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Epidemiological aspects of three *Trypanosoma cruzi* zymodemes in Bahia State, Brazil

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Summary

Culture forms of 104 stocks of *Trypanosoma cruzi* isolated in different regions of the State of Bahia were compared by electrophoresis of six enzymes. The three distinct combinations of isoenzyme patterns seen were designated Z1, Z2 and Z3. In an area of endemic Chagas's disease in eastern Bahia, *T. cruzi* Z1 was associated with sylvatic mammals and sylvatic triatomines, whereas *T. cruzi* Z2 was associated with a separate domestic cycle of transmission. *T. cruzi* Z1 was also found in sylvatic triatomines from other parts of the State. In contrast, in an area of the São Francisco Valley region of western Bahia, both *T. cruzi* Z1 and Z2 were isolated from man, domestic animals, and peridomestic rats. *T. cruzi* Z3 was isolated from an armadillo and from *Panstrongylus geniculatus*, a triatomine commonly found in armadillo burrows.

Both *T. cruzi* Z1 and Z2 appeared to be pathogenic in man: *T. cruzi* Z1 was isolated from patients with acute Chagas's disease and from a single patient with chronic cardiac manifestations. *T. cruzi* Z2 was isolated from some asymptomatic individuals but was also associated with acute disease and chronic cardiac and digestive syndromes.

Introduction

Wild mammals have been considered potential reservoirs of human Chagas's disease since the early work of CARLOS CHAGAS (1912). More recently, enzyme electrophoresis for the intrinsic characterization of *T. cruzi* (LUMSDEN, 1974; TOYE, 1974; MILES *et al.*, 1977; MILES *et al.*, 1978; MILES, 1979) has been used in attempts to assess the extent to which enzootic *T. cruzi* contributes to domestic cycles of transmission. Using this technique, MILES *et al.* (1977) compared 17 stocks of *T. cruzi* from São Felipe in the coastal region of eastern Bahia, and concluded that the parasites infecting man and the domestic vector *Panstrongylus megistus* circulated independently of *T. cruzi* infecting opossums (*Didelphis albiventris* Lund, 1841)† and a sylvatic vector *Triatoma tibiamaculata*.

In this paper we show that the distribution of zymodemes of *T. cruzi* in an area near São Felipe (Fig. 1) is similar to that described by MILES *et al.* (1977). In contrast, in the São Francisco Valley region of western Bahia, the zymodeme which in São Felipe was associated exclusively with sylvatic foci, was present in man and domestic animals and vectors.

To avoid the possibility of any confusion we give below the definitions of terms as used in this paper:

(i) *Stock*: "Population derived by serial passage *in vivo* and/or *in vitro* from a primary isolation, without any implication of homogeneity or characterization." (ANON., 1978).

(ii) *Zymodeme* (Z): "Trypanosome populations that possess like forms of specified enzymes." As used here, zymodeme is equivalent to "strain-group" (MILES *et al.*, 1977) and "type" (MILES *et al.*, 1978). Z1 = São Felipe sylvatic cycle strain group = type 1. Z2 = São Felipe domestic cycle strain-group = type II. Z3 = type III. (cf. MILES *et al.*, 1977, 1978). We now prefer to avoid this usage of "type", a word which already has alternative meanings in zoological nomenclature, and consider that terms such as "domestic" and "sylvatic" can only lead to bias and confusion when

* Consultants to the Pan American Health Organization.

† Previously referred to as *Didelphis azarae*, a name now considered unacceptable (HERSCHKOVITZ, 1972).

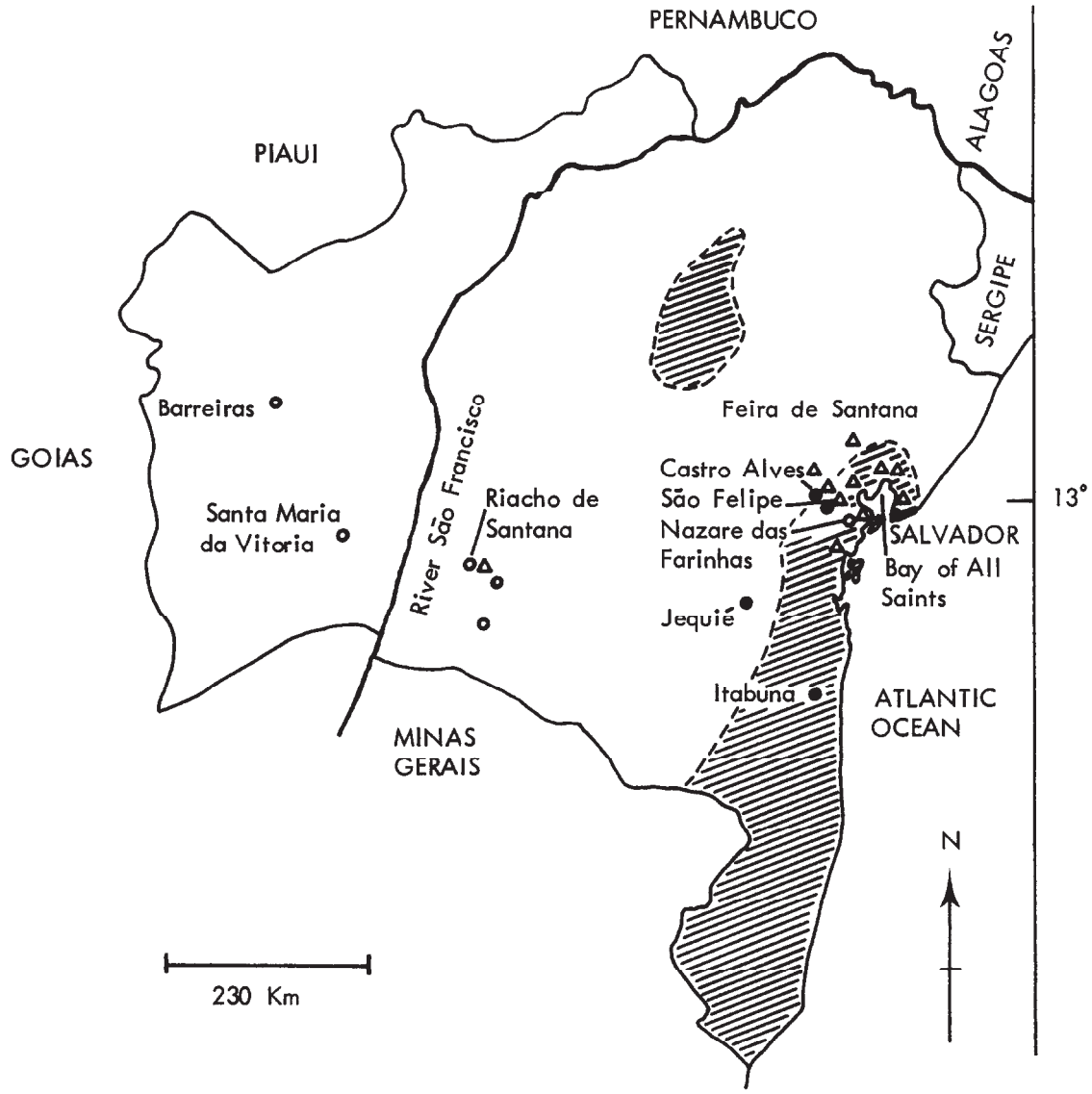

Fig. 1. The State of Bahia, to show the sources of the *T. cruzi* stocks examined.
○ zymodeme 1 domestic isolations ● zymodeme 1 sylvatic isolations △ zymodeme 2 domestic isolations
Shaded areas represent regions of high rainfall, where *Panstrongylus megistus* is the principal domestic vector (Based on SHERLOCK & SERAFIM, 1972).

used to describe intrinsic characters of trypanosomes. A word based on the "-deme" concept (GILMOUR & GREGOR, 1939; HOARE, 1967) was considered appropriate.

Materials and Methods

Origins of T. cruzi *stocks*

104 *T. cruzi* stocks were isolated from 57 persons, 24 wild and domestic animals and 13 triatomine bugs from 19 "municipios" (administrative districts, divisions) in the State of Bahia (Fig. 1). Most of these stocks were from the municipio of Castro Alves in eastern Bahia and from the municipio of Riacho de Santana in the São Francisco Valley region of western Bahia. Some of the ecological differences between these two areas are summarized in Table III: for further details see MOTT *et al.* (1976) and SHERLOCK & SERAFIM (1972). *T. cruzi* stocks from residents of other municipios were isolated in the course of diagnostic procedures from

Table I—*Trypanosoma cruzi* stocks examined

Origin	Host	Host's habitat	Number examined	Zymodeme
Eastern Bahia				
Castro Alves	*Didelphis albiventris*	sylvatic	6	1
Castro Alves	*Metachirus nudicaudatus*	sylvatic	1	1
Castro Alves	*Triatoma tibiamaculata*	sylvatic	1	1
Itabuna	*T. tibiamaculata*	sylvatic	2	1
Nazaré das Farinhas	man	domestic	1	1
Castro Alves	guinea-pig	,,	1	2
Castro Alves	man	,,	23	2
Cachoeira	man	,,	1	2
Candeias	man	,,	1	2
Capetinga	man	,,	1	2
Feira de Santana	man	,,	4	2
Maragojipe	man	,,	1	2
São Felipe	man	,,	3	2
São Francisco do Conde	man	,,	1	2
Santo Estevão	man	,,	1	2
Valença	man	,,	1	2
unknown	man	,,	4	2
Castro Alves	*Panstrongylus geniculatus*	sylvatic	1	3
Castro Alves	*Dasypus novemcinctus*	sylvatic	1	3
Jequié				
Jequié	*Triatoma melanocephala*	sylvatic	1	1
Jequié	*Panstrongylus geniculatus*	sylvatic	1	1
São Francisco Valley Region				
Barreiras	*Triatoma infestans*	domestic	1	1
Guanambi	man	,,	1	1
Iguapora	man	,,	1	1
Santa Maria da Vitoria	man	,,	1	1
Riacho de Santana	man	,,	8	1
Riacho de Santana	man	,,	4	2
Riacho de Santana	cat	,,	1	1
Riacho de Santana	cat	,,	2	2
Riacho de Santana	dog	,,	1	1
Riacho de Santana	dog	,,	1	2
Riacho de Santana	*T. infestans*	domestic	2	1
Riacho de Santana	*T. infestans*	peridomestic	1	1
Riacho de Santana	*Rattus rattus*	peridomestic	9×2	1
Riacho de Santana	*Rattus rattus*	peridomestic	1×2	2
Riacho de Santana	*Triatoma sordida*	peridomestic	3	1

Table II—Disease state in relation to zymodeme of *T. cruzi* isolated from man in Bahia

Disease state	Number of stocks examined	Zymodeme 1	Zymodeme 2
*Eastern Bahia**			
Acute Chagas's disease	7	0	7
Asymptomatic with normal ECG	12	0	12
ECG abnormality or symptomatic cardiomyopathy	21	1	20
Megaoesophagus and megacolon with normal ECG	1	0	1
Congenital Chagas's disease	1	0	1
Total	42	1	41
Western Bahia São Francisco Valley Region			
Acute Chagas's disease	15	11	4

* Four patients whose exact residence could not be determined were considered to be from this region.

Table III—Some features of the two main study areas (1976)

	Eastern Bahia : Castro Alves area (southern part)	Western Bahia : Riacho de Santana
Location	12° 45′ S 39° 25′ W	13° 37′ S 42° 55′ W
Annual rainfall	1300-1800 mm, no dry season	often <900 mm, distinct dry season
Vegetation	open farmland with patches of residual evergreen rain forest with epiphytes	xeromorphic deciduous woodland and scrub (caatinga), no epiphytes
Typical crops	cassava, tobacco	maize, cotton
Typical houses	mud-and-wattle unplastered walls, palm-thatch roof	mud-brick walls, often plastered, with tiled roofs
Domestic vector	*Panstrongylus megistus*	*Triatoma infestans*
Peridomestic vector	—	*Triatoma sordida*
Domestic *T. cruzi*	Z2	Z1, Z2
Sylvatic *T. cruzi*	Z1, Z3	?
Didelphis albiventris	present	present

patients at the Hospital Professor Edgard Santos in Salvador. For most of these patients we were able to determine the municipio of residence during childhood and early adulthood, the age at which most individuals become infected with *T. cruzi* (MOTT *et al.*, 1976).

Of the 57 stocks from man, 22 were from acute cases and 34 were from individuals considered to be in the chronic stage of infection. Of the latter, 22 had evidence of cardiac or digestive tract manifestations of Chagas's disease. One stock was from a case of congenital Chagas's disease (Table II).

Isolation and maintenance of T. cruzi *stocks*

Primary isolations from man and other mammals were made either by haemoculture using fortified blood agar with F29 medium as overlay (PAN, 1968) or by xenodiagnosis using fifth-instar nymphs of laboratory-reared *Triatoma infestans* or *Rhodnius prolixus*. Primary isolations from triatomines, and isolations from bugs used in xenodiagnosis, were made by inoculating triatomine faeces intraperitoneally into white laboratory mice and then culturing cardiac or tail blood when the infections became patent. *T. cruzi* from each of 10 *Rattus rattus* was isolated both by direct haemoculture, and by xenodiagnosis with subsequent mouse passage.

Prior to isoenzyme analysis the *T. cruzi* stocks were maintained in our laboratory in Salvador, Brazil, for one or more monthly passages using NNN blood agar with an overlay of Hanks' balanced salt solution. Lysates for isoenzyme analysis were prepared in Salvador from 11 of the *T. cruzi* stocks, from parasites grown in 200 ml of F29 medium. Lysates of the other stocks were prepared in London from parasites grown in enriched Maekelt's diphasic blood-agar medium (KETTERIDGE, 1975).

Electrophoresis

The production of lysates, electrophoretic methods and staining for the six enzymes (E.C. 1.1.1.40; E.C.1.1.1.49; E.C.2.6.1.1.; E.C.2.6.1.2.; E.C.2.7.5.1.; E.C.5.3.1.9.) were as previously described (MILES *et al.*, 1977). Cryopreserved lysates of Z1 and Z2 *T. cruzi* from São Felipe, previously characterized by MILES *et al.*, (1977), were included in each electrophoretic run as reference standards. Stocks which differed from these two reference standards in their isoenzyme mobilities were then compared with cryopreserved Z3 lysates characterized by MILES *et al.* (1978).

Results

Hosts and distribution of zymodemes

Three distinct groups of isoenzyme profiles were obtained, conforming with those described by MILES *et al.* (1977, 1978). Table I shows that in eastern Bahia, most stocks from sylvatic mammals and sylvatic triatomines were *T. cruzi* Z1. The exceptions were one stock from an armadillo, *Dasypus novemcinctus*, and one from the triatomine *Panstrongylus geniculatus*; both stocks were from Castro Alves and were identified as Z3. *T. cruzi* stocks isolated from man in this region were all Z2 with the exception of one Z1 stock from a 41-year-old woman who, as far as could be determined, had lived only in Nazare das Farinhas near Salvador.

In contrast, both Z1 and Z2 were represented among stocks of *T. cruzi* from man, dogs, cats and

peridomestic *Rattus rattus* in the São Francisco Valley region of western Bahia. Most stocks from man and *R. rattus* in this region were *T. cruzi* Z1.

Clinical correlations

Both Z1 and Z2 *T. cruzi* were isolated from patients with acute Chagas's disease in western Bahia (Table II). There were no evident clinical or pathological differences between patients infected with either zymodeme. No mixed infections were detected.

The patients with chronic Chagas's disease from whom we isolated parasites were all from eastern Bahia, where *T. cruzi* Z2 is the predominant domestic zymodeme and where it is associated with a wide spectrum of clinical manifestations (Table II). In this region we isolated *T. cruzi* Z1 from only one patient—a 41-year-old woman with complete atrioventricular heart block who died shortly after parasites were isolated.

Controls

The isoenzyme patterns of the stocks isolated by xenodiagnosis and subsequent mouse passage from the 10 *R. rattus* were identical to those of stocks isolated by direct haemoculture from the same animals.

Discussion

In Castro Alves, in eastern Bahia, it appears that three zymodemes of *T. cruzi* are associated with three independent host-vector systems or transmission cycles. Z1 infects opossums and *Triatoma tibiamaculata*, a vector which inhabits epiphytic bromeliads and *Attalea* palms. Z2 infects man and is transmitted by *Panstrongylus megistus*, which in this region has only been found in houses and chicken-houses. The use of isoenzyme markers to demonstrate the distinction between sylvatic and domestic *Trypanosoma cruzi* in this region confirms the findings of MILES *et al.* (1977) in the nearby municipio of São Felipe. Only two Z3 stocks were isolated but both were from animals associated with armadillo burrows.

That the observed distribution of these three zymodemes in eastern Bahia is due to ecological barriers and not to intrinsic factors restricting host range is indicated by the recent findings of Z1 and Z3 *T. cruzi* in cases of human acute Chagas's disease in Belém, State of Pará (MILES *et al.*, 1978), by the isolation of Z1 from a woman with chronic Chagas's disease in eastern Bahia, and by our results from Riacho de Santana.

The distribution of hosts and vectors of *T. cruzi* Z1 and Z2 in Riacho de Santana (Table I) is indicative of overlapping domestic and peridomestic cycles of transmission. The domestic vector in Riacho de Santana is *Triatoma infestans*, and *T. sordida* mediates a peridomestic transmission cycle. *T. infestans* was found in peridomestic habitats as well as in houses, and *R. rattus* is mobile between houses, the peridomestic area and the woodland. The peridomestic environment is in close contact with the woodland and, although we did not succeed in obtaining sylvatic isolates of *Trypanosoma cruzi* from Riacho de Santana, we found *T. sordida* and *T. pseudomaculata* in woodland habitats in which one infected *Didelphis albiventris* was captured. Regardless of how *Trypanosoma cruzi* Z1 and Z2 were introduced into the domestic environment in Riacho de Santana (with infected persons or *Triatoma infestans*, or from local forest mammals and vectors) our results indicate that domestic and peridomestic (and probably sylvatic) foci of *Trypanosoma cruzi* are now interlinked in this area and that *R. rattus* and *Triatoma sordida* must be regarded as potential reservoirs of the human disease.

T. infestans was unknown in Bahia in 1955 (BUSTAMENTE, 1957) and, according to local residents the very heavy and widespread household infestations we observed in 1976 were a relatively recent phenomenon. This may account for our failure to find cases of chronic Chagas's disease in Riacho de Santana.

Although the number of *Trypanosoma cruzi* stocks that have been characterized isoenzymically is at present small in relation to the wide distribution of the parasite and the many species of mammals and vectors involved in its transmission, the evidence so far available suggests that *T. cruzi* zymodeme 1 is particularly associated with marsupials. *T. cruzi* Z1 was the only zymodeme represented among stocks from seven didelphine marsupials in Castro Alves (Table I) and from five *D. albiventris* from São Felipe (MILES *et al.*, 1977). This zymodeme also predominated in stocks from *D. marsupialis* in Pará State (Miles, unpublished). Of the sylvatic bugs (*Triatoma tibiamaculata*, *T. melanocephala* and *Panstrongylus geniculatus*) from which we isolated this zymodeme, *T. tibiamaculata* is associated with *D. albiventris* and *D. marsupialis* in bromeliad epiphytes in eastern and south-eastern Brazil (MILES, 1976; MOURA *et al.*, 1969; SILVEIRA *et al.*, 1969), and *P. geniculatus* is known to feed on opossums as well as on armadillos (BARRETTO, 1968b, 1971). *T. melanocephala* is also associated with *D. albiventris* in Bahia (Barrett, unpublished). *D. albiventris* and *D. marsupialis* are recognized as important hosts of *Trypanosoma cruzi* (BARRETTO, 1968a) because of the high proportion found infected, their high population density in many areas, and the prolonged periods of patent parasitaemia seen in apparently healthy opossums, factors which suggest a long evolutionary association between *Didelphis* and *T. cruzi*.

We found one rat infected with *T. cruzi* Z2 in Riacho de Santana, but as yet no truly wild reservoir of this zymodeme has been detected. MILES *et al.* (1977) suggested that *T. cruzi* Z2 was introduced into eastern Bahia with *P. megistus* from South Brazil. However, *P. megistus* in south Brazil is principally a sylvatic bug (e.g. BARRETTO *et al.*, 1964; FORATTINI *et al.*, 1978) associated with *Didelphis* spp. and sharing the bromeliad habitat with *Triatoma tibiamaculata*, species which in Bahia are characteristically infected with *Trypanosoma cruzi* Z1. If enzyme electrophoretic mobilities reflect stable characters selected for optimal survival in particular host-vector systems, then *T. cruzi* Z2 is unlikely to have originated in the bromeliad habitat in south Brazil, which is connected to eastern Bahia by the coastal forest.

The stocks examined may not exactly reflect the prevalence of different zymodemes in nature, since

some hosts are more easily captured than others and, as with other methods of characterization, the process of isolation may be selective. In spite of these limitations, enzyme electrophoresis promises to be of great value in studies on *T. cruzi* and we have used this technique to demonstrate regional differences in the ecology of two zymodemes of *T. cruzi* infecting man in Bahia. Additional studies are now needed on the epidemiology of *T. cruzi* zymodemes in other regions and also to establish whether zymodeme differences are correlated with the clinical outcome of infection in man.

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Note from the Editor

The number of papers submitted for publication in the *Transactions* has increased steadily over the last three years and, in spite of increasing the number of pages in each part, first to 112 and then to 128 pp., the delay between acceptance and publication has crept up. The Council and the Editorial Board of the Society are acutely aware that this delay is undesirable and to alleviate the situation, each part of Volume 74 (1980) will contain 144 pages.