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Survey for *Trypanosoma cruzi* Infection in a Municipality in Northeast Brazil

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An epidemiological survey of *Trypanosoma cruzi* infection was carried out in Bodocó, located in the western part of the State of Pernambuco, Brazil. Two hundred and forty-one individuals were parasitologically and immunologically screened. Although hemoculture did not reveal the presence of parasites in the blood, the sera of 5 individuals were scored as positive by the indirect fluorescence antibody test and the enzyme-linked immunosorbent assay. Seropositivity in individuals above and below the age of 40 was 14.8 and 0.5%, respectively. These results indicate that recent infections with *T. cruzi* are rare in this area. However, since a *T. cruzi*-infected triatomid (*Triatoma brasiliensis*) was captured in a school classroom, this area must be considered endemic. When triatomid feces containing parasites were inoculated into a jird (mongolian gerbil), parasitemia appeared 10 days later. Immunohistochemical staining, using monoclonal antibody specific for *T. cruzi*, labeled organisms in jird tissues. These observations demonstrate that the jird is a suitable host for experimental *T. cruzi* infections and that monoclonal antibody is effective for detection of the parasite in host tissues.

Key words : *Trypanosoma cruzi*, Serology, Jird, Monoclonal antibody, Immunohistochemistry

INTRODUCTION

Chagas' disease, caused by the protozoan parasite *Trypanosoma cruzi*, is estimated to afflict over 24 million people in South and Central America [17]. In Brazil, Chagas' disease is a major public health problem in many rural and suburban areas and many epidemiological studies have confirmed the endemicity of the disease in Northeastern Brazil [3-5, 7, 8, 10, 11, 14, 15]. However, there still exists sizable gaps in our knowledge concerning the distribution of triatomine vectors and the prevalence of Chagas' disease in well-delineated geographic locations [18]. The present study, therefore, was undertaken to evaluate the prevalence of *T. cruzi*-infection in a circumscribed area in Pernambuco State, located in Northeastern Brazil. We also report the isolation of a strain of *T. cruzi*, and the application of a species-specific monoclonal antibody [20] for the detection of *T. cruzi* in animal tissues.

MATERIALS AND METHODS

Subjects

The study was carried out in Bodocó, located 560 km west of Recife, the capital of the State of Pernambuco. In October 1990, 241 blood samples were collected in 4 locations; Sipaúba, Lagoa do Salviano, Recurso, and Retronco, from primary school children and from individuals residing near the schools.

Hemoculture

Two or 3 drops of blood were immediate-

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ly inoculated into liver infusion-tryptose (LIT) medium overlaid on Novy-MacNeal-Nicolle agar slants containing 12.5% rabbit blood. The slants were checked weekly for 2 months after inoculation for the presence of parasites.

Indirect fluorescence antibody test (IFAT)

Sera were immunologically screened after heat inactivation for 30 min at 56℃. IFAT was carried out essentially as previously described [23]. Formalin-fixed epimastigotes of *T. cruzi* Tulahuen were smeared on glass slides and used as antigen. For screening, sera were diluted 20-fold, and fluorescein isothiocyanate-labeled goat anti-human IgG and IgM (Medical & Biological Laboratories, Nagoya, Japan) were used as second antibodies. Positive sera were further titrated by twofold stepwise dilutions.

Enzyme-linked immunosorbent assay (ELISA)

ELISA using sonicated antigen was performed as previously described [19]. Sera were used at 400-fold dilution and horseradish peroxidase-labeled goat anti-human IgG (Medical & Biological Laboratories) was used as the second antibody. After development by *o*-phenylenediamine and H_2O_2, the optical density was read at 492 nm. ELISA positivity was defined as 3 standard deviations above the mean for 20 negative control sera, obtained from healthy Japanese who had never been to South and Central America.

Isolation of *T. cruzi* from a vector

Feces of captured bugs were mixed with phosphate buffered saline (PBS), and examined under a microscope for active flagellates. A positive emulsion, in PBS, was injected intraperitoneally into an adult male jird (mongolian gerbil) weighing 65 g. Ten days later, its peripheral blood was examined for parasitemia and for cultivation in LIT medium.

Immunohistochemistry

Tissues removed from the jird infected with *T. cruzi* were fixed in phosphate-buffered formalin, embedded in paraffin, and cut at 4 μm. Sections were deparaffinized in xylene and rehydrated in a graded ethanol series. The slides were immersed for 30 min in 0.3% H_2O_2 in methanol to reduce endogenous peroxidase activity. After washing, sections were incubated with 10% normal rabbit serum for 30 min and then overnight with 100 μl of mouse ascites containing monoclonal antibody TCF87 [20], diluted 1:20 in PBS. After washing, the sections were treated with biotinylated antibody and Vectastain ABC reagents (Vector Laboratories, Inc., Burlingame, USA) according to the manufacturer's instructions. After washing, the reaction was developed by 3,3'-diaminobenzidine and H_2O_2. Finally, the sections were counterstained with hematoxylin.

RESULTS

Serological and parasitological screening of *T. cruzi* infection

Although hemoculture did not reveal the presence of parasites in 241 blood samples, IgG antibody to *T. cruzi* was detected by IFAT in 4 individuals in Sipaúba and 1 in

Table 1 Seroprevalence of *Trypanosoma cruzi* infection in 4 locations in Bodocó, Pernambuco State, Brazil

Location	No. samples	No. IFAT positives (%)		No. ELISA positives (%)
		IgG	IgM	
Sipaúba	171	4 (2.3)	0	4 (2.3)
Lagoa do Salviano	20	1 (5.0)	0	1 (5.0)
Recurso	30	0	0	0
Retronco	20	0	0	0
total	241	5 (2.1)	0	5 (2.1)

Lagoa do Salviano (Table 1). These 5 sera were also positive by ELISA. However, specific IgM antibody was not detected. In contrast, seropositives were not observed in the samples from Recurso and Retronco. Table 2 indicates the age distribution of the people examined. Four of the 5 positives were individuals over 40 years of age. Of the 214 individuals who were younger than 40 years of age, only a 3 year-old boy scored positive, although his IFAT titer was 20-fold, which is at the lower limit of positivity.

Detection of *T. cruzi* from a vector and experimental infection in a jird

During the collection of blood in Recurso and Retronco, a number of triatomines were captured in schoolrooms. The species and number of bugs were as follow: 4 adults and 5 nymphs of *Triatoma brasiliensis* and a nymph of *Panstrongylus megistus* in Recurso, and 21 nymphs of *Triatoma brasiliensis* and a nymph of *P. megistus* in Retronco. Metacyclic trypomastigotes were detected in one of the 4 *Triatoma brasiliensis* adults.

A fecal emulsion containing parasites was inoculated intraperitoneally into a jird. Ten days later, parasitemia was observed in the peripheral blood. The jird was sacrificed, and its liver, spleen, pancreas, intestine, heart, and skeletal muscle examined by immunohistochemistry. Positive immunoperoxidase staining using TCF87, a monoclonal antibody against the *T. cruzi*-specific M_r 25,000 antigen, demonstrated amastigotes in all the tissues examined (Fig. 1). In skeletal muscle, in addition to amastigotes, trypomastigotes with stained flagellae were also observed in an inflammatory lesion (Fig. 2).

DISCUSSION

The present study showed that the seroprevalence to *T. cruzi* in Bodocó was 14.8% (4/27) in individuals over the age of 40, but only 0.5% (1/214) in those below 40, indicating that recent infections by *T. cruzi* are rare in this area. A similar trend has also been observed in a community in the adjacent state of Piauí [10]. The fact that no positives were detected either by hemoculture or by specific IgM antibody demonstrates that the seropositives were in the chronic phase of the disease [6]. However, it seems unlikely that the 3 year-old boy with the low IFAT titer is chronically infected. When the single positive serum was examined by the competitive ELISA using *T. cruzi*-specific monoclonal antibody TCF87 [22, 24], it tested negative suggesting that the results of the IFAT and ELISA might be false-positives (data not shown).

In spite of the very low rate of seropositivity in the younger population, a *T. cruzi*-infected triatomid was captured in Recurso where no seropositives were detected. It may well be that *T. cruzi* is completing its life cycle in wild or domestic animals at present.

In this study, when metacyclic trypo-

Table 2 Age distribution of individuals examined and IFAT titers of positives to *Trypanosoma cruzi*

Age	No. samples	No. IFAT (IgG) and ELISA positives (%)	IFAT (IgG) titers
2-9	96	1 (1.0)	20
10-19	85	0	
20-29	21	0	
30-39	12	0	
40-49	15	2 (13.3)	320, 640
50-59	8	1 (12.5)	640
60-69	3	0	
70-76	1	1 (100)	80
total	241	5 (2.1)	

mastigotes in bug feces were inoculated into a jird, parasitemia was observed 10 days later. To our knowledge, this is the first report indicating that the jird is susceptible to *T. cruzi* infection. Although it is well known that the jird is a suitable host for filarial worms [2, 26], the present study shows that the jird may be a good model for experimental *T. cruzi* infection as well. The *T. cruzi* isolate axenized via the jird and designated the LIKA strain, has since been subcultured for further study.

The present study also indicated that TCF87 is capable of detecting not only amastigotes but trypomastigotes of *T. cruzi* in host tissues. It is not difficult to detect large clusters of amastigotes in tissue sections stained routinely with hematoxylin and eosin, but small clusters of parasites are easily overlooked under low magnification.

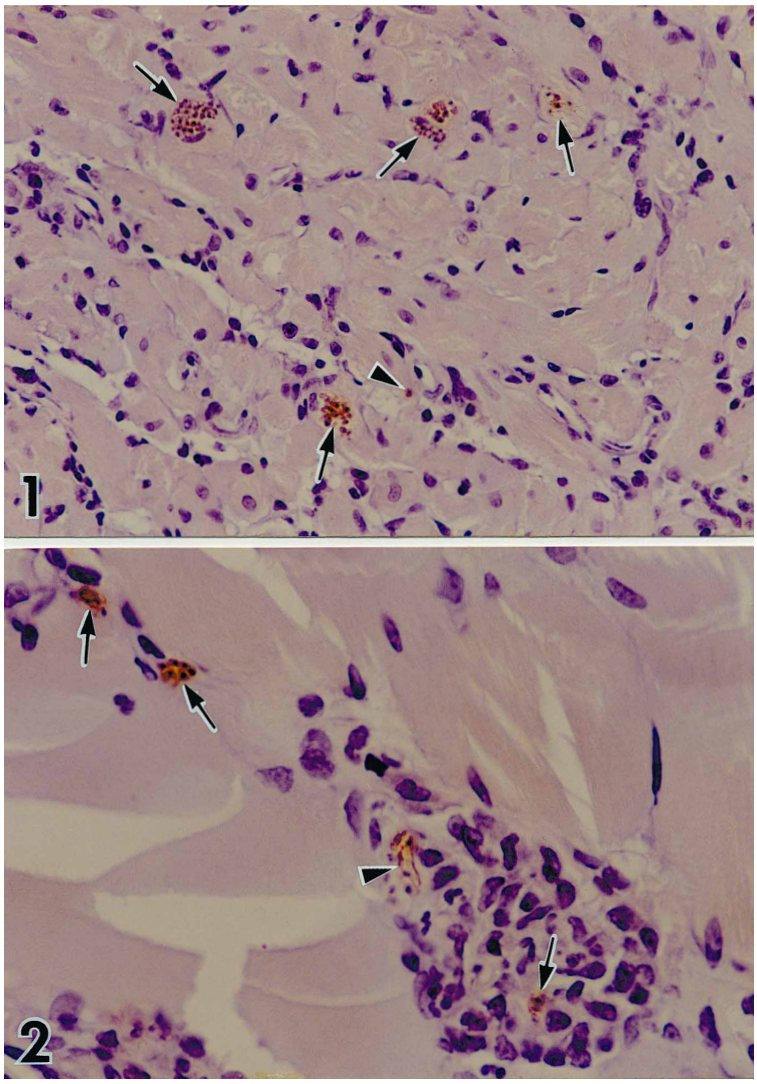

Fig. 1 Immunoperoxidase staining by monoclonal antibody TCF87 of *T. cruzi* in infected jird heart. Arrows indicate clusters of amastigotes. The arrowhead indicates a single amastigote. ×300.

Fig. 2 Immunoperoxidase staining by TCF87 of trypomastigotes (arrowhead) and amastigotes (arrow) of *T. cruzi* in an inflammatory lesion in infected jird skeletal muscle. ×600.

Recent studies have demonstrated that immunoperoxidase staining using polyclonal antibodies is useful for the detection of *T. cruzi* [9, 12, 16]. However, it has also been reported that *T. cruzi* shares cross reactive antigens with other trypanosomatids and mammalian tissues [1, 13, 25]. Since the TCF87 used in this study is reactive only with the species-specific antigens located on the plasma membrane and flagellum of *T. cruzi*, regardless of strain or developmental stage [20, 21], a reduction in non-specific binding and greater accuracy in diagnosis can be expected. Therefore, this method may be applicable on tissues obtained from patients with presumed Chagas' disease for an accurate diagnosis.

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