

## THE THREAT OF REINTRODUCTION OF NATURAL TRANSMISSION OF CHAGAS' DISEASE IN BAMBUÍ, MINAS GERAIS STATE, BRAZIL, DUE TO *PANSTRONGYLUS MEGISTUS*

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*In the Epidemiological Surveillance Program in the county of Bambuí, Minas Gerais, between August 1986 and December 1988, 154 Panstrongylus megistus were captured by the local population in both peridomicile and intradomicile environments. Fifteen (9.8%) of the P. megistus harboured Trypanosoma cruzi. Precipitin tests showed that the most frequent triatomine blood meal sources were birds, but other sources were dogs, men and cats. The isoenzyme characterization of 13 T. cruzi strains showed that six belonged to zymodeme Z1, corresponding to the wild cycle parasites, and seven belonged to zymodeme Z2, corresponding to parasites isolated from chronic chagasic patients (domestic cycle). As P. megistus were found to be naturally infected by parasites from both cycles, they are clearly able to transmit T. cruzi from the wild cycle to the domestic cycle. Furthermore the capacity of P. megistus in colonizing houses was observed in one residence, vacant for several years, in which 153 triatomines were captured. The data show the possibility of P. megistus reintroducing the natural transmission of Chagas' disease in the county if Epidemiological Surveillance is interrupted.*

Key words: *Trypanosoma cruzi* – *Panstrongylus megistus* – Chagas' disease – Epidemiological Surveillance – isoenzyme

The first human cases of Chagas' disease in Bambuí, Minas Gerais, were described in the 1930s by Martins et al. (1939-40). At the end of this decade, *Panstrongylus megistus* was found in 75% of the houses. Subsequently, *Triatoma infestans* became the predominant species and was found in 20% of urban and more than 60% of periurban dwellings (Dias, 1982). A pre-prophylact serological survey for Chagas' disease was undertaken in 1947 and showed a rate of 60.4% positivity among urban and rural inhabitants (Dias, 1950). From 1956 to 1969 the county has undertaken an intense campaign of vector control, using residual insecticides (Dias, 1982). This campaign eradicated the domiciliar vector, *T. infestans* and interrupted the transmission of Chagas' disease to man. Thereafter an Epidemiological Surveillance Program (Dias & Garcia, 1978) has been established which has reported no further occurrence of *T. infestans* and only a

sporadic appearance of *P. megistus* in rural houses (Dias, 1982).

Different methodologies have been used to study the relationships between vectors and *Trypanosoma cruzi* hosts. Precipitin tests have been used to identify triatomine food sources (Siqueira, 1960) and isoenzyme (Romanha & Brener, 1988) as well as kinetoplast DNA (K-DNA) patterns (Morel et al., 1980) have been used to characterize the parasites. Together, these methodologies have proved to be a valuable tool for a better understanding of the epidemiology of Chagas' disease (Gibson & Miles, 1985).

Within this context, using both precipitin tests and isoenzyme patterns, the present paper evaluates the threat of reintroduction of the natural transmission of Chagas' disease in Bambuí due to *P. megistus*.

### MATERIALS AND METHODS

Bambuí is a small city located at 46° longitude West and 20° latitude South, distant

260 km West from Belo Horizonte, capital of the Minas Gerais state, Brazil.

Between August 1986 and December 1988 triatomines were captured by the population throughout the district of Bambuí and sent to the René Rachou Research Center for classification and detection of *T. cruzi* infection. The triatomines were examined and the parasites isolated by inoculating the infected feces into LIT medium containing amplacyline (Bronfen et al., 1989). The triatomine food source was determined using the precipitin tests (Siqueira, 1960). Rabbit anti-serum against man, cat, dog, opossum, bird and rodent serum proteins were used.

In each case where *P. megistus* was captured on an intradomiciliar environment it was found to be infected by *T. cruzi*, xenodiagnosis was used with the residents with reactive Chagas' disease serology as well as with domestic animals. For xenodiagnosis third and fourth instar triatomines were used. Twenty and 10 *T. infestans* were used for each person and domestic animal, respectively, and 10 *P. megistus* and 10 *Rhodnius neglectus* for both hosts. Triatomine examination and parasite

isolation were undertaken according to Bronfen et al. (1989).

The isoenzyme patterns of the *T. cruzi* strains were determined according to Romanha (1982). Six soluble enzymes were studied: alanine aminotransferase (ALAT) [EC.2.6.1.2], aspartate aminotransferase (ASAT) [EC.2.6.1.1], glucose phosphate isomerase (GPI) [EC.5.3.1.9], phosphoglucomutase (PGM) [EC.2.7.5.1], glucose 6-phosphate deshydrogenase (G6PD) [EC.1.1.1.49] and malic enzyme (ME) [EC.1.1.40].

## RESULTS

*Triatomines captured by the local population* – Table I shows the rate of natural infection of *P. megistus* with *T. cruzi* in peridomicile and intradomicile environments. From August 1986 to December 1988, 154 *P. megistus* were captured by the population and 15 (9.8%) of them, were positive for *T. cruzi*.

In a rural house, vacant for several years, 153 nymphs and adults of *P. megistus* were captured. All triatomines were examined by dissection, and were found not to be infected

TABLE I

Rate of natural infection of *Panstrongylus megistus* with *Trypanosoma cruzi* in the peridomicile and intradomicile environments

<i>P. megistus</i>	Peridomicile		Intradomicile	
	Captured	Infected	Captured	Infected
Nymph	34	0	45	4 ( 8.9%)
Adult				
Female	21	1 (4.8%)	30	8 (26.7%)
Male	12	1 (8.3%)	12	1 ( 8.3%)
Total	67	2 (3.0%)	87	13 (14.9%)

TABLE II

Blood meal sources of *Panstrongylus megistus* determined by the precipitin test

<i>P. megistus</i>	Total	Man	Dog	Cat	Rodent	Bird	Opossum
Nymph	77	20 (25.9)	26 (33.7)	15 (19.5)	2 (2.6)	43 (55.8)	0 (0)
Adult	67	9 (13.4)	22 (32.8)	10 (14.9)	0 (0)	33 (49.2)	0 (0)
Total	144	29	48	25	2	76	0

The numbers within parentheses are percentages. The values total > 100% is due to multiple feeding.

TABLE III

Blood meal source and isoenzyme characterization of *Trypanosoma cruzi* strains from *Panstrongylus megistus*

<i>P. megistus</i>	Z	Blood meal source					
		Man	Dog	Cat	Bird	Rodent	Opossum
Intradomicile							
1) Adult	Z1	-	-	-	-	-	-
2) Adult	ND	+	+	+	+	-	-
3) Adult	ND	-	-	-	+	-	-
4) Adult	Z2	-	-	-	-	-	-
5) Adult	Z1	-	-	-	+	-	-
6) 4th instar	Z2	+	+	+	+	-	-
7) Adult	Z2	-	+	-	-	-	-
8) Adult	Z1	WB					
9) 4th instar	Z2	+	-	-	-	-	-
10) 5th instar	Z2	+	-	-	-	-	-
11) 5th instar	Z2	+	+	+	-	-	-
12) Adult	Z2	+	+	+	-	-	-
13) Adult	Z1	-	+	+	-	-	-
Peridomicile							
14) Adult	Z1	WB					
15) Adult	Z1	WB					
Total 12 (%)		6 (50)	6 (50)	5 (4.7)	4 (33.3)	0	0

Z = *T. cruzi* zymodeme.

- = Non reactive for the anti-sera teste.

+ = Reactive for the anti-sera tested.

ND = Not determined.

WB = Without blood in the stomach.

with *T. cruzi*. Three palm trees (*Acrocomia sclerocarpa*) located 15 m away from the house were cut down and searched for triatomines. In one of these 22 *R. neglectus* were captured, which were all negative for *T. cruzi*. No *P. megistus* were found.

**Triatomine blood meal sources – Precipitin tests** – Blood was detected in the digestive tube of 144 (93.5%) of the *P. megistus* captured. Most of the triatomines (45.9%) contained blood from only one host. The occurrence of multiple sources was 29.9%, of those, 15.9, 11.1 and 2.9% exhibited two, three and four different blood sources respectively. The most frequent food sources were birds, dogs and men (Table II). The results of blood meal source and the isoenzyme characterization of *T. cruzi* strains in the *P. megistus* naturally infected with *T. cruzi*, are shown on Table III.

The blood meal sources of the 153 *P. megistus* captured in the empty rural house were 47 (74.6%) from birds, 3 (4.8%) from birds and dogs, 8 (12.7%) from none of the hosts tested and 5 (7.9%) inconclusive.

**Xenodiagnosis of chronic chagasic patients and domestic animals** – In two localities where intradomiciliar *P. megistus* (nymph and adult) were found infected with *T. cruzi* zymodeme Z2, xenodiagnosis was applied to four residents, four cats and six dogs. One patient, produced two *T. cruzi* isolates, one via *P. megistus* and other via *R. neglectus*. Both isolates were of zymodeme Z2. The strain isolated from one cat, via *T. infestans*, was also of zymodeme Z2.

**Isoenzyme characterization of the strains of *T. cruzi* isolated from naturally infected *P. megistus*** – Table III shows that from the 13 *T. cruzi* strains isolated from *P. megistus*, 6 (46.2%) were of zymodeme Z1 and 7 (53.8%) of zymodeme Z2. These are the same zymodemes isolated from opossums (Fernandes et al., 1991) and the majority of the patients (Romanha, 1982) in the same region.

## DISCUSSION

People's notification and insecticide spraying have been the most important factors for

controlling the triatomines in Bambuí. By chance, in one residence, vacant for several years, therefore non notified, 153 *P. megistus* were captured. This shows the ability of *P. megistus* in colonizing houses if allowed. The focus was notified to the Program after the resident's return, reaffirming the importance of community participation in notifying the presence of the vector. The lack of *T. cruzi* infection in the *P. megistus* found in this focus was explained by the precipitin tests, which showed birds as their main source of food and an almost absence of mammalian blood.

In previous epidemiological studies carried out in Bambuí, the wild ecotopes of *P. megistus* have been difficult to determine, and they have been reported only sporadically in palm trees (Dias, 1982). The palm trees searched around the house in the present study contained no *P. megistus*.

There was no significant difference ( $p > 0.05$ ) between the number of nymphs and adults of *P. megistus* captured by the local population either in the peridomicile or in the intradomicile environments. These results plus the precipitin tests show that locally there is a clear tendency of *P. megistus* to colonize environments close to man. The precipitin tests showed that 29.9% of the *P. megistus* had fed on multiple sources. According to Forattini et al. (1982) this provides a high potential of *T. cruzi* transmission in this environment, particularly in view of the frequency of mammalian blood detected by the precipitin.

None of the samples examined by the precipitin tests, reacted with anti-opossum serum, even though a large number of these animals have been caught in the peridomicile (Fernandes et al., 1991). Nevertheless, some association between opossum-*P. megistus* in Bambuí was observed by Dias & Diotaiuti (personal communication). They verified that in 145 *P. megistus* captured in the peridomicile and intradomicile environments, 7 (4.8%) reacted with anti-opossum serum. Our data show that *T. cruzi* strains isolated from six triatomines, suggest a reservoir-vector-*T. cruzi* Z1 association. Their isoenzyme patterns were the same as the *T. cruzi* strains isolated from opossums in the same region (Fernandes et al., 1991). On the other hand the *P. megistus* parasitized by *T. cruzi* zymodeme Z2 may have been infected either in the peridomicile or intradomicile environments. *T. cruzi* strains

isolated from one patient and one cat living in the same house, where naturally infected *P. megistus* was captured, also had the same zymodeme Z2.

The remarkable participation of man as blood source for *P. megistus* makes the vectorial transmission feasible. A serological inquiry in the rural school area in Bambuí in 1973, revealed no children infected with *T. cruzi* among 315 examined (Dias, 1982). More recently, only one child was found positive among 650 rural students tested (Machado-Coelho et al., 1990), showing that although feasible only one recent human infection has occurred.

The small number of colonies in the intradomicile environment is due to the constant work of the Epidemiological Surveillance carried out by the population and by the "Centro de Estudos Emmanuel Dias"-FIOCRUZ, which has not allowed effective domiciliation of *P. megistus* in the county.

Epidemiological data suggest that the six triatomines from which *T. cruzi* Z1 strains have been isolated originated from the wild environment. All six were adults and three had no blood in their digestive tubes. Considering the possibility of identification of ingested blood for up to 120 days after feeding (Siqueira, 1960), we conclude that the three triatomines had been fasting for a long time. The six adult *P. megistus* were unique in the six different houses they were captured suggesting their recent introduction in this environment.

The peridomicile annexes and domestic animals attract triatomines from the wild environment, due to the resulting abundance of food (Forattini et al., 1984). The presence of *P. megistus* in the peridomicile environment presents an important link between the sylvatic and the domestic environments. Therefore *P. megistus* may act as a carrier of *T. cruzi* Z1 and maintain the transmission cycle of *T. cruzi* Z2 in the peridomicile and domicile environments, resulting gradual reinfestation of the county, if the Epidemiological Surveillance were to be interrupted.

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