

Eco-epidemiology of Chagas disease in northeastern Brazil: *Triatoma brasiliensis*, *T. pseudomaculata* and *Rhodnius nasutus* in the sylvatic, peridomestic and domestic environments

Otília Sarquis · Filipe Anibal Carvalho-Costa ·
Helena Keiko Toma · Ingebourg Georg ·
Marcelo R. Burgoa · Marli Maria Lima

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Abstract An entomological survey was carried out in four rural localities situated in the state of Ceará, assessing Chagas disease seroprevalence in man, focusing on the presence of vectors in natural foci contiguous to the domestic and peridomestic environments. Fifty-three *Triatoma brasiliensis*,

nine *T. pseudomaculata* and 71 *Rhodnius nasutus* were collected in their natural habitats as far as 10 m from the houses, and 663, 59 and 8 respectively were captured in peridomestic artificial structures, adjacent to the houses, including henhouses, pigpens, corrals, perches and piles of bricks, tiles and wood. Within the households, 37 *T. brasiliensis*, one specimen of *T. pseudomaculata* and one of *R. nasutus* were captured. Overall, *Trypanosoma cruzi* infection rates were 2.3% for *T. brasiliensis* and 11.3% for *R. nasutus*. Despite that the seroprevalence survey in man did not reveal positive results using two serological techniques, natural triatomine habitats are juxtaposed to man-made artificial ecotopes, resulting in overlapping habitats. The contiguity between natural ecotopes and human dwellings increases the interaction between vectors and humans, challenging continuous surveillance and vector control efforts.

O. Sarquis (✉) · M. M. Lima
Laboratório de Ecoepidemiologia da Doença de Chagas,
Instituto Oswaldo Cruz, Fiocruz,
Av. Brasil 4365,
Rio de Janeiro, RJ, Brazil
e-mail: otiliasarquis@ioc.fiocruz.br

F. A. Carvalho-Costa
Laboratório de Sistemática Bioquímica/Curso de Pós-graduação
em Medicina Tropical, Instituto Oswaldo Cruz, Fiocruz,
Av. Brasil 4365,
Rio de Janeiro, RJ, Brazil

I. Georg
Instituto de Pesquisa Clínica Evandro Chagas,
Instituto Oswaldo Cruz, Fiocruz,
Av. Brasil 4365,
Rio de Janeiro, RJ, Brazil

M. R. Burgoa
Secretaria Municipal de Saúde,
Morada Nova, Ceará, Brazil

H. K. Toma
Laboratório de Diagnóstico Molecular e Hematologia
Universidade Federal do Rio de Janeiro, Cidade Universitária,
Rio de Janeiro, RJ CEP 21941-901, Brazil

H. K. Toma
Laboratório Interdisciplinar de Pesquisas Médicas - IOC Instituto
Oswaldo Cruz, Fiocruz,
Rio de Janeiro, RJ, Brazil

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Introduction

Both vector-borne and transfusion transmission of Chagas disease's etiological agent, the protozoan *Trypanosoma cruzi*, have been successfully interrupted in Brazil. Control measures are based on insecticide chemical treatment of dwellings in endemic areas and rigorous blood bank control (Dias 2009). Nevertheless, Chagas disease remains a public health concern in many geographic areas, at least in part due to the potential invasion and re-colonization of dwellings by native triatomines after the insecticidal eradication of domestic species (Guhl et al. 2009). In this context, in many rural

areas the peridomestic environment, including corrals, pigpens and chicken coops, might represent a link between the sylvatic and the domestic cycle of Chagas disease transmission, once native triatomines are able to establish large peridomestic colonies (Sarquis et al. 2006). Domestic animals such as goats, chickens, pigs and dogs provide an excellent food source for triatomine colonies in the peridomestic environment. This eco-epidemiological scenario pertains to northeastern Brazil, the species *Triatoma brasiliensis* and *T. pseudomaculata* presenting potential epidemiological importance for Chagas disease transmission in this area (Oliveira Filho et al. 2000; Freitas et al. 2004; Carbajal-de-la Fuente et al. 2007, 2008). Both species have been captured in peridomestic environments and natural ecotopes in xerophytic semiarid ecosystems, often constituting large colonies with high rates of *T. cruzi* infection (Sarquis et al. 2004). *Rhodnius nasutus*, a native triatomine of northeastern Brazil which naturally inhabits Carnauba palm trees (*Copernicia prunifera*), has also been captured in the peridomestic and domestic environments (Sarquis et al. 2006; Dias et al. 2011). Furthermore, *R. nasutus* colonizes other wild ecotopes, such as the *Licania rigida* tree (Lima and Sarquis 2008), suggesting that this species presents some ecological eclecticism and may be readapting from its traditional habitat due to anthropic transformations.

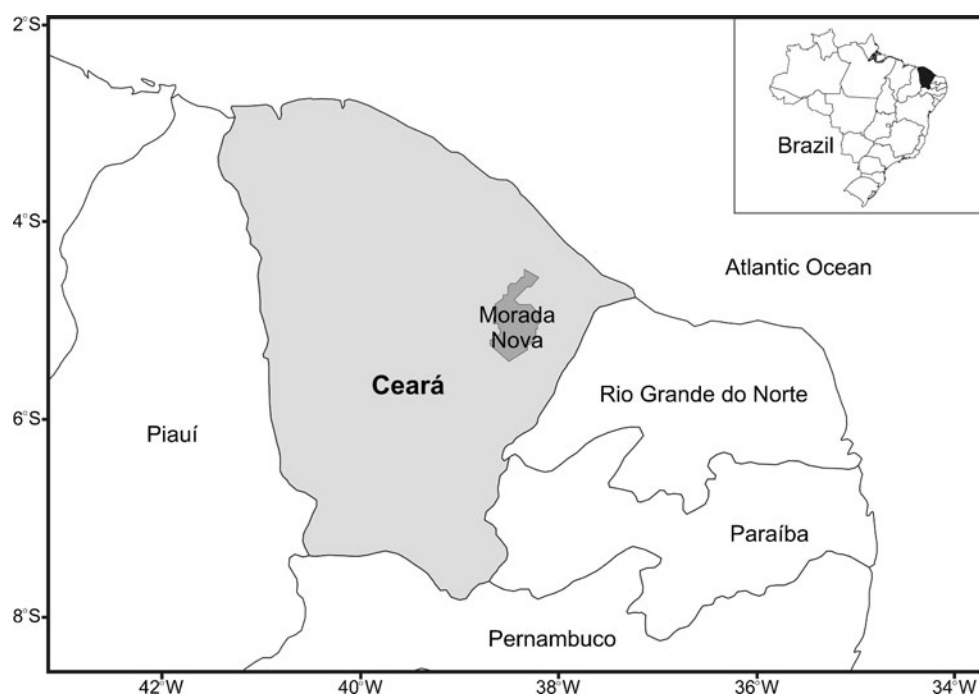
In this study, we carried out an entomological survey and assessed seroprevalence of Chagas disease in inhabitants of four rural localities of the Morada Nova municipality, situated in the State of Ceará, focusing on the presence of Chagas disease vectors in natural foci contiguous to the domestic and peridomestic environments.

Material and methods

The study was conducted in the localities of Lagoa do Frade (population= 131 residents), Alto do Camaleão (59 residents), Fazenda Nova (46 residents) and Cacodé (28 residents), all situated in the rural area of the municipality of Morada Nova (5° 06' 24" S; 38° 22' 21" W), state of Ceará, northeastern Brazil (Fig. 1). The average annual temperature ranges from 26°C to 28°C, and rainfall is approximately 742.5 mm per year with the rainy season from February to April. The Caatinga (scrublands), a characteristic vegetation of the semi-arid Brazilian northeast region, predominates in the landscape. Subsistence agriculture is the principal activity of the inhabitants, besides livestock, mainly goats, sheep and chickens, which all move free in the peridomestic environment.

The survey lasted a period of 5 consecutive days in both June and September 2009. Detailed domestic and peridomestic inspections in artificial and natural potential triatomine habitats were performed in every inhabited dwelling by two technicians, from 8:00 a.m. to 5:00 p.m., and no insect dislodging substances were used. Eighty-eight residences were assessed. In the peridomiciles, 226 artificial structures such as henhouses ($n=45$), pigpens ($n=43$), corrals ($n=37$), perches ($n=16$) and 77 others (piles of bricks, tiles and wood) were investigated. Additionally, we examined 178 natural habitats of triatomines such as rocks ($n=15$), trees of the species *Aspidosperma pyrifolium* (Pereiro), *Auxemma onocaly* (Pau-branco) and 135 *C. prunifera* palm trees. These natural ecotopes were located within 10 m of the houses. The *C. prunifera* palm trees

Fig. 1 Map of the Morada Nova municipality, Ceará, Brazil



were set with adhesive live-baited traps with a 7-day-old chick (Noireau et al. 2002). The traps were installed in the crown of the palm trees at 6:00 p.m. and recovered at 7:00 a.m. During the searches, temperatures of the houses and of the peridomestic appendices were registered with a data-loggers thermometer (Q-240, Quimis®, Diadema, Brazil). The mean temperature was 34°C inside the houses and 36°C in the peridomestic structures. All triatomines were initially stored in labeled plastic containers and forwarded to the laboratory.

In order to evaluate the natural *T. cruzi* infection rates, all specimens that arrived alive in the laboratory in Rio de Janeiro were examined. Rectal contents were obtained by abdominal compression and diluted in saline, and the presence of epimastigote and trypomastigote forms was assessed through light microscopy (400×).

To screen the prevalence of *T. cruzi* infection in man, finger-prick blood samples from 91 residents in Lagoa do Frade, 35 in Alto do Camaleão, 42 in Fazenda Nova and 7 in Cacodé who agreed to participate in the serological survey were collected on Whatman 1.5-mm filter paper (Clifton, NJ) for an initial trial assessment. After air-drying at room temperature, the samples were sealed in clean plastic wrap and sent to the laboratory, where they were kept dry in the refrigerator until analysis. In order to investigate the presence of antibodies (IgG) anti-*T. cruzi*, the blood samples were submitted to the indirect immunofluorescence assay (IFI) (Chagas-Biomanguinhos®, Rio de Janeiro, Brazil) and ELISA (Chagas ELISA III (Ebram Lab, São Paulo, Brazil)).

Results

Triatomines were captured in all habitats (Table 1). *T. brasiliensis* (53 specimens) was captured in rocks contiguous to a chicken coop situated 2 m from a residence. Seven specimens and eggs of *T. pseudomaculata* were collected in Pau-branco (*A. onocaly*) trees surrounding chicken coops, 2 were collected in Pereiro (*A. pyrifolium*) and 74 *R. nasutus* were obtained in *C. prunifera* palm trees within 10 m of the residences, in the backyards. In addition, a total of 663 *T. brasiliensis*, 59 *T. pseudomaculata* and 8 *R. nasutus* were captured in artificial peridomestic structures. In the houses, a total of 37 *T. brasiliensis* specimens (including 17 nymphs), 1 nymph of *T. pseudomaculata* and 1 *R. nasutus* adult were captured.

A total of 707 insects were examined for assessment of *T. cruzi*-infection. The overall *T. cruzi* infection rate in *T. brasiliensis* and *R. nasutus* was 2.8% and 11.6% respectively (Table 1). None of 57 *T. pseudomaculata* was infected. The seroprevalence survey of Chagas disease in man did not detect positive subjects through both serological techniques.

Table 1 Distribution and *T. cruzi* infection rates of *Triatoma brasiliensis*, *T. pseudomaculata* and *Rhodnius nasutus* in the peridomestic space and inside houses in Morada Nova, state of Ceará, Brazil, 2009

| | Triatoma brasiliensis | | | | | | Triatoma pseudomaculata | | | | | | Rhodnius nasutus | | | |
|------------------|-----------------------|--------------|--------------|--------------|--------------|--------------|-------------------------|--------------|--------------|--------------|--------------|--------------|------------------|--------------|--------------|--------------|
| | Nymphs (n) | | | Adults (n) | | | Nymphs (n) | | | Adults (n) | | | Nymphs (n) | | Adults (n) | |
| | Examined (n) | Infected (%) | Examined (n) | Infected (%) | Examined (n) | Infected (%) | Examined (n) | Infected (%) | Examined (n) | Infected (%) | Examined (n) | Infected (%) | Examined (n) | Infected (%) | Examined (n) | Infected (%) |
| Intradomiciles | 17 | — | 37 | — | 20 | — | 1 | — | 1 | — | — | — | — | — | 1 | — |
| Peridomiciles | | | | | | | | | | | | | | | | |
| Artificial | | | | | | | | | | | | | | | | |
| Henhouse | 51 | 4.1 | 49 | — | 13 | — | — | — | 4 | — | — | — | 6 | — | 1 | — |
| Pigpen | 42 | 5.7 | 35 | — | 5 | — | 1 | — | 1 | — | — | — | — | — | — | — |
| Corrals | 146 | 2.1 | 142 | — | 50 | — | — | — | — | — | — | — | — | — | — | — |
| Perches | — | — | — | — | — | — | 24 | — | 49 | — | 29 | — | 1 | — | 1 | — |
| Pile of tiles | 103 | 6.7 | 89 | — | 47 | — | — | — | — | — | — | — | — | — | — | — |
| Pile of wood | 172 | 1.34 | 149 | — | 34 | — | 1 | — | — | — | — | — | — | — | — | — |
| Natural ecotopes | | | | | | | | | | | | | | | | |
| Trees | — | — | — | — | — | — | 7 | — | 2 | — | 2 | — | — | — | — | — |
| Palm trees | — | — | — | — | — | — | — | — | — | — | — | — | 66 | — | 56 | 16.1 |
| Rocks | 31 | — | 48 | — | 22 | — | — | — | — | — | — | — | — | — | — | — |

Discussion

The eco-epidemiological scenario of Chagas disease in northeastern Brazil presents a fundamental characteristic, the risk of re-domiciliation of autochthonous triatomines following insecticide treatment (Alencar 1987; Silveira et al. 2001). This points to the necessity of continuous entomological surveillance in vast areas of the northeastern Brazilian outback (Silveira et al. 2001). Regarding Chagas disease vectors, their environments and habitats have been traditionally compartmentalized by authorities involved in disease control into three segments: i) sylvatic, including rock piles and trees, ii) peridomiciles, constituted by artificial structures like corrals and chicken coops and iii) intradomiciles, represented by human residences. In this scenario, it has been proposed that the peridomestic environment could be the key to the re-colonization of dwellings from sylvatic foci represented by natural habitats of triatomines (Borges et al. 2005). In the present study we demonstrate that natural triatomine habitats can actually be juxtaposed with man-made artificial ecotopes constituting a single environment in which *T. brasiliensis* and *T. pseudomaculata* benefit from a good food supply, provided by domestic animals, mainly chickens, and still living in their natural substrates. Furthermore, in northeastern Brazil, *R. nasutus* inhabits palm trees in close proximity to the residences. An adult specimen was captured inside a house, exemplifying the potential of this species to invade dwellings, probably attracted by light. In the studied region, *C. prunifera* palm trees, the natural habitat of *R. nasutus* and frequently situated in close proximity to the residences, represent an economic resource to the dwellers, providing palm wax (Brazilian wax), sold for utilization in the food and cosmetic industries. In addition, the lumber from the trunk of this palm tree is commonly used to construct houses, fences and animal shelters as are the leaves for local handicraft. Despite the evident tropism of the genus *Rhodnius* to palm trees (Diotaiuti et al. 1984; Romaña et al. 1999; Teixeira et al. 2001; Abad-Franch et al. 2005; Lima and Sarquis 2008), bugs of this genus have been captured in peridomestic habitats in the northeastern Brazil. *R. neglectus* (as well as *T. pseudomaculata*) has been captured in bird nests found in the cactus *Cereus jamacaru* (Emperaire and Romaña 2006). Although we have not found these species in this environment, this cactus is widely spread in peridomiciles of rural areas of Morada Nova.

Regarding the seroprevalence survey, we did not detect any positive reaction with either IIF or ELISA. Previous analyses in the Jaguaribe River valley estimated a seroprevalence rate of 3.1% in residents, not one under the age of 10 (Borges-Pereira et al. 2008). Despite the intense triatomine infestation of the peridomestic environments as

well as the presence of adult insects inside the houses, not one intradomiciliar colony was encountered. Improvements in the quality of the houses and periodical insecticide chemical treatment provided in the studied area are apparent for successful interruption of transmission. As demonstrated by Moncayo and Silveira (2009), the overlapping between natural ecotopes and human habitations potentially increases the possibility of interaction between the vectors and the inhabitants, increasing the risk of transmission of *T. cruzi*. Therefore, the results indicate that the region needs continuous entomological surveillance (Silveira and Dias 2011) and systematic vector control in order to prevent Chagas disease transmission in the investigated localities.

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