

## First report of *Panstrongylus megistus* sylvatic focus in municipality of Bambuí, state of Minas Gerais, Brazil

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*In 1943, the Center for the Study and Prophylaxis of Chagas Disease, Oswaldo Cruz Foundation, state of Minas Gerais (MG), was created in the municipality of Bambuí to carry out studies related to Chagas disease in the mid-western region of MG. Since that time, several investigations have been conducted to determine the natural habitat of triatomines, but Panstrongylus megistus colonies have never been found in this region. This paper records the first finding of a P. megistus sylvatic colony in 69 years of research. The characteristics of this ecotope and its implications for the epidemiology of Chagas disease are discussed.*

Key words: *Panstrongylus megistus* - sylvatic ecotope - Bambuí - Chagas disease

The municipality of Bambuí is located in the mid-western region of the state of Minas Gerais (MG), 270 km from Belo Horizonte. This region has been the focus of many studies related to Chagas disease. After Chagas was first described (Chagas 1909), the next reports of the disease occurred between 1930-1941, with 25 acute cases described in four MG municipalities; 20 cases were in Bambuí. At that time, *Panstrongylus megistus* was the predominant vector, present in 75% of home infestations, followed by *Triatoma infestans*, at 25% (Martins et al. 1942). In 1943, the occurrence of numerous cases of the disease prompted the founding of the Center for the Study and Prophylaxis of Chagas Disease (CEPMCh), Oswaldo Cruz Foundation/MG, under the management of Dr Emmanuel Dias, who performed several studies on the epidemiology, diagnosis, clinical and prophylaxis of Chagas disease.

When CEPMCh was founded, *T. infestans* was already dominant in the households of the region, overwhelming the native *P. megistus*. Having observed the importance of the insect vector in the emergence of new cases of the disease, Dr Emmanuel Dias worked to develop methods for triatomine control. In the late 1940s, the use of gamma-benzene hexachloride (Gammexane<sup>TM</sup>) led to an immediate reduction in acute cases and to the elimination of vector-borne Chagas disease in Bambuí by the early 1950s. Bambuí is recognised as a pioneer municipality in Chagas disease vector control in the Americas (Dias 1945, 1982, Dias & Pellegrino 1948, Silveira 2000).

These activities led to the elimination of *T. infestans*, a non-autochthonous species last found in 1978. After *T. infestans* was successfully controlled, however, the

native *P. megistus* began gradually to colonise homes and became the principal Chagas vector in the region, where it has persisted at low densities until today. The CEPMCh has since investigated the role of the sylvatic environment in the reinfestation process. These studies discovered *Psammolestes tertius* in Furnariidae bird nests and *Rhodnius neglectus* in palm trees, mainly in *Acrocomia aculeata* (*macaúba*). No *T. infestans* colony was found in the sylvatic environment, although nymphs of this species were observed in a *Didelphis albiventris* nest close to a residential area in Lagoa dos Monjolos, periurban area of Bambuí. The presence of adult *P. megistus* in the household environment was associated with shady ecotopes syvatics (Dias 1982).

This study aimed to identify the potential natural ecotopes of *P. megistus* and their role in the origin of household foci in Bambuí. To this end, nine field samplings were carried out between February 2008-May 2010. From Bambuí households with the highest number of notifications and proximity to residual natural vegetation were identified through Chagas Disease Control Program (PCDCH) data. These residences were investigated for the presence of triatomines in home environment and in the potential sylvatic habitats.

Peri-domiciliary and domiciliary areas were actively searched with the aid of flashlights and tweezers and the sylvatic environment was examined both by direct search in palm trees and other shelters and by seeking opossum nests by the spool-and-line technique (Miles 1976) with reels made of cylindrical plastic pots using Kooban<sup>TM</sup> line (Santos-Jr 2007). To evaluate possible natural reservoirs of *Trypanosoma cruzi*, small mammals were captured and checked for Chagas infection by xenodiagnosis and blood culture. Opossum burrows found by the spool-and-line technique were investigated using Noireau traps (Noireau et al. 2002), flashlights, tweezers and pirisa (a flushing-out agent). Material from inside the burrow was collected for screening in the laboratory. Temperature (T) and relative humidity (RH) inside the burrows and in the surrounding environment

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was monitored with HOBO H8 data loggers (Onset Computer Corporation) programmed to take readings every 15 min and data were processed using the Boxcar Pro 4.3 program. This study was carried out in accordance with Brazilian law (IBAMA written authorisation 16217-3/2008, CEUA P-0502/08). Using the data obtained in the PCDCH, it was possible to compare two distinct periods for *P. megistus*: 1974-1980 and 2005-2010 (Fig. 1). The reduction in the number of positive locations over the years is likely related to a large reduction in the number of notifications and a decrease in the dispersal of the species. Sylvatic habitats in 13 rural localities in Bambuí were also searched; these areas were chosen because they are close to houses where the presence of triatomines has previously been reported. We investigated 123 different natural ecotopes, in which Noireau traps were set daily. Material from these ecotopes (such as branches and leaves) was also collected when possible. In *A. aculeata* (*macaúba*) palm trees, which are abundant in the area, uninfected *R. neglectus* were found in *caatinga*

(20°32'24''S 46°01'48''W) and *capoeirão* (20°35'56''S 46°36'19''W). A natural focus of *P. megistus* was detected in only one ecotope (20°11'01''S 46°32'12''W): a living tree (*Dimorphandra mollis* Benth, popularly known as *faveira*) with a large natural hollow, in the village of Olhos D'água in May 2010 (Table). The tree contained nymphs and hatched eggs of triatomines; the nymphs were captured with the aid of the Noireau traps and the eggs by screening the material collected in the tree. Also found at the site were small lizards, a bat, and a large quantity of rodent and *D. albiventris* faeces, indicating that the tree was in frequent use as shelter for these wild animals (Fig. 2). Because *P. megistus* had not been reported in the area or in households in the last two years, the colony was characterised as sylvatic. The HOBO sensor was left installed inside the hollow tree for five days, with one sensor outside as a reference; results show a more constant T and RH inside the tree. Over the course of sampling in Bambuí, 74 wild or synanthropic animals, marsupials and rodents were collected. In sylvatic habitats surrounding the village of Olhos D'água, the following were captured: two marsupial species, *D. albiventris* (5) and *Micoureus* sp. (7) and three rodent species, *Nectomys* sp. (8), *Oecomys* sp. (2) and *Mus musculus* (1). No *T. cruzi* infection was detected in the captured animals or in the *P. megistus* nymphs. This area was searched because a female *P. megistus* had been found inside a house and vestiges of triatomine feces in the barn, which was also used as a henhouse five months previously. The natural focus was 54 m away from the house and the female most likely laid eggs in the natural environment before entering the building. According to Schlemper-Jr et al. (1985), triatomine sylvatic foci in the state of Santa Catarina were found between 15-300 m from the nearest residences, favouring incursions by adult triatomines. The discovery of *P. megistus* nymphs, rather than adults, is in agreement with the findings of Dias and Dias (1968), who found a greater abundance of early-stage nymphs between April-June, corresponding to oviposition in the late summer, when the number of adult insects in houses is

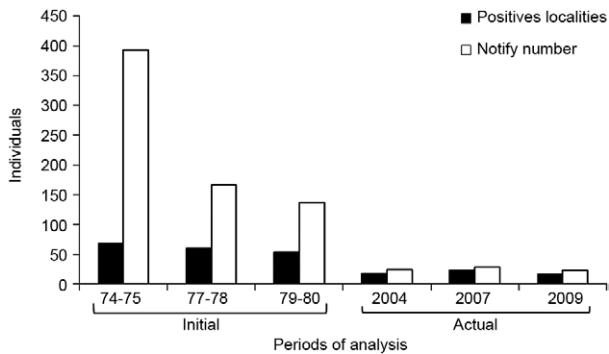


Fig. 1: triatomine reports and localities positive for *Panstrongylus megistus* in the municipality of Bambuí, state of Minas Gerais, in two periods of the Chagas Disease Control Program at the beginning of the epidemiological surveillance phase and in the last decade.

TABLE

Natural ecotopes investigated in 13 localities of municipality of Bambuí, state of Minas Gerais, indicating the triatomine<sup>a</sup> foci found

| Ecotopes investigated               | Quantity (%) | Ecotopes positives | Triatomine foci  |
|-------------------------------------|--------------|--------------------|--|
| Hollow trees and anfractuositities  | 46 (37)      | 1/46               | <i>Panstrongylus megistus</i><br>(4 nymphs of 2th and 3th stages and 4 hatched eggs) |
| Burrows in the floor                | 25 (20)      | -                  | -  |
| Palms ( <i>Acrocomia aculeata</i> ) | 21 (17)      | 3/21               | <i>Rhodnius neglectus</i><br>(1 ♀, 1 ♂ and 2 nymphs of 4th stage)                    |
| Bird nests                          | 15 (12)      | -                  | -  |
| Trellis of lianas                   | 10 (8)       | -                  | -  |
| Rock shelters                       | 6 (5)        | -                  | -  |
| Total                               | 123          | -                  | -  |

a: no triatomine showed *Trypanosoma cruzi* infection.

also reduced. Environmental parameters of the sylvatic focus of *P. megistus* showed smaller variations in T ( $T = 11 \pm 1^\circ\text{C}$ , max =  $15^\circ\text{C}$ , min =  $9^\circ\text{C}$ ) and RU (RU =  $88 \pm 4\%$ , max =  $95\%$ , min =  $62\%$ ) inside the breeding sites in comparison with the external conditions ( $T = 12 \pm 6^\circ\text{C}$ , max =  $24^\circ\text{C}$ , min =  $4^\circ\text{C}$ ; RU =  $78 \pm 16\%$ , max =  $94\%$ , min =  $41\%$ ). This damping effect probably provides greater protection for the triatomines, especially for early nymphs, and maintains relatively high humidity. The average RH in the sylvatic site remained close to the maximum observed. Pires (2003) showed that an RH of 73% leads to optimal rates of egg hatching and nymphal moulting in *P. megistus*. Since this species originates from the humid climate of the Atlantic Forest area, dryness may be a limiting factor for the dispersal of this species in Brazil (Forattini 1980, Cortez et al. 2007). Sylvatic *P. megistus* have been found in other Brazilian states, where they are often associated with nests of *Didelphis* sp. and show a preference for arboreal habitats (Fig. 3). Although the opossums tested here were not infested, Fernandes et al. (1991, 1994) demonstrated that these reservoirs play an important role in the interaction of the parasite's domestic and sylvatic cycles in Bambuí. Wildlife reservoirs are involved in the maintenance of *T. cruzi* in the natural environment and in its dispersal to domestic environments. The low frequency of natural triatomine colonies observed in the municipality corroborates the low numbers of domiciliary colonies currently reported. The reduction in triatomine colonies in households is attributed mainly to the extensive vector control program that has been established for decades in Bambuí. However, unlike *T. infestans*, which is restricted to the household environment in Brazil, *P. megistus* has a wide distribution in nature and frequently moves

between natural ecotopes and dwellings (Dias 1982, Fernandes et al. 1994). The low rates of re-infestation (10%) and domiciliary colonies (20%) observed in residences evaluated between 2004-2009 suggest that the sylvatic environment is the main source of household recolonisation. The expansion of monocultures such as beans, corn and especially sugarcane has reduced the original vegetation in the municipality over recent decades. Such land use change has led to a reduction in the natural resources used as shelter and food by the triatomines; these resources previously supported high rates of domestic invasion (Forattini et al. 1978). In an epidemiological study in Bambuí, Fernandes et al. (1992) observed that *P. megistus* captured inside dwellings showed higher feeding preference for humans and dogs and warned that vector-borne Chagas disease in the municipality could resurge if the triatomine control program were interrupted. The data presented here suggest that even with a low rate of infestation in sylvatic habitats, the colonisation of domiciliary environments has been extensive and ongoing throughout the municipality. We conclude that an active surveillance program is necessary to contain household infestations.



Fig. 2: natural ecotope of *Panstrongylus megistus* found in the municipality of Bambuí, state of Minas Gerais, Brazil (the white arrow indicates the entrance of the hollow).



Fig. 3: map of *Panstrongylus megistus* distribution (area in gray [modified from Carcavallo et al. (1999)]) and the principal findings of sylvatic foci in different Brazilian states. 1: state of Rio Grande do Sul (hollow trees) (Santos-Jr 2007); 2: state of Santa Catarina (hollow trees and Bromeliaceae) (Leal et al. 1961, Schlempler-Jr et al. 1985, Grisard et al. 2000); 3: state of São Paulo (hollow trees, anfractuositities, bromeliads, palms and rock shelters) (Barretto et al. 1964, Barretto 1967, Forattini et al. 1970, 1977, 1978); 4: state of Rio de Janeiro (hollow trees) (Miles et al. 1982); 5: state of Minas Gerais (hollow trees, tree holes, palms and burrows in the floor) (Barretto et al. 1978, Neves 1979, Santos-Júnior et al. 2011, the present paper).

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