ORIGINAL ARTICLE

doi: 10.5216/rpt.v42i1.23596

USING CAPTIVE SENTINELS TO COLLECT WILD TRIATOMINES IN THE REGION OF MARÍLIA-SP, BRAZIL

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ABSTRACT

In order to collect wild triatomines in the municipality of Marilia, two captive sentinel sites were installed in a remnant riparian zone in the Atlantic Forest comprising 34 hectares located on the Nossa Senhora Aparecida farm. One enclosure was built on the margin and another inside the forest, about 200 meters from the edge, using rabbits as bait to attract wild triatomines. In addition to the caged rabbits, active searches for triatomines were performed in tree holes, bird nests and armadillo burrows by spraying Pirisa 1080-L1E1 aerosol dislodger. The sentinels were maintained for one year and four months. During this period six active searches were performed, but without success in attracting or colonizing wild triatomines. One factor that has probably intervened in the triatomine colonization is the periodic application of insecticides formulated with pyrethroid in farms neighboring the forest to fight *Diaphorina citri* in orange plantations.

KEY WORDS: *Trypanosoma cruzi*; wild triatomines; pyrethroid; captive sentinel; *Diaphorina citri*.

RESUMO

Ensaio experimental para captura de triatomíneos silvestres na região de Marília-SP

Com o objetivo de coletar triatomíneos silvestres na região do município de Marília foram instalados dois cativeiros experimentais em uma mata ciliar remanescente de Mata Atlântica, com 34 hectares de extensão localizada no sítio Nossa Senhora Aparecida. Um cativeiro foi construído às margens e outro no interior da mata a aproximadamente 200 metros da borda, utilizando-se coelhos como atrativos para triatomíneos silvestres. Além dos cativeiros experimentais foram realizadas buscas ativas de triatomíneos em ocos de árvores, ninhos de pássaros, habitáculos de tatu com pulverização do desalojante Pirisa 1080-L1E1. Os cativeiros foram mantidos por um ano e quatro meses e seis

1 Received for publication in: 3/7/2012. Reviewed in: 12/12/2012. Accepted in: 27/2/2013.
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Received for publication in: 3/7/2012. Reviewed in: 12/12/2012. Accepted in: 27/2/2013.

buscas ativas foram realizadas nesse período, porém sem sucesso na colonização de triatomíneos silvestres. Um dos fatores que provavelmente tenham interferido para a não colonização de triatomíneos seja a aplicação periódica de inseticida a base de piretróide nas fazendas vizinhas da mata para combater o *Diaphorina citri* nas plantações de laranja.

DESCRITORES: *Trypanosoma cruzi*; triatomíneos silvestres; cativeiro experimental; *Diaphorina citri.*

INTRODUCTION

The area of natural transmission of American trypanosomiasis by triatomine vectors includes the Neotropical Region from the south of the United States to the Chubut province in Argentina. However, the presence of triatomines is not limited to the American continent (Silveira, 2000).

Due to the immense size of the area considered as endemic in Brazil, which comprises more than three million km^2 (Fernandes et al., 1997) and the social gravity of the disease, in 1975 the health authorities introduced the vector transmission control program at national level, which implemented the application of insecticide with residual action in homes, targeting *Triatoma infestans* (Silveira & Rezende, 1994).

This action to control vector transmission achieved full coverage of the endemic area in 1983 and since then has been maintained on a regular basis (MS, 2005). As a consequence of these control measures, in addition to the restructuring of the agrarian space, modernization of agriculture and consequent rural exodus, there has been a significant change in the epidemiological picture of Chagas disease in the country (Silva, 1986; Wanderley, 1991).

In São Paulo State, Chagas disease, with *T. infestans* as the main vector, was a considerable public health problem, being found in virtually all municipalities in the western plateau until the 1970s, excluding part of the Paulista Peripheral Depression zone (Silva, 1986).

With the fight against *T. infestans*, the secondary native species *Triatoma sordida* and *Panstrongylus megistus* became the targets of triatomine surveillance, because they were harder to control than the intradomiciliary vector (Wanderley, 1991).

In 1985, *T. sordida* represented 77.4% and *P. megistus* 15.2% of the insects captured around homes, and the infection rate was 0.8% and 7.9% respectively. In addition to these two species, *Rhodnius neglectus*, *Triatoma arthurneivai*, *Panstrongylus diasi, Panstrongylus geniculatus, Rhodnius domesticus and Triatoma tibiamaculata* were also found (Wanderley, 1991).

According to Silva et al. (2003), in the State of São Paulo during the 1990s *T. sordida* was the most frequently captured species in the regions of Ribeirão Preto, São Jose do Rio Preto and Araçatuba cities; the second species was *P. megistus*, captured in forested areas of the regions of Sorocaba and Campinas,

municipalities bordering the region of Ribeirão Preto with the Minas Gerais State and the coastal region.

Recent studies conducted from 1990 to 2006 by Silva et al. (2008) in 645 municipalities of São Paulo State showed an increase in infestation by triatomines from 1.2% in 1990 to 2.9% in 2006. However, the studies of Silva et al. (2003, 2008) did not find high levels of infestation in the city of Marília, a region that was part of the endemic area in the State, with large numbers of patients affected by Chagas disease, where a Y strain (Silva & Nussenzweig, 1953) was isolated from a patient. More recently, the FAMEMA strain (Martins, 2003) was isolated by artificial xenodiagnosis from a chronic chagasic patient from that same region.

Thus, the search and collection of secondary native triatomine species became necessary, to ascertain the current nature of triatomine fauna in the region of Marilia city, considered an endemic area of Chagas disease before effective vector control.

MATERIALS AND METHODS

Local allocation of traps for wild triatomines in the region of Marilia / SP

The installation of experimental captive sentinels occurred in a riparian remnant of the Atlantic Forest (Figure 1), a 34 hectare area located on the Nossa Senhora Aparecida farm, County Road JLM, nº 430, Sol Nascente district (GPS co-ordinates 22°00'24.80 "S and 49°50'14.40"), belonging to the Julio Mesquita municipality, 51 km from Marilia city. This project was approved by the Ethical Committee for Research at FAMEMA under nº 508/09.

Experimental captive sentinels

Two experimental enclosures were built, one located at the edge of the forest (Figure 2) and another inside, approximately 200 meters from the edge (Figure 3). The enclosure at the forest edge measured 3.0 m x 3.0 m x 3.0 m and was constructed following the technique of Forattini et al. (1973) and adjusted in accordance with Ceretti (2002), using wire mesh with 14 BWG and 2.1 / 2" of mesh opening. The enclosure, covered with asbestos cement tiles, was placed 2 meters above ground and trellis wires were placed on leaves and coconut palms. Three standardized metal cages were placed inside, where we kept three rabbits as a food source for wild triatomines. Piles of bricks and tiles were placed beside the rabbit cages.

The rabbits used in this research were provided by the Central Animal Facility of UNESP, Botucatu and kept in the experimental cages for a period of one year and four months.

The enclosure inside the reserve had smaller dimensions, measuring 1.50 m x 1.50 m x 1.50 m, housed one rabbit, was built out of wood and wire and covered with ceramic tiles.

Active search

Active searches for bugs were performed in possible wild animal shelters, such as armadillo burrows and nests of opossums, rodents, birds and also in shells, in hollow tree trunks and palm trees. Searches were performed by spraying these sites with Pirisa 1080-L1E1, a dislodging insecticide. Four traps following the Obara et al. (2002) model and adapted according to Noireau et al. (2002) were also placed in branches of trees (Figure 4) for 24 hours, using "Swiss" mice aged 30 days as bait to attract wild triatomines.

Search for bugs in experimental enclosures

The search for wild triatomines occurred every two months. Rabbit cages were removed from the interior of the enclosure and piles of bricks, tiles and palm leaves searched for triatomines.

RESULTS

The experimental sentinel sites (Figure 1) were maintained for one year and four months, but during this period there was no success in colonizing them with wild triatomines.

During this period, six active searches were carried out in the woods with the installation of the Obara traps and Pirisa 1080-L1E1 spraying, but these actions also proved negative.

Figure 1. Location where the experimental sentinel sites were built. The arrow shows the enclosure located at the edge of the forest.

Figure 2. Sentinel enclosure located at the edge of the forest.

Figure 3. Sentinel enclosure inside the forest.

Figure 4. Obara traps adapted according to Noireau.

DISCUSSION

The region of Nossa Senhora Aparecida farm, the area where the experimental sentinel sites were installed, belongs to a remnant of the semi deciduous forest, Aguapeí Basin, which includess 1-2% of the remaining vegetation of the entire State (Kronka et al., 2005). There is a predominance of livestock and citrus agriculture.

The difficulty in attracting and finding sylvatic triatomines in the locations where the experimental sentinels were installed was partly due to the changes in the natural habitat of these vectors, because due to the formation of forest fragments, there were changes in the microclimate, the heterogeneity and diversity of species, that could lead to some being reduced or becoming locally extinct (Datilo et al., 2011).

The results obtained from this study do not reflect the reality found in other municipalities belonging to SR11 (Serviço Regional 11, Marília,- São Paulo State), because during the year 2011 there was an increase of 78.8% in the capture of triatomines when compared to 2010, corroborating the studies of Silva et al. (2008), who also found an increase between 1990 and 2006. Although there has been an increase in the number of insects captured, these triatomines were found to be negative for *T. cruzi*. Research by Silva et al. (2011a) in São Paulo State showed that about 1% of *T. sordida* and 9% of *P. megistus* were positive for *T. cruzi*.

In the area covered by SR11, 461 triatomines were collected during the year 2011, comprising of 90.67% *T. sordida*, 6.07% *R. neglectus* and 3.25% *P. megistus*. When compared with the catches of the year 2010, when 258 insects, comprising of 94.18% *T. sordida*, 4.65% *R. neglectus* and 1.16% *P. megistus* were collected, the 2011 catches revealed a decrease in the prevalence of *T. sordida* and an increase in *R. neglectus* and *P. megistus* populations. This increase in the catches of *P. megistus* in the region serves as a warning, because the species is currently the most important vector of *T. cruzi* in São Paulo State, with high rates of natural infection (Silva et al., 2011b).

Documents left by reliable organizations in the residences of the farm showed that the last capture of *T. infestans* occurred in 1980 and although this triatomine species has been eradicated in Brazil, wild triatomines threaten to reintroduce Chagas disease in São Paulo State, showing the need to keep the population engaged in surveillance programs, while stressing the possibility of changes in susceptibility to deltamethrin and providing guidance if there is a need for control actions.

Deforestation for agriculture and the predominant citrus culture on farms surrounding the state have probably influenced the capture as well as the colonization of sylvatic triatomines in the experimental sentinel sites, because the orange plantations harbor a large number of pests (Yamamoto et al., 2001), such as *Diaphorina citri*, a Hemiptera, Psyllidae, vector of "greening" or "huanglongbing" (Silva et al., 2009), with a wide geographic distribution, found in citrus crops in the State of São Paulo (Paiva, 2009). This insect occurs mainly in new shoots (Yamamoto et al., 2001), such as those of the crops from the farm next to the site. To prevent their colonization and prevent them from spreading to other farms, the citrus growers perform aerial sprayings with deltamethrin and thimetoxam insecticides (Paiva, 2009) with a 90-day periodicity (Sanches et al., 2009).

Deltamethrin is a pyrethroid, and since 1983 the National Program for Control of Chagas Disease established the use of this group of insecticides in

the fight against triatomines (Obara et al., 2011). Although research has shown resistance in several species of triatomine originating from Argentina, Venezuela and Bolivia (Vassena et al., 2000; Audino et al. 2004), recent studies performed by Sonoda et al. (2009) with *T. infestans* and Obara et al. (2011) with *T. sordida* showed that these species coming from Brazil are susceptible to this insecticide.

Although chemical control of the environment is not so effective due to climate changes that hinder its residual effect (Dias, 2001), the periodic fumigations made by the farmers in current citrus plantations possibly interfered with the colonization of wild triatomines.

Reinforcing the above hypothesis, it was observed through recent data provided by SR11 that the triatomine density is low in the cities of Marilia, Julio Mesquita and Guaimbê, and in the last two years there was only one notification in Marilia and none in the Julio Mesquita and Guaimbê municipalities, bordering the forest reserve where the sentinels were installed.

According to Dias (2000), the new spaces formed by human action are unfavorable to triatomine fauna and the natural reservoirs of *T. cruzi*, because they are deforested areas with pasture and monoculture plantations introduced that have extensive and intensive use of pesticides.

Although searches for animal reservoirs were not performed in this study, we can infer a trend reducing wild trypanosomiasis in this micro-region of São Paulo State.

ACKNOWLEDGMENTS

To Dra. Maria Teresa Marcoris Andrighetti and Priscila Fernanda de Oliveira Coneglian of SUCEN-SR11 for support given to this research. Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). To Global Science Editing Ltd. for English language editing services.

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