Identification of bloodmeal sources of triatomines captured in the Paraguayan Chaco region of South America by means of molecular biology analysis

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ABSTRACT

The Paraguayan Chaco is an isolated environment with its own unique ecosystem. In this region, Chagas disease remains a health problem. Chagas disease is caused by the parasite *Trypanosoma cruzi*, and it is primarily transmitted by triatomines. In order to identify the blood meal sources of triatomines, specimens of the vector were collected in domestic and peridomestic areas and the PCR-RFLP method was implemented. Cytochrome b was amplified from the samples and later subjected to digestion with two restriction enzymes: Hae III and Xho I. It was possible to generate distinct restriction patterns on the amplified material to identify several blood meal sources for the vectors. We employed the blood from several species as positive controls: human, chicken, canine, feline, and armadillo blood. However, we identified only 3 sources for the blood meals of the insect vectors: human, chicken and canine blood. In total, 76 triatomines were captured. *T. cruzi* was not found in any of them. In 61% of the captured specimens, the blood meal sources for the vectors could be identified. In 30% of these cases, the presence of DNA from more than one vertebrate was detected in the same triatomine. The most common blood meal source found was chicken blood. The presence of human and chicken blood in triatomines captured in domestic and peridomestic areas strongly suggests that the parasite can freely move amongst both areas regardless of food availability. Free vector movement in these areas constitutes an epidemiological threat for the inhabitants of the community under study.

KEYWORDS

Trypanosoma cruzi; chagas disease; cytochrome b; PCR-RFLP

Introduction

Chagas disease is a zoonotic parasitism caused by the hemoflagellate *Trypanosoma cruzi* and is mainly transmitted by triatomines. The parasite is introduced into mammals when the vectors feed and later deposit their infected feces on the skin of the host. The infected feces, in turn, come into contact with the mucous membranes of the host, or alternatively, enter the host through the open wound left by the insect bite. By these mechanisms, the metacyclic parasites reach the host and invade the nucleated cells of different organs, where they ultimately differentiate into amastigotes and form pseudocysts [1].

According to conservative estimates, there are between 7 and 8 million people diagnosed with Chagas disease, most of those are in South America. Of this total number of diagnosed cases, approximately 150,000 cases occur in Paraguay. In Paraguay *Triatoma infestans* constitutes the most common vector [2,3]. Individuals afflicted with the disease usually come from a challenging socioeconomic context in which access to proper health care is restricted. Moreover, factors such as precarious housing, presence of adjacent chicken coops, stables or other

animal housing facilities all promote triatomine proliferation. Taken together, all these factors conspire in favor of the disease's prevalence in impoverished communities [4].

A number of indigenous communities populate the Paraguayan Chaco Region, as these communities were the original inhabitants before European immigrants settled in the Americas. Natives of the Paraguayan Chaco Region keep their traditional building methods, making use of mudbrick and/or plywood walls, soil floors, thatched roofs, palm roofs and, more recently, zinc roofs.

As is their traditional way of life, chicken coops are often placed near houses. These building practices, and in particular the practice of using thatched roofs, allow Chagas disease vectors to proliferate in domestic and peridomestic areas of such indigenous communities [4,5].

The Paraguayan government implemented the National Program for the Control of Chagas Disease, under a special branch of the Ministry of Health Affairs called National Service for the Eradication of Malaria (SENEPA, in Spanish). As a result of vector elimination measures undertaken by SENEPA, there was a significant decrease in the triatomine infestation

index, and Paraguay was able to obtain the certification of interruption of *T. cruzi* transmission by *T. infestans* [6]. Nevertheless, recent data indicates that less commonly known vectors, such as *Triatoma sordida* and *Triatoma guasayana*, are gaining ground as emerging vectors of the disease. Consequently, there is a growing concern in the Paraguayan health-care community that Chagas disease could quickly reemerge to become a national health crisis if not managed properly [7].

Triatomine vectors feed mainly on bird and mammal blood. However, the parasite *T. cruzi* can only develop inside the mammal cells. Several mammal species are known to serve as hosts for the parasite, and the list includes: domestic, synanthropic and wild mammal species [8,9]. Due to the sheer number of parasite reservoirs as well as the lack of an effective vaccine against Chagas to date, the disease remains difficult to eradicate completely. For this reason, the best strategies to prevent the disease's incidence are: the improvement of the living conditions of the most vulnerable communities, and vector control by means of vector elimination programs [2].

Blood meal identification is relevant because it provides information on the insect's vector capacity, its food preferences under natural conditions, the biological cycles of the disease, and all this information in turn allows the development of more efficient strategies for national vector control programs [10,11].

Originally, the determination of the source of the vector's blood meals was based on antigen-antibody assays. However, this assay requires species-specific antibodies and also has sensitivity problems [12,13]. In the last two decades, new molecular biology techniques were developed which greatly enhanced the accuracy and sensitivity required to properly identify the source of the hematophagous insects' blood meals [14–16].

Specifically, the use of Polymerase Chain Reaction with Restriction Enzymes (PCR-RFLP) constitutes an effective and widely accepted method employed in studies aiming at identifying hematophagous insects' blood meal sources [11,17–19]. The PCR-RFLP method has the added advantage of being less expensive than other molecular biology methods, as it only necessitates basic molecular biology equipment such as thermal cyclers and gel electrophoresis chambers.

The primary aim of this study was to identify the blood meal sources of domestic and peridomestic triatomines captured in the Paraguayan Chaco Region by means of the PCR-RFLP method. The secondary aim of the present study was to examine whether there was a blood meal preference for the vectors, and to examine the triatomine dynamic dispersion among the domestic and peridomestic areas.

Material and methods

Study area

The country of Paraguay is located in the center of South America, and it is divided into 17 departments across 2 Regions. The 2 Regions are divided in turn by the Paraguay River. The Paraguayan Chaco Region is located on the north-western part of the country and it encompasses 3 departments. The Chaco Region is outlined by river boundaries with Argentina and Brazil, and by a land boundary with Bolivia. Numerous indigenous communities populate the 3 departments on the Chaco Region. Although indigenous communities in this region of the country keep aspects of their traditional way of life, in more recent times, they have been influenced by Mennonite communities in the Chaco Region, as well as modern Paraguayan society to the degree that they have gradually come to have a more modern lifestyle. Nevertheless, indigenous people in Paraguay remain a disadvantaged minority in that they have less opportunities to access proper social services such as health services and public education, amongst others.

The study area was located in the Paraguayan Chaco Region, in the Department of Presidente Hayes. The exact geographic coordinates of the surface area for the study were as follows: parallels 22°36' and 22°56' south latitude, and meridians 59°50' and 59°53' west of the Greenwich meridian. This geographical site corresponds to a surface area of approximately 500 km² (Figure 1). The geographical area of the study is home to the 10 Leguas tribe, an indigenous population of the Maskoy ethnicity, who are the direct descendants of the original human inhabitants of the area that is today Paraguay.

This geographical area constitutes the current living area of the tribe, as they were originally a nomad tribe in the times before European settlers first explored what is now Paraguay.

The insects were collected in 3 villages of the 10 Leguas tribe, named: 12 de Junio, Tiberia and Karandillas respectively. The area where the vectors were collected corresponds to the xeromorphic forests of the Paraguayan Chaco ecoregion, with a climate characterized by extreme heat in the summer and mild temperatures in the winter. Lastly, according to our environmental measurements in the study area, the wind blows at an average speed of approximately 11.9 km/h in the fall, and that can increase to 14.0 km/h in the winter [20].

Capture of triatomines

Triatomines were collected in domestic and peridomestic areas of the villages of 12 de Junio, Tiberia and Karandillas between January and June 2017. Collection

Figure 1. Indigenous Maskoy community 10 Leguas of Presidente Hayes department. 1: Tiberia village, 2: 12 de Junio, 3: Karandillas.

in these months was performed in order to obtain the highest possible insect numbers, since during winter season the reproductive cycle of the vector comes to a halt [21]. Collection of the insect vector in domestic areas was made by the capture/hour/man method, collecting insects at places such as: walls, ceilings and beds of the residences. In peridomestic area, triatomines were collected mainly from chicken coops. Additionally, sticky traps with pheromones were placed in the residences and in the poultry houses in the peridomestic area. Collected triatomines were placed in plastic jars, identified according to the collection area and later transported to the laboratory.

Once in the laboratory facilities, more detailed identification parameters, such as: species confirmation, sex and stage of the triatomines were determined following the taxonomic key of Lent & Wygodzinsky [22].

DNA extraction from triatomines' feces

To determine blood meal sources and for the assessment of the presence of *T. cruzi*, intestinal contents were recovered by exerting gentle abdominal pressure on the collected insects, in order to stimulate the expulsion of feces. During the process special attention was given to avoid any external contamination of the sample. In this study adults and nymphs of 4th and 5th stage were used because of their larger size, thereby facilitating handling and feces extraction. The samples were collected in 1.5 ml sterile tubes with 200 µl of PBS and stored at -20°C until DNA extraction.

Due to the fact that feces samples have a number of naturally occurring PCR inhibitors, five DNA extraction methods were evaluated: three conventional methods (CTAB, phenol chloroform precipitation with isopropanol and phenol chloroform precipitation with ethanol) and two commercial methods (Gene Jet Genomic DNA Purification kit

from Thermo Scientific® and the QIAamp DNA Mini Kit from Qiagen®). Regardless of the method, DNA was extracted in a biological safety cabinet and adequate preventive measures were used to avoid contamination of the sample with human DNA.

PCR-RFLP analysis

In order to identify different species that serve as a source of bloodmeal for the triatomines, a 358 bp fragment of the mitochondrial Cytochrome b (Cyt b) gene of vertebrates was amplified, using the Forward and Reverse primers previously published by Boakye *et al.* [10]. Subsequently, the amplified fragments were independently digested with two restriction enzymes to obtain different restriction patterns of each species studied. The restriction enzymes selected for the digestions were the Hae III enzyme [11,17,23] and Xho I enzyme [18].

To implement the PCR/RFLP technique, positive DNA controls extracted from human (*Homo sapiens*), dog (*Canis lupus familiaris*), cat (*Felis catus*), chicken (*Gallus gallus domesticus*) and armadillo (*Dasypus novemcinctus*) were used (samples were given as donations to the Center for the Development of Scientific Research (CEDIC, in Spanish).

The Cyt b fragment was amplified under the conditions described by Oshaghi *et al.* [18], with some modifications, such as an increase in the initial DNA concentration (40 ng/µl), final concentration of MgCl_2 (3 mM), and the increase in the hybridization time of cycling to 1 minute.

Equal amounts of digested DNA were electrophoresed on 3% agarose gel. Gels were stained with Sybr Safe (Invitrogen®) and DNA fragments were visualized under UV light. The differential restriction pattern obtained from the positive controls was compared with the results obtained from DNA samples extracted from triatomine feces.

Detection of *T. cruzi* in triatomines' feces by PCR

TCZ Forward and TCZ Reverse primers published by Schijman *et al.* [24] were used to determine the presence of *T. cruzi* in the feces of the captured triatomines. The mix Maxima Sybr Green qPCR (Thermo Scientific®) was used on the amplification reaction and the two-step cycling proposed by the manufacturer was followed. *T. cruzi* DNA extracted from a parasite culture and DNA isolated from one sample of triatomines feces (to which 1 µl with 5×10^6 parasites was added) were used as positive controls.

Ethics statement

The study was carried out within the territory of indigenous communities, and was approved by the local Ethical Committee of a nonprofit organization that safeguards the welfare of such communities (Fundación Moises Bertoni, in Spanish).

Furthermore, the community leaders of the indigenous communities from the indigenous villages of: 12 de Junio, Karandillas and Tiberia all approved of the study goals and procedures that took place in their villages. Informed consent was then obtained by these community leaders, who represented all 3 indigenous villages in this study.

Results

As mentioned in the methods section, in order to establish an effective DNA extraction method that eliminates the naturally occurring PCR inhibitors present in the triatomines feces, five methods of DNA extraction were evaluated. For the DNA extraction, three conventional extraction methods and two commercial kits were evaluated. In the 3 conventional DNA extraction methods, very high concentrations of DNA were obtained. However, the ratio of purity of such extractions was lower than 1.8, thereby indicating a high concentration of ring structures that are not nucleic acids. With the commercial Kit Genomic DNA Purification (Thermo Scientific®) most of the extractions had purity values higher than 1.8, indicating a high concentration of RNA. The optimal extraction method was the commercial Kit QIAamp DNA mini (Qiagen®), previously used by Buitrago *et al.* and Kjos *et al.* [14,15] for DNA extraction from triatomine feces. With this kit, a good DNA concentration was obtained with values close to the optimum for purity.

As a means to standardize the PCR/RFLP technique, two independent digestion reactions were performed with the restriction enzymes Hae III and Xho I. The restriction profile obtained with the Hae III enzyme generated four fragments for armadillo blood (sizes: 153, 240, 131 and 64 bp respectively), three fragments for chicken blood (sizes: 159, 126 and 74 bp

respectively) and four fragments for cat blood (sizes: 330, 273, 74 and 20 bp respectively). However, digestion with the same enzyme showed the same restriction pattern of two fragments for human blood and dog blood (sizes: 233 and 125 bp respectively).

Therefore a second round of enzymatic digestion, with the Xho I enzyme, was employed to differentiate both species. This enzyme generated two fragments in the human blood Cyt b sequence (sizes: 214 and 144 bp respectively) and a single fragment for the dog blood DNA (size: 358 bp) which represents the intact fragment of Cyt b amplified. The differential digestion patterns for the identification of the blood meal sources in the selected species can be seen in Figure 2.

In total, 76 insect vectors of the genus *Triatoma* were captured, 17 were captured in the domestic areas and 59 in the peridomestic areas. Specimens of 3 different species of triatomines were captured: 27 correspond to *T. infestans*, 45 to *T. sordida* and 4 to *T. platensis*. The species most frequently captured at domestic areas was *T. infestans* while *T. sordida* was the species most commonly captured in the peridomestic area. For detailed records on each captured specimen, the data is shown in Table I of the Supplementary Information section.

In 46 of the captured triatomines (60,5%) the Cyt b fragment was successfully amplified and the source of the bloodmeal was identified. Figure 3 shows the enzymatic digestion patterns obtained that allowed us to identify the source of the bloodmeal of these specimens. Figure 4 and Table I of Supplementary Information summarizes the sources of the bloodmeals identified for each specimen. In the remaining 30 triatomines (39,5%), the Cyt b fragment was not amplified.

All the triatomines captured in the peridomestic area corresponded to the village 12 de Junio sample sites. Insect vectors consisted of 13 specimens of *T. infestans* (7 nymphs V, 3 males and 3 females) and 4 specimens of *T. sordida* (1 male, 2 females and 1 nymph V).

In 15 of the collected specimens, the source of the bloodmeal was identified. In 8 of the collected specimens the exclusive presence of human blood was identified, while the remaining 7 collected specimens had mixed bloodmeal sources consisting of human and chicken blood (Figure 4). A mixed source of blood meal was found for the nymphs, and the adults of *T. infestans*. In regards to the source of the blood meal for *T. sordida*, we did not find the same pattern. A mixed source of bloodmeal was found in the adults of *T. sordida*. However, we did not find a mixed source of bloodmeal for the nymphs of *T. sordida* (Supplementary Table I).

The 59 triatomines captured in the peridomestic areas corresponded to 28 specimens from the Tiberia village, 24 from 12 de Junio village and 7 from Karandillas village. The collected specimens belong to

Figure 2. PCR/RFLP restriction profiles of Cyt b obtained from potential triatomines food sources with the enzymes Hae III and Xho I. MM: Molecular Marker. PC: Positive Control, undigested Cyt b fragment. Rails 1, 3, 5, 7, 9: restriction patterns with Hae III in human, armadillo, chicken, cat and dog samples respectively. Rails 2, 4, 6, 8, 10: restriction patterns obtained with Xho I in human, armadillo, chicken, cat and dog samples respectively.

Figure 3. Identification of triatomines bloodmeal by PCR/RFLP using Hae III and Xho I on Cyt b sequences.

41 specimens of *T. sordida* (4 nymphs IV, 10 nymphs V, 15 males and 12 females), 14 specimens of *T. infestans* (3 nymphs IV, 6 nymphs V, 1 male and 4 females) and 4 specimens of *T. platensis* (4 nymphs V). In 31 of these collected specimens, the source of the bloodmeal was identified (15 of 12 Junio village, 11 of Tiberia village and 5 of Karandillas village) (Supplementary Table I).

Out of the 15 specimens captured in 12 de Junio village, 7 fed exclusively on human blood and 1 specimen fed exclusively on chicken blood. The remaining 7 collected specimens had mixed sources of bloodmeals, with 6 of them being human and chicken blood and 1 with human and dog blood. The specimens captured in the Tiberia village (3 nymphs IV, 1 nymph V, 4 males

Figure 4. Bloodmeal sources from triatomines of different stages captured in the indigenous community 10 Leguas of the Paraguayan Chaco. ♂: males, ♀: females, N: nymphs.

and 3 females) and Karandillas village (5 nymphs V) were fed only with chicken blood (Figure 4). In all of the stages of *T. infestans* and in adults of *T. sordida*, captured in the village 12 de Junio, mixed sources of bloodmeals were identified (Supplementary Table I).

It is important to emphasize that 12 de Junio was the only village in which triatomines were collected inside domiciles and in peridomestic areas and the only locality in which mixed blood meals were detected. In the other two villages, triatomines were only captured in the peridomestic areas and all the specimens from these localities fed exclusively on chicken blood (Supplementary Table I).

A little more than half of the triatomines captured in domestic areas (only in the village 12 de Junio) fed exclusively on human blood (53,3%). While the remaining 46.7% fed on both human and chicken blood.

100% of the specimens captured in Tiberia and Karandillas in which the food sources could be identified were fed exclusively with chicken blood. Importantly, no *T. infestans* were captured in these villages. However, in the village 12 de Junio the scenario was very different. 46.6% of the triatomines fed exclusively on human blood, 40% of human and chicken blood, 6.7% fed on human and dog blood and 6.7% fed exclusively on chicken blood. In the

domestic and peridomestic areas the presence of a higher number of nymphs V was observed, followed by females, males and nymphs IV (Figure 5(b)). No *T. cruzi* DNA was detected in any of the captured triatomines (Supplementary Table I).

Discussion

Triatomines are hematophagous insects that are not known to exhibit exclusivity when selecting a host to extract a blood meal. Many Chagas disease epidemiological factors revolve around feeding preferences and behavior of these insects. Therefore, a proper identification of triatomine blood meal sources allow the recognition of possible *T. cruzi* reservoirs. Moreover, the proper identification of the blood meal source improves our current understanding of the biological cycles of the disease, which in turn, provide important information for the optimization of vector control mechanisms [11].

In this study, the molecular biology assay employed to identify the source of the bloodmeal was the PCR/RFLP assay. In our assay, blood samples from human, dog, cat, chicken and armadillo were used as positive controls. After the amplification of a Cyt b fragment of these samples, two independent digestion reactions on the amplified product were performed with the restriction enzymes Hae III and Xho I. The enzyme Hae III was chosen because it was

Figure 5. Number of triatomines captured in domestic and peridomestic areas of the Paraguayan Chaco: (a) Feeding source (b) Stage and sex of the specimens.

previously used by Chena *et al.*, Maleki-Ravasan *et al.* and Soares *et al.* [11,17,23], to generate differential restriction patterns of the Cyt b amplicons. This development allows for the identification of several vertebrate species. This specific digestion enzyme yields differential patterns for amplicons of the gene of interest from samples that come from armadillo, chicken and cat blood.

However, this enzyme produced the same restriction pattern for amplicons originating in human and dog blood. For this reason, a second enzyme was used to differentiate these 2 species. The restriction enzyme Xho I has a specific restriction site for the Cyt b fragment of human DNA and has no restriction site for other vertebrates [18], thereby allowing to differentiate human and dog DNA.

We have also tested a simultaneous reaction with both enzymes, however the major problems observed were: the restriction patterns were difficult to interpret and the concentration of isolated DNA was insufficient for both reactions (data not shown).

Once the differential restriction patterns for the selected vertebrate species were obtained, the feces of the captured triatomines in the Paraguayan Chaco were analyzed. It was possible to identify the blood-meal sources in 61% of the captured triatomines. For the remaining 29%, it was not possible to amplify the Cyt b segment, this may be due to several reasons, such as: the presence of high levels of naturally occurring PCR inhibitors on the triatomine feces, the amount of vertebrate DNA was insufficient for the PCR reaction or the degradation of the blood source DNA. A similar phenomenon can be noted on reports from other research projects on the identification of triatomines bloodmeal sources [14,15,19], in which similar percentages of detection in feces samples were obtained.

Higher percentages for a single feeding source of the blood meal vs a mixed feeding source of the blood meal was detected in triatomines of the peridomestic area in the village 12 de Junio the percentage of single feeding source vs mixed feeding source was of 53% vs 47%, while on the villages Tiberia and Karandillas,

100% of the triatomines captured were fed exclusively on chicken blood. In domestic areas of 12 de Junio, the same pattern remained between a homogeneous vs a heterogeneous blood meal source (53% vs 47%). Interestingly, the percentage of triatomines that fed exclusively on human blood at domestic and peridomestic areas in the village 12 de Junio was very similar.

Human blood was found on all mixed blood meal cases, identifying human-chicken mixtures in domestic triatomines, and human-chicken and human-dog in peridomestic areas.

In light of the presence of mixed human-chicken blood in triatomines collected in domestic areas as well as human-dog blood in insects collected in poultry houses, a high rate of movement for these insects between the domestic and peridomestic areas can be noted. Recently, morphometric studies of triatomine wings [25,26] have shown the coexistence of domestic insects and wild and/or peridomestic insects, supporting the findings in this research project.

Considering the fact that nymphs do not have wings, their mobility is restricted compared to adults. Furthermore, it should be noted that in 2 nymphs collected in a domestic area of the 12 de Junio village, the presence of human and chicken blood was positively identified. Additionally, in 7 nymphs collected in poultry houses of the peridomestic area of the same village, the presence of human blood was identified. Interestingly, in 4 of these nymph specimens, human blood was the only source for the blood meals. These findings indicate that even nymphs are mobilized between areas, regardless of blood meal availability.

In the Tiberia village, the chickens are found confined in chicken coops that are elevated above ground level. However, in 12 de Junio and Karandillas villages, the chicken coops are not housed in such facilities, and during the day the chickens are free, being able to move around and inside the residences. These conditions could explain the presence of human blood that was found in insects that were captured in the peridomestic area and the chicken blood found on specimens collected in the domestic area. In this case, the

movement of the triatomines takes place in a passive manner, using the chickens as a vehicle in order to move between the domestic and peridomestic areas.

Nevertheless, it is important to recognize that the possibility of mobility of the insects by their own means by flying or walking cannot be dismissed [27]. Studies have shown that triatomines colonies are blood-dependent, meaning that an excessive increase of the insect's population by easy access to food effectively forces adult insects to scatter looking for new food sources [28].

In 96% of the triatomines captured in the village 12 de Junio, the presence of human blood was detected regardless of the area of collection and availability of food. Only one specimen coming from the peridomestic area was shown to have exclusively fed on chicken blood. The high rate of identification of human blood in triatomines captured in the peridomestic area raises the need for further studies that will allow us to confirm whether this observation is due to anthropophilic behavior of these insects or some other extraneous factor. The clinical significance of such follow up studies would lie in the ability to determine whether the transmission of *T. cruzi* from the vector to the host increases if there was a preference for human blood as a food source. In this study, none of the captured triatomines were infected with *T. cruzi*.

There are previous studies about the triatomines preferences of blood meals, however, no conclusive observations have been achieved. In 1977 Rocha e Silva *et al.* [29] observed that in laboratory conditions nymphs of 1st stage of *T. sordida* preferred to feed from blood of birds than of humans. However, Crocco & Catalá [30] observed that nymphs of the 5th stage of the same species preferred guinea pig blood to pigeon blood. Additionally, Jirón & Zeledón [31] concluded that under laboratory conditions nymphs of the 5th stage of *T. infestans*, *T. dimidiata* and *Rhodnius prolixus* did not present food preferences among dog, chicken and opossum blood meal sources.

Lastly, Gurtler *et al.* [32] observed that *T. infestans* preferred to feed from dog blood than of chicken blood, and they stated that this preference can be exploited to reduce domestic insect populations by using topical lotions or insecticide-impregnated collars so as to turn the dogs into baited lethal traps.

Even though chickens cannot be reservoirs of *T. cruzi* and they are not the main food source of the triatomines in the village 12 de Junio, they could be playing an important role in the maintenance of the triatomine infestation in the domestic and peridomestic areas. Furthermore, chicken blood serves as a blood meal source for the vector, allowing the establishment of large populations of these insects. These findings indicate that the triatomines of the peridomestic areas represent an

epidemiological threat for the local population, while posing as a potential source of domestic re-infestation.

Furthermore, Rolón *et al.* [33] reported that sylvatic colonies of *T. infestans* populate the same area in which the present study was performed. The presence of these sylvatic colonies also poses a direct threat of infestation for the nearby domestic areas. For this matter, a recent study shows the similarity of domestic and peridomestic populations of *T. infestans* with wild specimens, indicating a relationship between them [25].

Lastly, the country of Paraguay is experiencing considerable levels of deforestation due to the demand for wood on the illegal market of neighboring Brazilian States. The dispersive behavior of the sylvatic colonies, that is seen as a direct effect of deforestation, can cause the displacement of wild triatomines into domestic and peridomestic areas. The complex relationship between the environment, the insect vector of the disease, and the diverse animal hosts all play an essential role in our understanding and eradication efforts of Chagas disease.

Conclusions

The PCR-RFLP assay, employing 2 digestive enzymes: Hae III and Xho I generates differential restriction patterns of the amplified material. Such distinctive restriction patterns allow for the molecular identification of the blood meal source from human, chicken, dog, cat and armadillo blood origins.

The dynamic dispersion in between the domestic and peridomestic areas represents an epidemiological threat for the inhabitants of the villages that we studied. Colonies of triatomines on peridomestic areas also serve as a clear and present source of re-infestation of the insect vectors in the domestic areas.

Studies regarding the feeding preferences of triatomines are of great importance, especially in countries where the insect vector is endemic, since they allow to establish the degree of dispersion and adaptation of these insects.

Taken together, our research findings contribute to the efforts to accurately assess the epidemiological dangers that the Chagas Disease vectors pose for the diseases' high risk communities.

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