were placed into an incubator at 27°C and microscopically examined for the presence of motile trypanosomes two weeks later. Cultures were maintained by passaging in LIT media. These cultures were mixtures of abundant epimastigotes and rare trypomastigotes as determined by microscopy. The *T. cruzi* DTU in each culture were confirmed by a multiplex qPCR targeting the spliced leader intergenic region (SL-IR) for determination of strain type, according to previously described protocols [47].

2.2.4. Experimental Infections

To calculate parasite concentration of each culture, we agitated the culture flask and pipetted 10 µL of parasite in media into 90 µL of formalin (VWR, Radnor, PA, USA) and counted individual parasites in a hemocytometer (Reichert, Buffalo, NY, USA) to determine an approximate density of parasites in the culture while accounting

for the formalin dilution. To concentrate the parasites, we centrifuged samples from the same culture flask in microcentrifuge tubes for 10 minutes at 3,000 rpm, poured off the culture medium, and replaced it with sterile phosphate-buffered saline (PBS) solution (VWR, Radnor, PA, USA). After resuspending the parasite in PBS, we again centrifuged and poured off the PBS and culture medium.

To prepare blood, we transferred the washed parasite into a measured quantity of defibrinated rabbit blood to a final estimated concentration of 3 x 10^6 parasites per mL of blood, a concentration similar to peak parasitemia in laboratory mice and produces a very high probability of insect infection [48-51]. Infected blood was offered to 4^{th} and 5^{th} instar triatomine nymphs through an artificial membrane feeder for two hours in an ACL2 facility. These instars were chosen not only because of robust availability in the insect colony, easy handling, and high visibility under the surveillance cameras used in the trials, but also these life stages were potentially considered better defecators, at least for *T. gerstaeckeri* and *T. sanguisuga* [19]. The control groups were offered blood without parasites. The following treatments were prepared: *T. gerstaeckeri* (control); *T. gerstaeckeri* (TcI); *T. gerstaeckeri* (TcIV); *T. sanguisuga* (control); *T. sanguisuga* (TcI); *R. prolixus* (control); *R. prolixus* (TcI); *R. prolixus* (TcIV). There was no treatment group for *T. sanguisuga* TcIV because there were limited numbers of *T. sanguisuga* nymphs in the triatomine colony. The insects were starved for 2-6 weeks after feeding before being used in the trials, with individual periods of starvation included in the analysis. Insects that did not feed on the infected bloodmeal, or fed then molted into adults prior to use, were removed from the study.

2.2.5. Confirmation of Infection Status

We used up to three different methods to confirm the infection status of the insects. For abdominal compression, insects at 2 weeks post feeding on infected blood were gently compressed to obtain any fecal material that was directly expelled into 5 mL of LIT culture media. The cultures incubated at 27°C and were checked weekly for presence of *T. cruzi.* If the presence of at least one *T. cruzi* parasite was observed, the insect was confirmed for infection. The final call on the infection status was made at the 1-month mark [52]. For fecal spot testing, we collected feces from individuals held in 50 mL-conical tubes containing filter paper (Whatman Filter Paper, Sigma-Aldrich, Darmstadt, Germany). Following defecation on the filter paper, the fecal spot was cut using sterile scissors and placed into a microcentrifuge tube using sterile forceps. Fecal spots were extracted using the KingFisher Cell and Tissue kit (Thermo Fisher Scientific, Waltham, MA, USA), and the DNA were run through real-time qPCR [9]. After the insects were used in trials and if their infection status was not confirmed with the former two methods, then the insects were dissected to obtain gut material, which was subjected to DNA extraction and tested using qPCR using protocols previously described [9].

2.2.6. Feeding and Defecation Trials

The trials were conducted over a 1-year period (August 2019─September 2020) in an ACL2 negative air pressure biocontainment unit (bioBUBBLE, Fort Collins, CO, USA). Temperatures ranged from 18-26°C. For each trial session, we set up four arenas, each consisting of a 17.6 in x 11.5 in x 7.8 in, clear, polycarbonate Sous Vide container (Lipavi, United Kingdom) with the bottom surfaces lined with white bench paper, onto

shelf racks. The bench paper was taped down using white laboratory tape. One Yi Lite camera (YI Technology, Pudong District, Shanghai, China) was docked above each arena to allow recording of the trials (Figure 1). After the first several trials were done in ambient light, two 25-watt red, light bulbs were set up above the containers to allow for observations to be made with the low-light surveillance cameras.

Figure 1: Experiment arena with polycarbonate containers lined with white bench paper and Yi Lite cameras docked above

Four guinea pigs were used for each trial session, one per arena. Each guinea pig was restrained into a 2 inch-mesh, cotton stockinette (Rolyan, Warrenville, IL, USA) with both ends tied. For optimal restraint, the guinea pigs were in a curled position to restrict excessive mobility in the stockinette after the ends were tied. Both ends of the stockinette were secured with white duct tape to the sides of the containers, and the guinea pigs were positioned on their feet. Each insect was weighed before and after the

trial period and randomly assigned to be placed with a guinea pig for the first 60 minutes of the trial period [52]. One insect was released in each assigned container with a guinea pig at the start of the trial.

During the initial hour of the trial in which the insect and guinea pig were together in the arena, both insect and guinea pig behaviors were monitored. Insect behavioral scoring was based on: (i) whether the insect was sedentary or walking; (ii) feeding attempts, feeding events, interrupted feedings; (iii) and defecation events. The color of each defecation was also observed (clear, light, medium, or dark). Guinea pig behavioral scoring was based on: (i) reaction to probing by an insect (strong, weak, no reaction); (ii) movement inside the stockinette (yes or no); (iii) and other general observations (such as chewing on stockinette or defecating or urinating in stockinette).

After the 60 minutes, the guinea pigs were removed from the arena and returned to group housing, and the insects stayed in the arena for an additional 60 minutes for observations of defecation events. In cases that an insect was still feeding on a guinea pig at the 60-minute mark, the guinea pig stayed in the arena until the insect finished feeding and then the guinea pig was returned to group housing. After the two-hour trial period, the insects were weighed again and a unique colored marking with nail polish was painted over their lower abdomen. Any fecal spots on the bench paper from the trials were collected.

2.2.7. Statistical Analyses

Of the 91 triatomines that were experimentally exposed to and fed on *T. cruzi*-infected blood, all were subjected to confirmatory testing of infection status, and *T. cruzi*

was found via culture or PCR methods in 85 (93.4%) (Table 1). Of the 69 triatomines that were in the control category, 64 (92.8%) were subjected to confirmatory testing of infection status, and 63 (98.4%) were confirmed as uninfected. Insects that had test results congruent with their assigned treatment groups were used for statistical analysis, which resulted in a total of 148 (92.5%) individuals.

Table 1: Number of triatomines of each species by infection group that were used in the trials and were confirmed for their assigned treatment group.

Species	**Control**	**TcI**	**TcIV**	**Total**
T. gerstaeckeri	26	21	17	64
T. sanguisuga	16	11	0	27
R. prolixus	21	19	17	57
Total	63	51	34	148

A feeding event started when an insect inserted its proboscis into the guinea pig and initiated feeding; the feeding event ended once the insect removed its proboscis and walked away from the location of the bite [24]. A defecation event occurred when an insect excreted either urinal or fecal material at any time in the two-hour trial period. The time at which the insect defecated was also noted.

We adopted the defecation index from Zeledon [2] to allow for a standardized index of infection capacity to compare with other studies [17, 21, 22], where DI= (% of insects that defecated up to 10 minutes post feeding x average number of defecations up to 10 minutes post feeding)/100. We also recorded the time intervals between an insect's most recent bloodmeal to the time of its first defecation [20].

For any insect that fed on a guinea pig and gained weight after the two-hour trial, the volume of blood ingested was calculated using a proportion of 1 mg of weight gained after feeding equal to 1 μL of blood [24]. The percent weight gain was also calculated by dividing the ingested blood volume by the pre-trial weight of the insect [19].

We tested for differences among treatment groups (triatomine species and infection status) using a generalized estimating equation models, GEE [53]. Models were fit using the "geepack" package in R version 4.1.1. We employed GEE models given the nature of the data, where the performance of experiments using different guinea pigs and done over different days, constrained the use of simpler regression tools that assume full replication [54]. Given that we analyzed different outcomes we employed GEE models with different distributions for the response variables. To analyze whether triatomines fed or defecated we employed logistic GEE models, a suitable modeling strategy for dichotomous variables [55]. To analyze variables associated with the number of times a triatomine fed, or defecated/urinated, we employed models with a Poisson distribution [55]. To analyze the total feeding time and the post-feeding defecation intervals, we used a model with a Gaussian distribution [53].

In all models we considered the triatomine species and the infection status as the main explanatory variables. As covariates, we considered the illumination conditions for the experiment (with lights on or off), the number of days the triatomine was starved before the experiment, and the nymphal instar. This basic structure was used in the model looking at factors associated with vector feeding; in all other models, we included additional covariates depending on the response variable studied. For the model studying

whether triatomines defecated or not, we considered whether the triatomines fed when offered the guinea pig. For models studying the number of feedings, number of defecations, total feeding time, and PFDIs, we compared their goodness of fit considering either the initial insect weight or the pre- and post-feeding weight difference at the end of the experiment. After running the models, we found that the pre- and post-feeding weight difference was the best predictor. For the model of number of defecations, we also added the number of feedings as a covariate.

For the inference we used a sandwich estimator to obtain robust standard errors, since naïve standard errors are appropriate only when the correlation structure is correct [55]. When fitting the GEE models we fitted alternative models that either considered an independent or a correlated, a.k.a. exchangeable, error structure as function of the clustering factor [53]. We also fitted alternative models considering the day of measurement or guinea pig as clustering factor. Among these alternatives we chose the best model for each of the responses based on the minimization of the quasi-likelihood information criterion (QIC), a goodness of fit function that trades-off deviance and number of parameters in GEE models, and whose minimization can be used to choose the best model [56].

2.3. Results

For each species and infection group, we recorded the number of triatomines that fed, defecated, defecated after feeding, and the proportion of those that fed and defecated out of the number of triatomines that fed (Table 2). Out of 148 triatomines, 59 (40.0%)

fed on guinea pigs during the trial periods. Of those 59 insects, 42 (71.2%) defecated at least once after feeding. Forty-one (64.1%) *T. gerstaeckeri* fed on the guinea pigs, while only eight (29.6%) *T. sanguisuga* and ten (17.5%) *R. prolixus* fed on the guinea pigs. For defecation, 33 (51.6%) *T. gerstaeckeri* defecated during the observation period, while there were eight (29.6%) for *T. sanguisuga* and 15 (26.3%) for *R. prolixus*. Surprisingly, none of the *R. prolixus* insects in the TcI infection group fed.

Table 2: Descriptive summary of triatomine species feeding and defecation behaviors. No. Fed indicates the number of triatomines that fed within 60 minutes of exposure to the guinea pig. No. Defecated indicates the number of triatomines that defecated within the 2-hour observation period. Results are presented by triatomine species and *T. cruzi* DTU (TcI and TcIV) infection status

Species	Infection Group	No. of Insects	No. Fed (%)	No. Defecated (%)[a]	No. Fed + Defecated (%)[b]	% Defecated After Feeding
T. gerstaeckeri	Control	26	17 (65)	15 (58)	13 (50)	76
	TcI	21	14 (67)	7 (33)	7 (33)	50
	TcIV	17	10 (58)	11 (65)	8 (47)	80
T. sanguisuga	Control	16	6 (38)	5 (31)	3 (19)	50
	TcI	11	2 (17)	3 (25)	2 (17)	100
R. prolixus	Control	21	9 (47)	8 (42)	8 (42)	89
	TcI	19	0 (0)	2 (11)	0 (0)	0
	TcIV	17	1 (6)	5 (29)	1 (6)	100

[a]This is the number of insects that defecated at least once in the 2-hour period, including insects that defecated before feeding or did not feed at all.
[b]This number represents insects that fed on a guinea pig either with simultaneous defecation or defecation following feeding.

For the logistic GEE model that looked at predictors of whether or not an insect fed, the best fit model had an independent correlation structure considering the guinea pig as the clustering factor (Table 3). We found the odds of *T. gerstaeckeri* feeding on a

guinea pig were 9.21 times higher (P<0.001) than in *R. prolixus*, the reference species in the analysis. In contrast, *T. sanguisuga* feeding odds were not different to those of *R. prolixus*. If an insect was infected with TcI, it had 1/3 the odds of feeding on the guinea pig (P=0.025) than that of an uninfected insect (Table 4). There were no differences in feeding odds if the insect was infected with TcIV when compared to uninfected triatomines.

For the model looking at variables associated with whether or not an insect defecated, the best fit model had an independent correlation structure considering the guinea pig as the clustering factor (Table 5). We found that there were no significant differences between species nor infection status, but if an insect was observed in the dark (with the red lights), it had an odds of defecating two times higher than the odds of an insect in ambient light (P=0.045) during the two-hour trial period (Table 6). If an insect fed on the guinea pig, the odds of defecating was 17.99 times higher than that of an insect that did not feed on the guinea pig (P<0.001).

Table 3: Model selection for the best fit logistic generalized estimating equation model to predict whether or not a triatomine fed on a guinea pig

Correlation Structure	Cluster	Variables	QIC
Independent	Guinea Pig	Illumination Environment, Starvation Period, Life Stage, Triatomine Species, *T.cruzi* DTU	**173.52**
Independent	Date of Trial	Illumination Environment, Starvation Period, Life Stage, Triatomine Species, *T.cruzi* DTU	177.15
Exchangeable	Guinea Pig	Illumination Environment, Starvation Period, Life Stage, Triatomine Species, *T.cruzi* DTU	179.78
Exchangeable	Date of Trial	Illumination Environment, Starvation Period, Life Stage, Triatomine Species, *T.cruzi* DTU	178.27

QIC: Quasi-likelihood Information Criterion

Table 4: Parameter estimates for the best logistic generalized estimating equations studying whether or not a triatomine fed on a guinea pig

Parameter	Odds Ratio	Estimate (± S.E.)	P-value
Intercept	1	—	—
Illumination Environment—Lights On	1.68	0.52 (± 0.40)	0.196
Starvation Period	1.00	0.001 (± 0.01)	0.911
Life Stage—5th Instar	0.48	-0.73 (± 0.78)	0.350
T. gerstaeckeri	9.21	2.22 (± 0.44)	<0.001*
T. sanguisuga	1.17	0.16 (± 0.72)	0.825
TcI Infected	0.34	-1.09 (± 0.49)	0.025*
TcIV Infected	0.34	-0.94 (± 0.58)	0.109

*Statistically significant (P<0.05)

Table 5: Model selection for best fit logistic generalized estimating equation model to predict whether or not a triatomine defecated during the trials

Correlation Structure	Cluster	Variables	QIC
Independent	Guinea Pig	Fed, Illumination Environment, Starvation Period, Life Stage, Triatomine Species, *T. cruzi* DTU	**151.43**
Independent	Date of Trial	Fed, Illumination Environment, Starvation Period, Life Stage, Triatomine Species, *T. cruzi* DTU	151.76
Exchangeable	Guinea Pig	Fed, Illumination Environment, Starvation Period, Life Stage, Triatomine Species, *T. cruzi* DTU	153.16
Exchangeable	Date of Trial	Fed, Illumination Environment, Starvation Period, Life Stage, Triatomine Species, *T. cruzi* DTU	153.03

QIC: Quasi-likelihood Information Criterion

Table 6: Parameter estimates for the best logistic generalized estimating equation model studying whether or not a triatomine defecated during the trials

Parameter	Odds Ratio	Estimate (± S.E.)	P-value
Intercept	1	—	—
Fed	17.99	2.89 (± 0.61)	<0.001*
Illumination Environment—Lights On	0.52	-0.65 (± 0.32)	0.045*
Starvation Period	0.98	-0.02 (± 0.01)	0.213
Life Stage—5th Instar	2.75	1.01 (± 0.64)	0.116
T. gerstaeckeri	1.16	0.15 (± 0.44)	0.725
T. sanguisuga	1.86	0.62 (± 0.59)	0.294
TcI Infected	0.64	-0.45 (± 0.58)	0.444
TcIV Infected	1.99	0.69 (± 0.43)	0.106

*Statistically significant (P<0.05)

2.3.1. Feeding

For the model explaining the predictors that determined the number of feedings, the best fit model had an exchangeable correlation structure using the date of trial observation as the clustering factor (Table 7). Of the insects that fed, the mean number

(± S.E.) of feeding events per insect within the first 60 minutes with the guinea pig were 6.49 (± 0.56) for *T. gerstaeckeri*; 6.50 (± 0.68) for *T. sanguisuga*; and 3.50 (± 0.76) for *R. prolixus*. The control group had an average of 5.34 (± 0.61) feeding events per insect, while TcI had 7.44 (± 0.91) and TcIV had 5.73 (± 0.73) feeding events per insect. Figure 2 shows the distribution of number of feeding events per insect by infection status and species. On average, *T. gerstaeckeri* had more feeding events than *R. prolixus* (P=0.017). As expected, if an insect gained more weight when feeding, it correlated with a higher number of feedings (P<0.001) (Table 8).

Table 7: Model selection for the best Poisson generalized estimating equation model to predict the total number of feedings on the guinea pig during the first 60 minutes of the trials

Correlation Structure	Cluster	Variables	QIC
Independent	Guinea Pig	Illumination Environment, Starvation Period, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	-453.02
Independent	Date of Trial	Illumination Environment, Starvation Period, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	-455.39
Exchangeable	Guinea Pig	Illumination Environment, Starvation Period, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	-453.22
Exchangeable	Date of Trial	Illumination Environment, Starvation Period, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	-453.23
Independent	Guinea Pig	Illumination Environment, Starvation Period, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	-468.15
Independent	Date of Trial	Illumination Environment, Starvation Period, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	-469.60
Exchangeable	Guinea Pig	Illumination Environment, Starvation Period, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	-468.73
Exchangeable	Date of Trial	Illumination Environment, Starvation Period, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	**-469.95**

QIC: Quasi-likelihood Information Criterion

Table 8: Parameter estimates for the best Poisson generalized estimating equation model studying the total number of feedings on the guinea pig during the first 60 minutes of the trials

Parameter	Estimate (± S.E.)	P-value
Starvation Period	0.00 (± 0.005)	0.991
Illumination Environment—Lights On	0.02 (± 0.18)	0.899
Insect's Weight Change (g)	2.76 (± 0.67)	<0.001*
Life Stage—5th Instar	-0.12 (± 0.43)	0.786
T. gerstaeckeri	0.71 (± 0.30)	0.017*
T. sanguisuga	0.60 (± 0.42)	0.154
TcI Infected	0.15 (± 0.22)	0.495
TcIV Infected	-0.57 (± 0.34)	0.093

*Statistically significant (P<0.05)

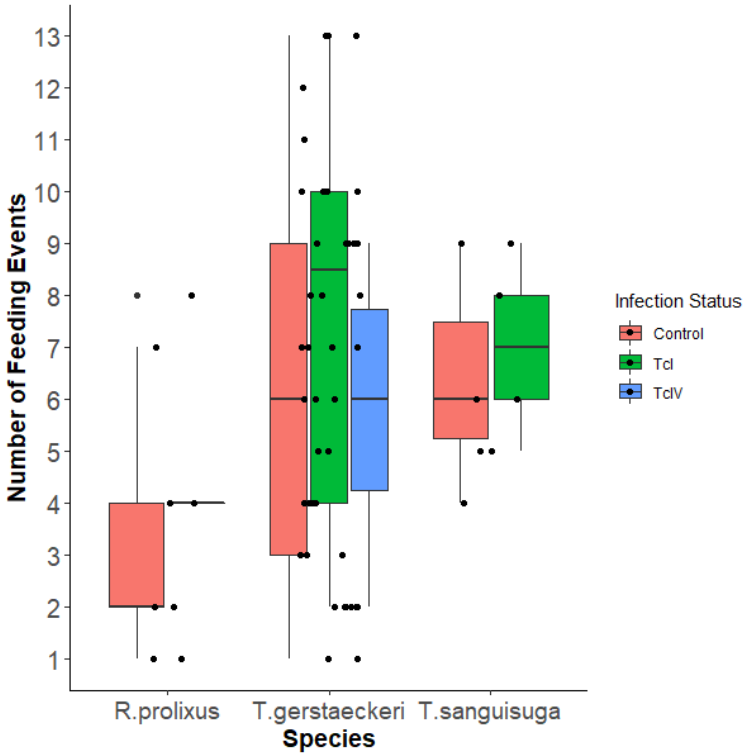

Figure 2: Boxplots of mean number of feeding events per triatomine species: *Rhodnius prolixus* (Control, TcIV); *Triatoma gerstaeckeri* (Control, TcI, TcIV); *Triatoma sanguisuga* (Control, TcI)

The best fitted Gaussian model explaining total feeding time per insect had an independent correlation structure using the date of trial observation as the clustering factor (Table 9). The mean total feeding times per insect were 14.6 (± 1.51) minutes for *T. gerstaeckeri;* 15.2 (± 3.34) minutes for *T. sanguisuga*; and 14.8 (± 3.86) minutes for *R. prolixus*. Uninfected insects fed for a mean of 13.67 (± 1.80) minutes; TcI fed for 14.42 (± 2.67) minutes; and TcIV fed for 18.13 (± 2.35) minutes. Figure 3 shows the distribution of total feeding times (min) for each *T. cruzi* DTU infection status and triatomine species. On average, *T. sanguisuga* fed 10.55 minutes longer than *R. prolixus*

(P<0.001) (Table 10). The model also showed the covariates of life stage and illumination environment were significant: 5th-instars took an average of 10.43 minutes more to feed than 4th-instars (P<0.001), and insects that fed in ambient light fed 4.37 minutes less than those with red lights (P=0.021) (Table 10). An insect fed for 0.065 minutes more for every 1 mg increase in its weight (P<0.001).

Table 9: Model selection for the best Gaussian generalized estimating equation model to predict the total feeding time (min) on the guinea pig

Correlation Structure	Cluster	Variables	QIC
Independent	Guinea Pig	Illumination Environment, Starvation Period, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	4971.97
Independent	Date of Trial	Illumination Environment, Starvation Period, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	4971.95
Exchangeable	Guinea Pig	Illumination Environment, Starvation Period, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	5090.40
Exchangeable	Date of Trial	Illumination Environment, Starvation Period, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	5113.30
Independent	Guinea Pig	Illumination Environment, Starvation Period, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	2869.63
Independent	Date of Trial	Illumination Environment, Starvation Period, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	**2868.98**
Exchangeable	Guinea Pig	Illumination Environment, Starvation Period, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	2879.04
Exchangeable	Date of Trial	Illumination Environment, Starvation Period, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	2887.92

QIC: Quasi-likelihood Information Criterion

Table 10: Parameter estimates for the best Gaussian generalized estimating equation model studying the total feeding time (min) on the guinea pig

Parameter	Estimate (± S.E.)	P-value
Starvation Period	-0.09 (± 0.05)	0.077
Illumination Environment—Lights On	-4.36 (± 1.89)	0.021*
Insect's Weight Change (g)	64.92 (± 11.89)	<0.001*
Life Stage—5th Instar	10.43 (± 2.70)	<0.001*
T. gerstaeckeri	-3.14 (± 3.50)	0.370
T. sanguisuga	10.55 (± 2.96)	<0.001*
TcI Infected	3.86 (± 2.65)	0.145
TcIV Infected	-3.46 (± 2.38)	0.146

*Statistically significant (P<0.05)

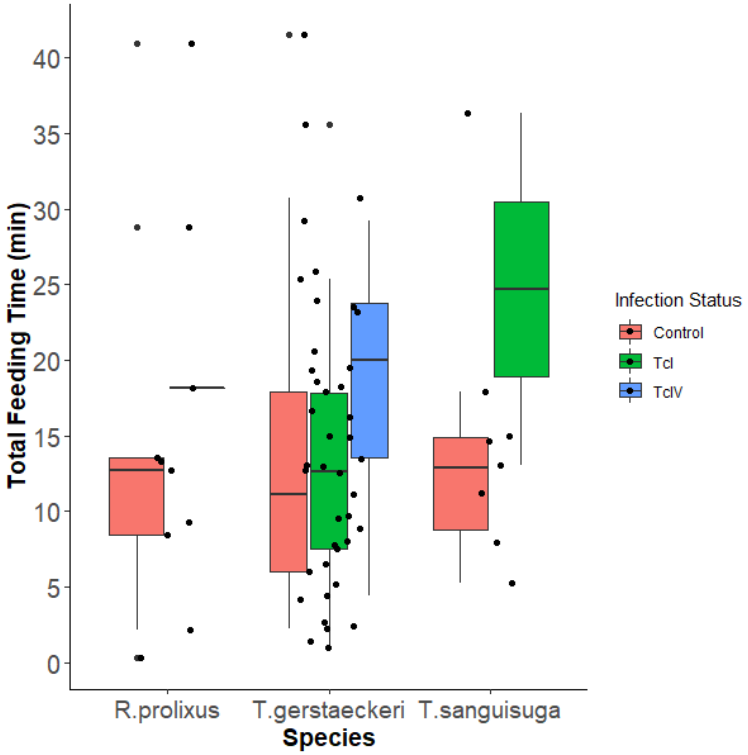

Figure 3: Boxplot of total feeding times (min) per triatomine species: *Rhodnius prolixus* (Control, TcIV); *Triatoma gerstaeckeri* (Control, TcI, TcIV); *Triatoma sanguisuga* (Control, TcI)

2.3.2. Defecation

A total of 56 (37.8%) insects defecated during the trials, and 42 of those defecated after feeding. The remaining 14 insects defecated without feeding during the trial. We recorded the mean number of defecations per insect during the 2-hour trial and PFDIs to the first defecation [20, 57]. The best fit model for number of defecation events had an independent correlation structure using guinea pigs as the clustering factor (Table 11). Both *T. gerstaeckeri* and *T. sanguisuga* had, on average, fewer defecation events than *R. prolixus* ($P<0.001$ for both) (Table 12). For every 1 mg increase in an insect's weight, there was a 6.65% increase in the number of defecations ($P=0.002$). Infection status did not yield significant differences in the number of defecation events. The other covariates (lights on/off, starvation period, number of feedings, and life stage) also did not yield significant differences. The mean (± S.E.) total number of defecation/urination events per insect were 1.80 (± 0.29) for *T. gerstaeckeri*; 0.91 (± 0.21) for *T. sanguisuga;* and 2.56 (± 0.59) for *R. prolixus.* In regards to infection status, controls had 1.81 (± 0.32) defecation events per insect; TcI had 1.53 (± 0.50); and TcIV had 2.22 (± 0.43). Figure 4 shows the distribution of total number of defecation events by infection status and species. It is noted that although the *R. prolixus* TcI group did not feed at all, there were some insects that still defecated.

Table 11: Model selection for the best Poisson generalized estimating equation model to predict the total number of defecation and urination events during the trials

Correlation Structure	Cluster	Variables	QIC
Independent	Guinea Pig	Illumination Environment, Starvation Period, Number of Feedings, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	85.85
Independent	Date of Trial	Illumination Environment, Starvation Period, Number of Feedings, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	91.60
Exchangeable	Guinea Pig	Illumination Environment, Starvation Period, Number of Feedings, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	94.15
Exchangeable	Date of Trial	Illumination Environment, Starvation Period, Number of Feedings, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	89.86
Independent	Guinea Pig	Illumination Environment, Starvation Period, Number of Feedings, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	**61.00**
Independent	Date of Trial	Illumination Environment, Starvation Period, Number of Feedings, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	64.89
Exchangeable	Guinea Pig	Illumination Environment, Starvation Period, Number of Feedings, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	69.08
Exchangeable	Date of Trial	Illumination Environment, Starvation Period, Number of Feedings, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	63.19

QIC: Quasi-likelihood Information Criterion

Table 12: Parameter estimates of the best Poisson generalized estimating equation model studying the total number of defecation and urination events during the trials

Parameter	Estimate (± S.E.)	P-value
Number of Feedings	0.07 (± 0.04)	0.080
Starvation Period	-0.00 (± 0.004)	0.950
Illumination Environment—Lights On	0.18 (± 0.13)	0.169
Insect's Weight Change (g)	4.21 (± 1.38)	0.002*
Life Stage—5th Instar	0.14 (± 0.22)	0.507
T. gerstaeckeri	-1.01 (± 0.15)	<0.001*
T. sanguisuga	-0.94 (± 0.14)	<0.001*
TcI Infected	-0.16 (± 0.26)	0.549
TcIV Infected	0.06 (± 0.20)	0.755

*Statistically significant (P<0.05)

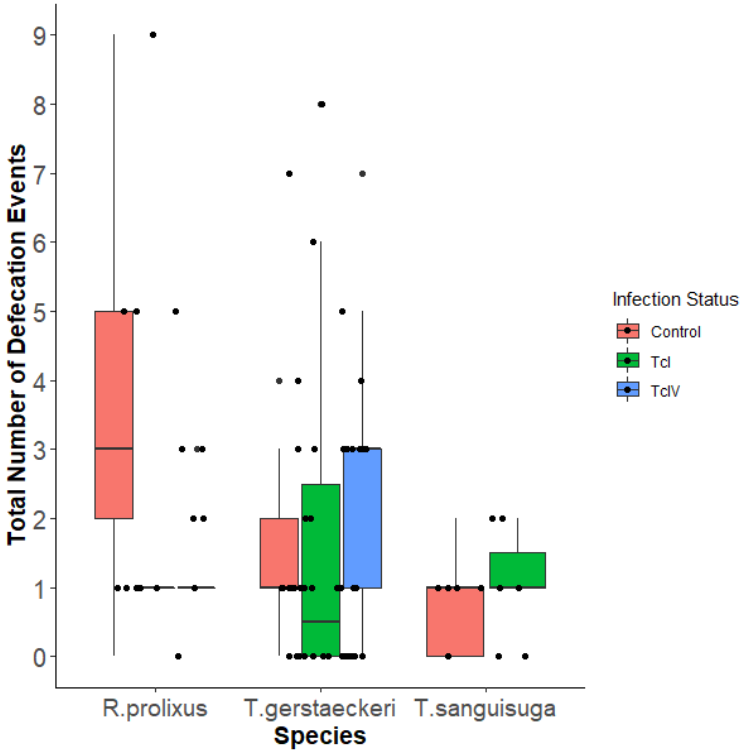

Figure 4: Boxplots of total number of defecation events per triatome species: *Rhodnius prolixus* (Control, TcI, TcIV); *Triatoma gerstaeckeri* (Control, TcI, TcIV); *Triatoma sanguisuga* (Control, TcI)

2.3.3. Post-Feeding Defecation Intervals

We measured the post-feeding defecation interval (number of minutes from the end of a blood meal to the first defecation) using GEE models with a Gaussian distribution. The best fitted model had an independent correlation structure using guinea pigs as the clustering factor (Table 13). The mean (±S.E.) interval between feeding to the first defecation was 9.75 (± 2.52) minutes for *T. gerstaeckeri*; 20.69 (± 8.98) minutes for *T. sanguisuga*; and 4.54 (± 2.46) minutes for *R. prolixus*. The PFDIs for the control group was 11.82 (± 3.17) minutes; TcI group was 8.19 (± 3.26) minutes; and TcIV group was 6.68 (± 4.02) minutes. Comparing to *R. prolixus*, we found that on average *T. gerstaeckeri* would defecate 11.45 minutes later post feeding ($P<0.001$), and *T. sanguisuga* would defecate 19.52 minutes later post feeding ($P<0.001$) (Table 14). We did not see a significant difference between the infected and uninfected groups (TcI: $P=0.087$; TcIV: $P=0.389$) for the PFDIs to the first defecation; however, when we fitted the model to include multiple PFDIs (since many of the insects had multiple defecation events post feeding), we saw that TcI-infected insects were faster than the uninfected controls in defecating after feeding ($P=0.019$). We also observed insects with a bigger weight gain had shorter PFDIs ($P<0.001$). Figure 5 shows the distribution of the PFDIs of the first defecation broken down by infection status and species. There is no boxplot for the *R. prolixus* TcI since none of those insects fed, and for *R. prolixus* TcIV, only one insect is represented.

Table 13: Model selection for the best Gaussian generalized estimating equation model to predict the post-feeding defecation intervals (min) to the first defecation

Correlation Structure	Cluster	Variables	QIC
Independent	Guinea Pig	Illumination Environment, Starvation Period, Number of Feedings, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	6422.22
Independent	Date of Trial	Illumination Environment, Starvation Period, Number of Feedings, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	6426.44
Exchangeable	Guinea Pig	Illumination Environment, Starvation Period, Number of Feedings, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	6587.01
Exchangeable	Date of Trial	Illumination Environment, Starvation Period, Number of Feedings, Initial Weight, Life Stage, Triatomine Species, *T. cruzi* DTU	6437.36
Independent	Guinea Pig	Illumination Environment, Starvation Period, Number of Feedings, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	**5243.38**
Independent	Date of Trial	Illumination Environment, Starvation Period, Number of Feedings, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	5248.51
Exchangeable	Guinea Pig	Illumination Environment, Starvation Period, Number of Feedings, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	5400.46
Exchangeable	Date of Trial	Illumination Environment, Starvation Period, Number of Feedings, Weight Change, Life Stage, Triatomine Species, *T. cruzi* DTU	5359.62

QIC: Quasi-likelihood Information Criterion

Table 14: Parameter estimates of the best Gaussian generalized estimating equation model studying the post-feeding defecation intervals (min) to the first defecation

Parameter	Estimate (± S.E.)	P-value
Starvation Period	0.18 (± 0.13)	0.384
Illumination Environment—Lights On	3.13 (± 2.37)	0.187
Insect's Weight Change (g)	-58.98 (± 12.08)	<0.001*
Life Stage—5th Instar	6.10 (± 7.56)	0.420
T. gerstaeckeri	11.45 (± 2.08)	<0.001*
T. sanguisuga	19.52 (± 1.60)	<0.001*
TcI Infected	-8.26 (± 4.83)	0.087
TcIV Infected	2.87 (± 3.33)	0.389

*Statistically significant (P<0.05)

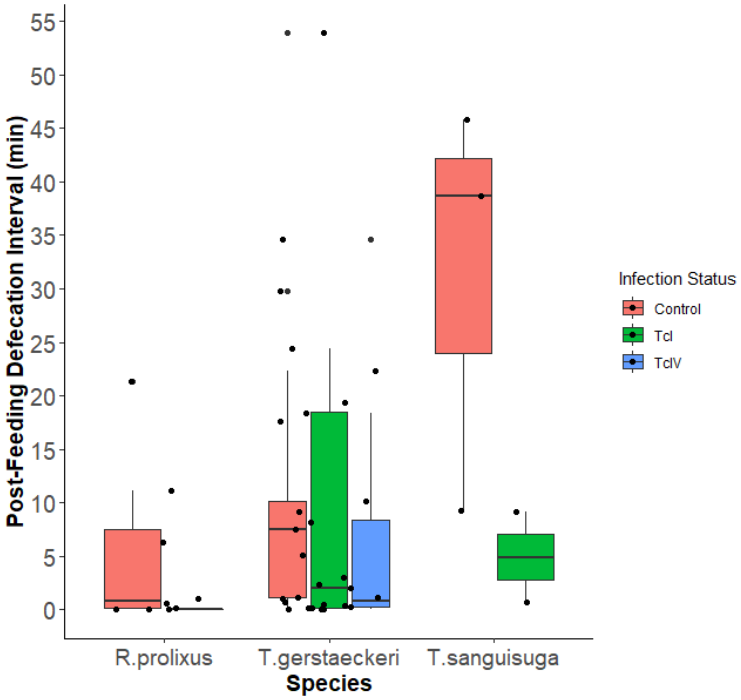

Figure 5: Boxplot showing the post-feeding defecation interval (min) of the first defecation per triatomine species: *Rhodnius prolixus* (Control, TcIV); *Triatoma gerstaeckeri* (Control, TcI, TcIV); *Triatoma sanguisuga* (Control, TcI)

Of the 148 triatomines that were analyzed, we observed six insects (4.05%) simultaneously feed and defecate (Figure 6). At least one insect in each species and infection group were represented. Three of these insects also simultaneously fed and defecated twice. These insects had a total feeding time range from 13 to 40 minutes and defecated a total of 29 times, of which 21 defecations were within 10 minutes post feeding. For both *T. gerstaeckeri* and *T. sanguisuga*, only infected insects simultaneously fed and defecated, and they accounted for 14 total defecation events—10 of which were within 10 minutes post feeding. We also observed that when insects were placed at the top of the guinea pigs at the start of the trials, most insects immediately crawled off of the animals and preferred to feed while standing on the bench paper. Only a couple stayed on the guinea pigs, including one that fed and also simultaneously defecated. This insect was a *R. prolixus* in the control group.

Figure 6: Triatomines simultaneously feeding on a guinea pig and defecating. Left to right: *Triatoma gerstaeckeri, Triatoma sanguisuga, Rhodnius prolixus*

2.3.4. Defecation Index

A defecation index (DI= (% of insects that defecated up to 10 minutes post feeding x average number of defecations up to 10 minutes post feeding)/100)) was calculated to measure an insect's potential infection capacity [17]. Figure 7 shows the defecation indices for each infection status of *T. gerstaeckeri, T. sanguisuga,* and *R. prolixus.* For *R. prolixus,* both the uninfected and infected groups had higher DIs compared to the two North American species. The high DI for *R. prolixus* (TcIV) is most likely due to only one insect included in the calculation. The DI for *R. prolixus* (TcI) could not be calculated since none of the insects in that group fed on a guinea pig, which was needed to determine if an insect defecated within 10 minutes post feeding. Given that some studies emphasize the importance of defecation at an interval less than 10 minutes [17, 20], we also report the percent of individuals defecating within 1-minute post-feeding: 11 (39.3%) of *T. gerstaeckeri*, 1 (20%) of *T. sanguisuga*, and 6 (66.7%) of *R. prolixus*.

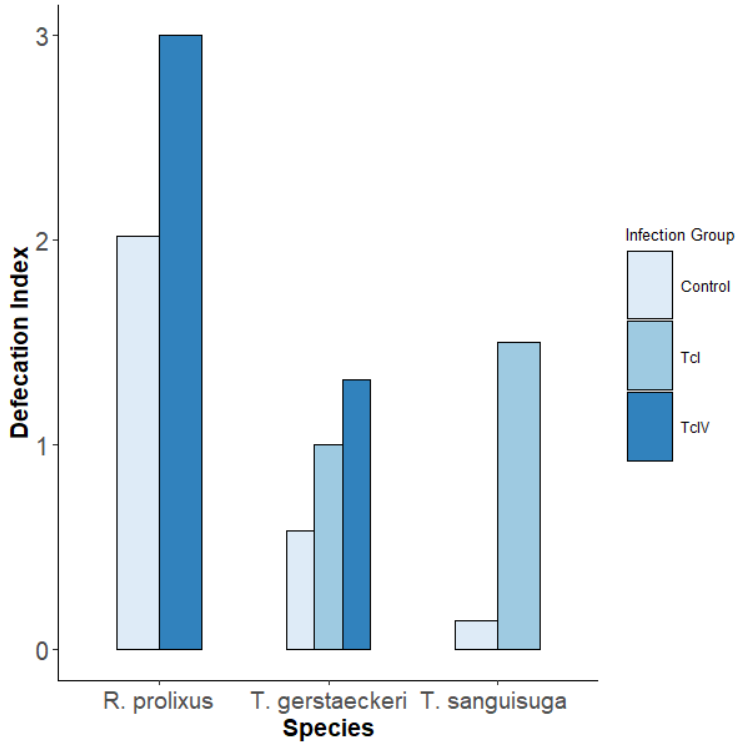

Figure 7: Defecation indices (DI= (% of insects that defecated up to 10 minutes post feeding X average number of defecations up to 10 minutes post feeding)/100)) of each infection group

2.3.5. Weight Gain

We calculated the mean weight gain (mg), mean volume of blood ingested (µL), and percent weight gain for insects that fed on the guinea pigs and gained weight (Table 15). There was a total of five insects that we recorded feeding events for, but they did not gain weight and were excluded in the tabulation, so the total number of insects that fed and gained weight was 54. The lack of weight gain could possibly be due to scale error or insufficient blood to detect a difference on the scale. Overall, the majority of the infected groups gained more weight and ingested more blood. *T. gerstaeckeri* (TcI) had lower mean weight gain and mean blood volume ingested, but had a higher percent

weight gain than the control group. Both *T. cruzi*-infected groups (TcI and TcIV) for all three species had larger mean weight gain percentages than the control groups, with the exception of *R. prolixus* (TcI) since none of those insects fed.

Table 15: Mean weight gain, mean blood volume ingested, and percent weight gain of triatomine insects that fed and gained weight for each species by infection status.

Species	Infection Group	No. Insects that Gained Weight	Mean Weight Gain (mg)	Mean Blood Volume Ingested (µL)	% Weight Gain
T. gerstaeckeri	Control	15	121	120.7	98.8
	TcI	13	102	102.2	104.8
	TcIV	8	265	264.9	164.0
T. sanguisuga	Control	6	16	16.0	45.1
	TcI	2	64	63.7	88.9
R. prolixus	Control	9	71	70.7	222.3
	TcI	0	0	0	0
	TcIV	1	160	159.5	406.9

[a] There were five insects that fed on guinea pigs, but did not gain any weight. These data points were excluded from the calculations.

2.4. Discussion

We observed the feeding and defecation behaviors of two important triatomine species found in the U.S. and compared them with a highly competent vector species from South America. We found that in the uninfected (control) groups, all three species had similar mean total feeding times to each other. *Rhodnius prolixus* had fewer feeding events per insect than *T. gerstaeckeri* and *T. sanguisuga*, which may be attributed to *R.*

prolixus taking a longer time to feed per feeding event. When it comes to the defecation behaviors, *R. prolixus* had significantly more defecation events and had shorter PFDIs than the two U.S. species. This was expected since *R. prolixus* has been shown to be a more efficient defecator than other U.S. and South American species [17-19, 31]. *T. gerstaeckeri* had the highest weight gain at 121 mg of the three species, which could be explained by that this species has a larger body size than that of *T. sanguisuga* and *R. prolixus*.

We also experimentally infected a subset of triatomines for each species to determine any differences in behaviors between *T. cruzi*-infected and uninfected insects. Many studies have investigated the influence of *T. cruzi* on various aspects of triatomine behavior, such as development, fecundity, and fitness [58-61]. Some of these studies and others have seen changes in infected triatomine's biting rates, weight gains, and defecations [20, 24-26], suggesting that *T. cruzi* may modulate vector competence. One study showed infected *Mepraia spinolai* were twice more likely to bite and defecated sooner than uninfected triatomines [25], and another study showed that *T. cruzi* infection increased triatomine's vector activity [26]. Both studies came to conclusions that suggested the manipulation by *T. cruzi* of feeding and defecation behaviors led to increased parasite transmission. Interestingly, while we saw the infected groups had slightly more feeding and defecation events and longer feeding times, none of these metrics were significantly different for infection status. Both infected groups also had shorter PFDIs to the first defecation, which can also be seen in a study with *Triatoma infestans*, where the infected insects defecated more and faster post feeding than

uninfected insects [20]. Our study did not see a significant difference in infection groups for PFDIs to the first defecation; however, since we observed multiple insects defecate multiple times, we also ran a model considering the multiple defecation events post feeding and found that TcI-infected insects had significantly shorter PFDIs than uninfected insects. This finding could suggest that infection with *T. cruzi* makes a difference in the timing of multiple defecation events. We saw insects that had more than one defecation event generally fed longer and had higher blood intake, on average, than those that defecated once or did not defecate at all. This could also account to these insects having multiple PFDIs and defecated faster. The model showing that TcI-infected insects had lower odds of feeding on the guinea pig than uninfected insects was the only one that showed a difference based on infection status.

The defecation index (DI) was first proposed as a measurement to estimate an insect's infection capability that accounted for defecations within 10 minutes post feeding [17]. A higher DI generally equates to a higher capability of infection. Zeledon calculated the DIs for 4^{th} and 5^{th}-instars of *R. prolixus* to be 3.8 and 1.8, respectively. Our DI calculations were based on infection status, but we saw higher DIs in both the control and TcIV groups for *R. prolixus.* Therefore, we could say the DIs for *R. prolixus* in our study were fairly similar to those in Zeledon's study. The DIs for most of the *T. gerstaeckeri* and *T. sanguisuga* groups were relatively higher than that found in *Triatoma protracta* and *Triatoma rubida* [17, 21, 23], with one study showing adult female *T. rubida* having a DI of 1.3 [22]. This suggests that both *T. gerstaeckeri* and *T. sanguisuga* may potentially be better vectors than *T. protracta* and *T. rubida.* However,

it should be noted that some of the DI calculations were based off from data of one or two insects, and thus may not accurately represent the species' infection capability. We found that the infected groups had higher DIs compared with their own control groups, so it seems *T. cruzi* may be playing a role in increasing the vector competence of triatomines with respect to their defecation behaviors, as explained in a previous study [20]. With the exception of *T. gerstaeckeri* (TcI), all infected groups had a higher mean for blood volume ingested than the control groups, which was found in *Triatoma rubrovaria* infected with DTU TcIV [24]. Not only that, we also observed the more blood an insect ingested—indicating a bigger weight gain—the PFDIs were shorter, corroborating that blood intake had a negative correlation with the time of appearance of the first defecation [57]. While our study did not measure the effect of *T. cruzi* on the triatomine's development, survival, and fecundity at the end of the trials, we still observed how *T. cruzi* could potentially influence the feeding and defecation behaviors of the U.S. triatomines like it did in other species.

A total of six insects fed and defecated simultaneously, in which all three species were represented. These insects had multiple defecation events, of which a majority was within 10 minutes post feeding. If an insect fed to repletion, then it is possible that they will still be in close proximity to the host because the added weight may slow down its mobility [17], and the chances to defecate or urinate multiple times is likely. We also observed 14 insects that defecated without feeding. These insects were considered in the logistic GEE models because they could still potentially infect a host by being attracted to the host and subsequently defecate on or near it even without feeding. Thus, these

insects are still in close contact with the host, and may feed later when the host is not active, which increases contact time with the host despite not having defecations post feeding [17, 62].

The three species we used in the trials were the same that were used in Pippin's study in 1970 with triatomines feeding on laboratory mice and rats. His findings showed the mean total feeding times of 4th- and 5th-instar nymphs were 33 and 39 minutes, respectively, for *T. gerstaeckeri*; 25 and 31 minutes for *T. sanguisuga*; and 17 and 19 minutes for *R. prolixus* [19]. Our mean total feeding time data shows that all three species had similar mean feeding times to each other as well, although we broke it down by species and not by life stage. Pippin stated that *T. gerstaeckeri* and *T sanguisuga* fed more than twice as long as *R. prolixus*, which we did not see in our study; however, we saw a significant difference in total feeding times for *T. sanguisuga* as it fed longer than *R. prolixus*. Pippin also founded that a larger percentage of 4th- and 5th-instar nymphs of *R. prolixus* defecated within two minutes of feeding than that of *T. gerstaeckeri* and *T. sanguisuga*, indicating the two U.S. species were less efficient stercorarian vectors than *R. prolixus*. In our study, we did not see significant differences determining which species was more likely to defecate, but both *T. gerstaeckeri* and *T. sanguisuga* had fewer defecation events than *R. prolixus.* It was also noted that in Pippin's study, about 1/4th of the late instar nymphs of *T. gerstaeckeri* and *T. sanguisuga* were able to defecate within two minutes post feeding, making them potential efficient vectors for *T. cruzi* [63]. If we applied the same method of reporting for our insects that defecated, we would have seen 42.9% of *T. gerstaeckeri* and 20% of *T. sanguisuga* defecating within two

minutes after feeding, which is similar to *T. sanguisuga* (23.3%) but higher than *T. gerstaeckeri* (25%) observed in Pippin's study [19]. Another study compared feeding and defecation behaviors of *T. gerstaeckeri* with two other North American species and found that late stage *T. gerstaeckeri* took a longer time to feed and had a PFDI at around 13 minutes [28], which is slightly longer than what we observed.

Our study design posed some limitations. A previous study reported that *T. sanguisuga* were more likely to carry DTU TcIV [9], yet we did not have a TcIV infection group for that species. *Triatoma sanguisuga* is more challenging to colonize [64] than *T. gerstaeckeri* which thus, we could not properly make comparisons between TcIV-infected and uninfected insects in the trials. Surprisingly, our results showed none of the *R. prolixus* (TcI) insects feed on the guinea pigs. This is most likely because the *R. prolixus* we have in our insect colony have been removed from wild populations for over 15 years, and therefore may exhibit different feeding and defecation behaviors. Also, the DTU TcI is known to be mostly associated with *R. prolixus* [50, 65, 66], so due to the lack of data for that infection group, we could not assess if *T. cruzi* can influence the feeding and defecation patterns nor could we corroborate that parasite manipulation did not occur in *R. prolixus* [67].

The results from this study will help to gain a better insight of the disease risk of Chagas disease in the U.S. in comparison with Latin America. Although *T. gerstaeckeri* and *T. sanguisuga* had fewer defecation events and longer PFDIs than *R. prolixus*, a highly efficient vector, our observations in this study suggest that these two species are capable vectors of *T. cruzi* in the U.S. through the stercorarian form of biological

transmission. While considering results on PFDI, DI, and the percent defecating within 1-minute following feeding, *T. gerstaeckeri* appears to have higher transmission potential compared to *T. sanguisuga* based on feeding and defecation behavior. Although this does not consider intrinsic factors associated with vector competence for *T. cruzi* that could exist between these two species. We present evidence that *T. cruzi* infection in triatomines might influence feeding and defecation behavior in ways that would facilitate *T. cruzi* transmission. We observed a decreased PFDI with *T. cruzi* infection which was only significant when models considered multiple defecation events. This observation warrants further research investigating the influence of *T. cruzi* on feeding and defecation behavior and mathematical models to determine the importance at the population level. Additionally, up to 11 species of triatomines exist in the U.S. and more studies should be conducted to compare the feeding and defecation behaviors among these species and from multiple geographic populations. The scientific and lay-community perspective that prolonged PFDIs results in less efficient transmission of *T. cruzi* in the U.S. is not supported by the observations in this current study. Instead, we hypothesize that other factors of triatomine ecology and contact with humans contributes to the lower disease burden in the U.S. compared to other regions in Latin America.

CHAPTER III

CONCLUSION

Chagas disease is becoming a growing public health concern, and we are currently trying to find innovative strategies to control triatomine populations. In Latin America, initiatives were implemented which drastically reduced the number of infected people as well as eliminated highly competent species that posed as public health threats [11-13]. However, when one species population is eliminated or reduced as a public health threat, there are concerns that another species may take over, and they could be just as efficient vectors as their predecessors. In the U.S., species have established populations—primarily in the sylvatic environment—but this still makes control measures difficult to implement. There have been reports that triatomines can be found in peridomestic environments, such as dog kennels, as well as inside homes where people may be in close contact with these vectors [68]. With a high infection prevalence in some species [9], there is a need to learn triatomine behavior so that efforts in creating innovative control measures can be successful.

In the study comparing the feeding and defecation behaviors of two U.S. species with a South American species, we found that the U.S. species fed more which indicates more contact time with the host, but they also defecated less than the South American species. This may account for the low disease risk of Chagas disease in the U.S.; however, we observed that *T. gerstaeckeri* and *T. sanguisuga* are still capable of defecating when nearby a host, which may lead to increased parasite transmission. There

also may be evidence that *T. cruzi* infection could play a role in the feeding and defecation behaviors for the U.S. species, but further research is needed. For those reasons, we believe that other factors in triatomine ecology contribute to the low disease burden in the U.S. and not solely the feeding and defecation behaviors.

By understanding the feeding and defecation patterns of triatomines, we are able to identify which species are of most epidemiological importance and can use that knowledge to determine their vector competence, or ability to transmit a disease. We will also gain insightful knowledge that can help us with assessing the disease risks in the Americas, as well as developing preventive measures in eliminating those species. However, in order to fully combat against Chagas disease, we need to continue to learn more of triatomine ecology and behavior, raise educational awareness of the vectors [41], and improve public health interventions and control methods.

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