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Short communication

A novel association between *Rhodnius neglectus* and the *Livistona australis* palm tree in an urban center foreshadowing the risk of Chagas disease transmission by vectorial invasions in Monte Alto City, São Paulo, Brazil

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ABSTRACT

After several public notifications of domiciliary invasions, palm trees were investigated in downtown Monte Alto City, São Paulo State, Brazil, in proximity to the city hall building, the main church, condominiums and marketing establishments. One hundred seventy four palm trees of 10 species were investigated, in which 72 specimens of *Rhodnius neglectus*, a potential Chagas disease vector, were captured via manual methods. All insects were collected from dead leaves, organic debris and bird nests in the only three *Livistona australis* palm trees in the central park square. This was the first record of *R. neglectus* colonizing this palm species. Although no *Trypanosoma cruzi* was found by abdominal compression followed by light microscopy, the poor nutritional status of the bugs hampered the examination of gut contents for parasite detection. Furthermore, the central crowns of the trees, which shelter bats (Chiroptera: Mammalia), could not be carefully searched for insects due to difficult access. This new finding highlights the sudden alteration in insect behavior, probably as a result of man's interference. This report aims to warn those involved in the health system about this new threat, justifying detailed research of the area to evaluate the magnitude of this emerging public health issue.

Chagas disease afflicts millions of people throughout Latin America, yet no vaccine has been developed. This disease is caused by a protozoan parasite, *Trypanosoma cruzi*, transmitted to man mainly by infected blood-sucking bugs, the triatomines. It is estimated that between seven to eight million people carry Chagas disease, placing this illness among the most serious parasitic disease in the southern hemisphere (WHO, 2010). The epidemiological scenario for this disease is continuously changing, and recently, Abad-Franch et al. (2013) questioned the certification of the absence of Chagas disease transmission by native vectors in Brazil. In fact, in Ceará State, a domestic transmission cycle was disclosed as 20% of all dogs in one periurban locality were *T. cruzi* positive (Lima et al., 2012).

The establishment of endemic Chagas disease requires man/triatomine cohabitation in homes (Schofield and Galvão, 2009). Some species of *Rhodnius* adapt to domiciles (e.g. *Rhodnius prolixius*), whereas the majority are strictly sylvatic. *Rhodnius neglectus* may be in between these adaptation statuses, because colonies of this species have already been reported in domiciles of rural areas (Abad-Franch et al., 2009). However, the consensus is that even a sylvatic species represents a threat, as a population portion of these insects will naturally end up invading homes or landing on food, usually attracted by artificial lights (Abad-Franch et al., 2009; Gurgel-Gonçalves and Cuba, 2007). There is one hypothesis to explain the high indexes (in some spots over 10%) of human *T. cruzi* serological prevalence in the Amazon basin, as people may become infected by biting or accidentally, through infected food (e.g. açaí or sugar cane juice) contaminated by *T. cruzi* infected triatomines (Coura & Borges-Pereira, 2012), which has been responsible for outbreaks in some spots other than the Amazon (e.g. Santa Catarina State; Steindel et al., 2008).

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In February 2012, the municipal health surveillance office of Monte Alto City, São Paulo State, Brazil, was notified by the local population of dwellings invaded at night by insects, supposed Chagas disease vectors. These notifications came from the urban center, more specifically from the 14th floor of a building located in front of a small wooded park (~11,500 m^2) in the central square of the city. Therefore, a task force involving (i) the city health surveillance service, (ii) the city hall and (iii) the local superintendence of endemic diseases (SUCEN) made it possible to carry out an investigation of the palms in the central square.

Monte Alto city (21° 10′ 34″ S, 48° 33′ W) is located 374 km from the capital of the state of São Paulo and has 46,642 inhabitants within an area of 347 km^2 (IBGE, 2012). The annual rainfall rate is 1441 mm (Agritempo, 2012) and the annual average temperature is 21.5 °C (Prefeitura Municipal de Monte Alto, 2012). There have not been any triatomine reports in human dwellings since 2003 (Wanderley et al., 2007). Therefore, since the local population as well as vector and medical surveillance systems are no longer used to dealing with Chagas disease and its vectors, the initial search was performed superficially, characterized by an extreme care to avoid any damage to the palm trees (e.g. pulling off or tearing live leaves). Therefore, this inspection was restricted to dead palm leaves, bird nests and vegetation residue. Unfortunately, it was impossible to carefully investigate the crowns of the palms due to difficult access. As a result, 174 palm trees of 10 species were investigated: *Caryota urens*, *Coccothrinax barbadensis*, *Dypsis lutescens*, *Dypsis madagascariensis*, *Livistona australis*, *Livistona chinensis*, *Phoenix roebelenii*, *Syagrus romanzoffiana*, *Roystonea oleracea* and *Roystonea regia*. The searching labor took three working days (a total of ~24 h). Five men, shared the task: one man climbed a mechanized mobile ladder to remove dead leaves, bird nests and vegetation debris while the others searched for bugs in everything that fell to the ground.

Of all the palms trees present in the square, only the three *L. australis* (Fig. 1) were positive for triatomines with this methodology, providing the 72 collected bugs (25 males, 22 females, 13 fourth stage and 12 fifth stage nymphs), all of which were identified as *R. neglectus*, according to Lent and Wygodzinsky (1979). It was impossible to determine how many bugs came from each tree because leaves and organic debris got mixed after falling down. However, live insects were surely seen in all of these three trees during the inspection. The distances between the infested palms and the invaded building were measured, as well as those from the palms to the closest residual forests, which shall be investigated regarding the presence of triatomines (Figs. 2 and 3).

An important feature of *L. australis* is that its leaves remain attached to the trunk for a long time, even after dying and drying, which favors the maintenance of bird nests and organic debris. This was the first record of *R. neglectus* associated with *L. australis* palms. Hitherto, *R. neglectus* had been only found in palms of the genera *Mauritia* spp. and *Acrocomia* spp. (Abad-Franch et al., 2009; Dias, 2000; Gurgel-Gonçalves and Cuba, 2007; Batista and Gurgel-Gonçalves, 2009). In Ceará State, Lima et al. (2008) established for the first time an unusual association between *R. nasutus* and the *Licania rigida* (Oiticica) tree, a large leafy tree of the Chrysobalanaceae family. Valenca-Barbosa et al., in press reported also for the first time *Triatoma brasiliensis* colonizing cacti. *R. nasutus* usually inhabits palm trees, specially *Copernicia prunifera*, whereas *T. brasiliensis* had been considered exclusive for rocky outcrops. Almeida et al. (2009) claimed that this behavior alterations might be a result of man's environmental interference and has been increasingly evident for many triatomine species. This might be a synanthropization process, such as *T. sherlocki*, a species previously considered exclusively sylvatic, however recently detected colonizing homes in a quarry mining community. This event might be also applied to the novel occurrence of *R. neglectus* in *L. australis* in a very urbanized area.

Fig. 1. *Livistona australis* specimens from which *R. neglectus* specimens were collected.

Professionals from SUCEN, Ribeirão Preto (SP) staff, examined the insects by abdominal compression, followed by light microscopy in order to verify the natural infection by *T. cruzi*, but all specimens proved negative for the protozoan. However, most of the insects were with poor gut contents, which compromises a reliable parasite detection. Furthermore, molecular methods are required to assure the natural infection, because some insects may present a low parasitic burden (Ramsey et al., 2012). In addition, bugs collected might have been negative because they were all encountered in proximity to bird nests, birds being refractory to *T. cruzi* infection. However, bats (Chiroptera: Mammalia) inhabit the crown of the trees (A. Vedoveli, municipal department of epidemiological surveillance; personal communication, September 10, 2013), and opossums (Mammalia: Didelphimorphia), the most common *T. cruzi* synanthropic reservoir, are naturally abundant in urban areas, especially parks.

An inspection in the crown of the trees is still required, and we suspect that only a portion of the triatomine population was caught, as those supposed to be in the central crown of the trees evaded this capture effort. We consider the possibility that the crowns of other palm tree species are also infested. To answer this question, live-bait adhesive traps adapted for triatomine capture (for details, see: Noireau et al., 1999; Abad-Franch et al., 2000) will be applied for all palm trees in the area. Lima et al. (2008) collected an average of 3.9 triatomines per infested palm with this kind of trap in Ceará State.

Some Monte Alto neighboring cities (e.g. Araçatuba e Birigui) have already presented records of *R. neglectus* forming domiciliary colonies. Of special interest, a *R. neglectus* colony was discovered on the 10th floor of an apartment building in Araçatuba, where 20 first and second stage nymphs were captured (the precise date was

Fig. 2. Aerial picture of Monte Alto city taken by Google Earth, accessed on October 8, 2012. Distances from the infested palms 1 and 2 (PL1 and PL2) and the building invaded (B) by triatomines: PL1 – B: 83.70 m; PL2 – B: 50.94 m.

Fig. 3. Aerial picture of Monte Alto city taken by Google Earth, accessed on October 8, 2012. Distances from palms (PL) and the closest residual forests (F1, F2 and F3): PL 1 – F1: 429.00 m; PL1 – F 2: 464.00 m; PL1 – F 3: 1146.00 m. For PL1 and PL2 definition, go to Figure 2.

not available; Rodrigues et al., 2009). Given that the epidemiological scenario is not static, the fact that *R. neglectus* specimens were present in an urban center from Monte Alto city is, per se, a reason for concern (Costa et al., 2002; López-Cárdenas et al., 2005; Rodriguero and Gorla, 2004). Micro-foci of Chagas disease have been attributed to either residential triatomine invasions or food contamination, incriminating several species of native triatomines, which may result in sporadic transmissions or even outbreaks (Abad-Franch et al., 2013).

As stressed above, the lack of natural infection for this capture technique does not mean there are no infected bugs in this area. The last national serological survey for Chagas disease, performed from 2001 to 2008 (Ostermayer et al., 2011), declared that there was no acute Chagasic case in children under five years old (V.M. Costa, Brazilian Department of Epidemiological Surveillance; personal communication, September 09, 2013) in the entire São Paulo State. However an element of the epidemiological channel (the vector in this case) arrives in a non-endemic place, an unprepared population is vulnerable at several levels: (i) to recognize the vector, (ii) to diagnose acute cases of the disease, (iii) to apply basic control measures and (iv) to prevent the dissemination of this new hazard. A future project will involve vector search/surveillance, health education and collection as well as handling of wild mammals with the participation of specialized staff (mammalogists, medical veterinarians and doctors). Therefore, this finding warrants multidisciplinary research efforts in this area to evaluate this potential emerging public health issue.

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