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THE IMPORTANCE OF THE OPOSSUM *(DIDELPHIS ALBIVENTRIS)* AS A RESERVOIR FOR *TRYPANOSOMA CRUZI* IN BAMBUÍ, MINAS GERAIS STATE

ALEXANDRE JOSÉ FERNANDES; EGLER CHIARI*; RODRIGO RIBEIRO RODRIGUES; JOÃO CARLOS PINTO DIAS & ALVARO JOSÉ ROMANHA

Centro de Pesquisas René Rachou – FIOCRUZ, Caixa Postal 1743, 30190 Belo Horizonte, MG, Brasil
*Instituto de Ciências Biológicas, Departamento de Parasitologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

In a survey realized on the sylvatic and peridomestic environments at Bambuí county, Minas Gerais State, 44 (37.9%) out of 116 opossums (Didelphis albiventris) *captured were found to be naturally infected with* Trypanosoma cruzi. *One hundred and forty three parasite samples were obtained from 43 infected opossums using simultaneously hemoculture, xenodiagnosis* (Triatoma infestans, Panstrongylus megistus *and* Rhodnius neglectus) *and examination of anal glands contents. The parasite samples were characterized according to six isoenzyme patterns. All samples, independently of the method of isolation, presented an isoenzyme pattern similar to the standard* T. cruzi *Z1, showing that either xenodiagnosis or hemoculture can used without selecting parasite subpopulation from naturally infected opossums. Previous isoenzyme patterns reported for human* T. cruzi *isolates from the same region were completely different. This isoenzyme dissimilarity between sylvatic and domiciliar environments suggests the existence of two independent* T. cruzi *transmission cycles in Bambuí. The epidemiological implications of these results are discussed.*

Key words: Chagas' disease – *Trypanosoma cruzi* – reservoir – opossum – isoenzymes

The isoenzymatic characterization of *Trypanosoma cruzi* strains has made it possible to establish the relationship between zymodemes and Chagas' disease transmission cycles in many areas. Miles et al. (1977), identified two distinct groups of parasites isolated from man, domestic animals, sylvatic reservoirs and triatomines, in São Felipe, in the east of the State of Bahia. The zymodeme Z1 parasites were associated with the sylvatic transmission cycle while the Z2 parasites were associated with the domiciliary transmission cycle. The distinction between the independently circulating groups suggests that the sylvatic and domiciliary transmission cycles do not overlap in São Felipe. However, Barrett et al. (1980) demonstrated an overlap in transmission cycles by the isolation of *T. cruzi* zymodemes Z1 and Z2 parasites from man, domestic animals and peridomiciliar rats, in Riacho de Santana, in the west of the State of Bahia.

Due to their sinantropic behavior, some reservoirs are important links between the sylvatic and domiciliary transmission cycles. Opossums of the genus *Didelphis* present high levels of *T. cruzi* infection, a long period of patent parasitemia and are frequently found near domestic areas (Barretto et al., 1964). These attributes indicate an important epidemiological status for this genus.

Trypanosoma cruzi strains are frequently isolated by hemoculture and/or xenodiagnosis, and it is important to determine the influence of these methods in the selection of parasite subpopulations. Deane et al. (1984) demonstrated experimentally by isoenzymes and electrophoretic analysis of k-DNA that, in mice infected with two distinct strains, the isolation period favors one strain or the other.

In this paper, isoenzyme patterns of *T. cruzi* isolated from opossums were compared to those of previously characterized strains from chronic chagasic patients, so as to understand the interaction between the domestic and sylvatic *T. cruzi* transmission cycles in Bambuí, Minas Gerais. Furthermore the parasite isolation procedures used, hemoculture and xenodiagnosis were evaluated in terms of isoenzymes for their ability to select parasite subpopulations.

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MATERIALS AND METHODS

Areas studied – Bambuí county is located in the western region of the State of Minas Gerais, Brazil. Since 1974 the county has been under epidemiological surveillance for the control of Chagas' disease (Dias, 1982). At first all houses were sprayed with residual insecticide and thereafter application was only made in houses where the population had reported the presence of triatomines. Ten rural areas and the urban area of the county were chosen for the capture of opossums. The areas were selected according to the number of reports of triatomines in artificial ecotopes.

Opossum capture – Opossums were captured with wire traps (20 x 20 x 60 cm) distributed in selected areas, in sylvatic, rural and urban peridomiciliar surroundings. The forest residues 1 km far from the nearest residence were considered sylvatic.

Trypanosoma cruzi *strain isolation* – Captured animals were examined using fresh blood. The blood was collected from the caudal marginal vein by puncture and parasite isolation was performed by xenodiagnosis and hemoculture.

The xenodiagnosis was undertaken with thirty 3rd and 4th instar triatome nymphs, 10 from *T. infestans,* 10 from *P. megistus* and 10 from *R. neglectus.* Isolation and amplification of *T. cruzi* strains were undertaken according to Bronfen et al. (1989). The parasites were isolated, cultivated and amplified in LIT medium (Camargo, 1964). The culture flasks were incubated at 28 °C and examined 15, 30, 45 and 60 days after incubation. The contents of the anal glands, independently of being positive, were inoculated in LIT and/or NNN medium with LIT as overlay containing with 3.3 μg/ml ampicilin. When positive part of the glandular material was inoculated i. p. into albino mice.

Isoenzyme electrophoresis – The isoenzyme patterns of *T. cruzi* strains were determined according to Romanha (1982). The six enzymes assayed were: alanine aminotransferase (ALAT) (E.C.2.6.1.2), aspartate aminotransferase (ASAT) (E.C.2.6.1.1), glucose phosphate isomerase (GPI) (E.C.5.3.1.9), phosphoglucomutase (PGM) (E.C.2.7.5.1), glucose 6-phosphate dehydrogenase (G6PD) (E.C.1.1.1.49) and malic enzyme (ME) (E.C.1.1.1.40).

RESULTS

Forty-four out of 116 opossums *(D. albiventris)* captured in Bambuí (37.9%) were found to be infected with *T. cruzi.* The rate of infection of animals captured in sylvatic, rural and domestic peridomiciliar surroundings is presented in Table I.

TABLE I

Trypanosoma cruzi infection rate in opossums *(Didelphis albiventris)* captured in different environments in Bambuí

Environment	Opossums		
	Examined	Infected	% infected
Sylvatic	66	22	33.3
Peridomiciliar			
rural	37	21	51.3
urban	13	01	7.7
Total	116	44	37.9

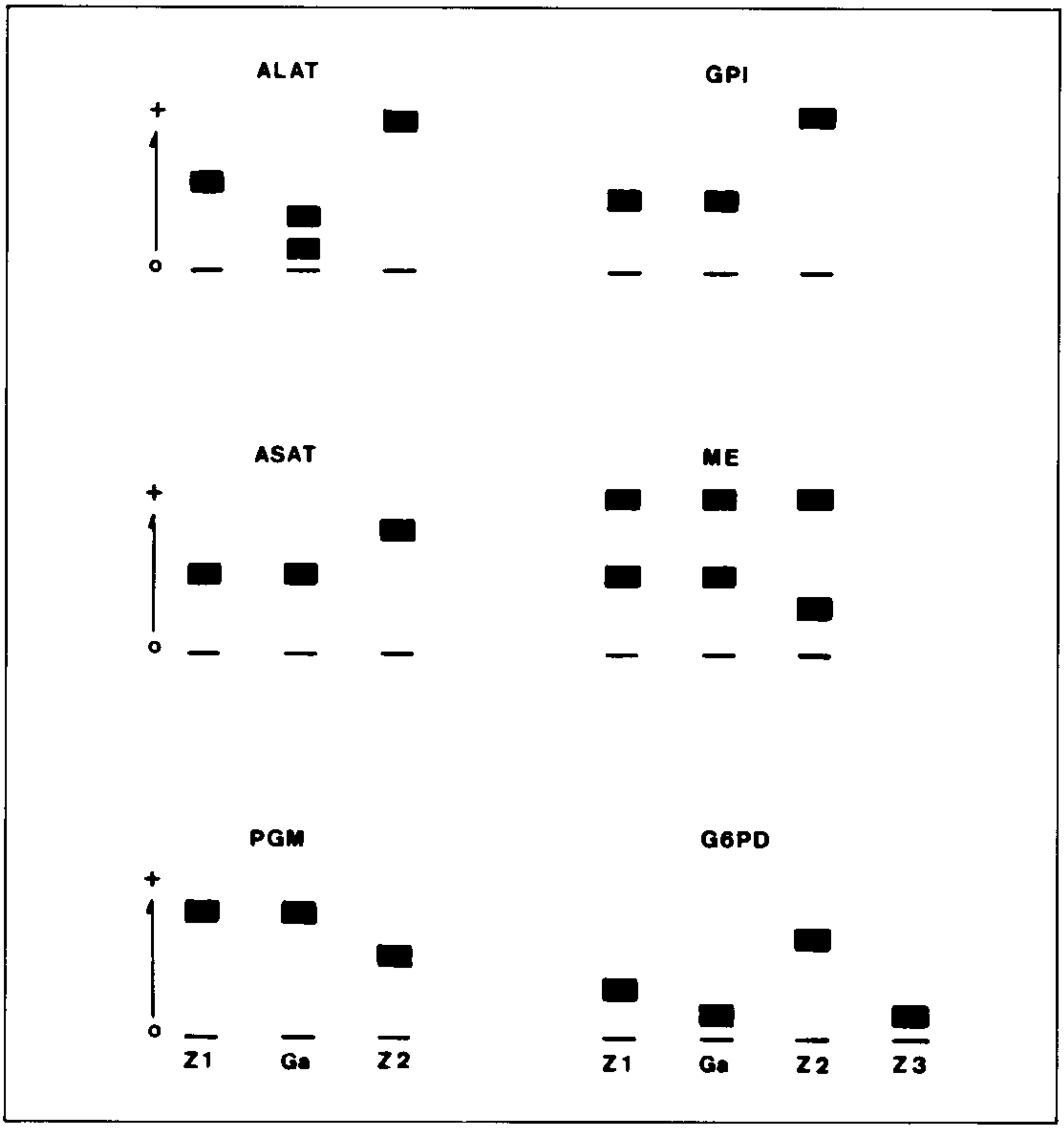
Xenodiagnosis and hemoculture were similarly efficient for detecting *T. cruzi* in opossums (c. a. 35%) whereas blood examination showed only 24.1% positivity (Table II). Only 2 out of 30 (6.7%) opossums infected with *T. cruzi* presented parasite in their anal glands.

TABLE II

Evaluation of parasitological methods for the detection of *Trypanosoma cruzi* infection in naturally infected opossums from Bambuí-MG

Parasitological methods	Opossums		
	Examined	Infected	% infected
Xenodiagnosis	116	43	37.1
Hemoculture	112	37	33.0
Fresh blood	116	28	24.1

One hundred and forty five *T. cruzi* samples were isolated from 43 of the infected opossums by different methods: (a) 35 by hemoculture; (b) 110 by xenodiagnosis, 28 in *T. infestans,* 37 in *P. megistus* and 42 in *R. neglectus* and (c) 3 isolated from anal glands material, 1 by direct cultivation in LIT medium, 1 via infected mice hemoculture and 1 via infected mice xenodiagnosis using *R. neglectus.*



Isoenzyme patterns of *Trypanosoma cruzi* samples isolated from naturally infected opossums from Bambuí. O = electrophoretic origin; Ga = a representative *T. cruzi* sample from opossum; Z1, Z2 and Z3 standard *T. cruzi* zymodemes.

All strains, independently of the method of isolation presented the same isoenzyme pattern for each enzyme (Fig.). The strains showed zymodeme Z1 patterns for enzymes GPI, ASAT, PGM and ME. G6PD presented zymodeme Z3 pattern while ALAT presented a new pattern, formed by two close proeminent bands with slow migration (Fig.).

DISCUSSION

Isoenzyme patterns of *T. cruzi* strains isolated from opossums *(D. albiventris)* from Bambuí, showed a homogeneous parasite population, circulating among those animals in sylvatic and peridomiciliar areas. The *T. cruzi* strains isolated from opossums, were closer to Miles et al. (1977) zymodeme Z1, differing in only 2 out of 6 enzymes. ALAT present a new pattern and G6PD was identical to the pattern of zymodeme Z3. Isoenzyme studies of *T. cruzi* isolated from different regions of Brazil, also presented mixed zymodemes (Miles et al., 1981; Povoa et al., 1984). Tibayrenc & Ayala (1988) identified 43 zymodemes among 524 *T. cruzi* strains isolated from various hosts in

different regions. Their zymodemes Z1 to Z25 were considered similar to Miles zymodeme Z1.

The *T. cruzi* strains isolated from opossums in Bambuí, were considered to belong mainly to Miles zymodeme Z1 group. Since Tibayrenc & Ayala (1988) did not show the isoenzyme patterns observed in the 43 zymodemes they proposed, the Z1 pattern herein observed could well be included amongst the above mentioned 25 zymodeme Z1 variants.

Xenodiagnosis and hemoculture were similarly efficient for parasitological diagnosis and *T. cruzi* isolation. On the other hand direct blood examination revealed lower infection rates. These results are related to opossum's infection characteristics in that they present a rather low parasitemia for long periods. We observed that among the 44 opossums with either positive hemoculture and/or xenodiagnosis, 16 (34.4%) presented repeatedly negative direct blood examination. The opossums have an important epidemiological role in *T. cruzi* sylvatic and domestic cycles because they are widely distributed, sinantropic and constitute a continuous source of infection for Chagas' disease vectors.

The different methods used for *T. cruzi* isolation did not select subpopulations of the parasite, since the 145 isolates obtained from the opossums showed identical isoenzyme patterns in the six enzymes irrespective of the parasite isolation procedure used. Similar results were found by Barrett et al. (1980) and Povoa et al. (1984). Recently, Romanha et al. (1989) have also demonstrated that xenodiagnosis, hemoculture and one subsequent *T. cruzi* passage through mice did not select subpopulations of parasites deriving from chronic chagasic patients. Thus, according to the above results, both xenodiagnosis and hemoculture can be used for parasite isolation from naturally infected opossums without selection. Furthermore, the parasite populations of the anal glands did not differ isoenzymatically from the bloodstream parasites in the opossum.

The results of isoenzyme characterization of the parasite strains isolated from opossums suggest the existence of a sylvatic transmission cycle in the region. The *T. cruzi* which circulate in this environment is isoenzymatically different from that observed in the domiciliar transmission cycle. The heterogeneity among *T. cruzi* strains isolated from chronic chagasic patients (Romanha, 1982) and the homogeneity of the sylvatic strains isolated from opossums in the same region constitute a puzzle. Since Chagas' disease is a zoonosis and the sylvatic *T. cruzi* Z1 a homogenous population in Bambuí, how can the fact that chronic chagasic patients present 4 different zymodemes be explained? The answer to such question may come from the understanding of the dynamics of the *T. cruzi* vectors *T. infestans* and *P. megistus* in that region. In the expectation of a better understanding of *T. cruzi* transmission cycle in Bambuí, studies have been carried out with parasites isolated from *P. megistus*, the only triatomine yet captured in peridomiciliar and domiciliar environments. Another possibility to explain such a difference in parasite population between the domestic and sylvatic transmission cycles may be selection exerted by the host. One important epidemiological finding in Chagas' disease is that most patients in the acute phase are infected by *T. cruzi* Z1 and Z2 whereas chronic patients are found infected almost exclusively with Z2 strains (Barret et al., 1980; Luquetti et al., 1986). Furthermore some authors have already reported zymodeme changes on parasites isolated twice from the same patient (Lana, 1981; Romanha, 1982; Romanha et al., 1987) and from the same experimental host (Bahia, 1985; Romanha et al., 1987; Carneiro et al., 1990) at different times.

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