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EPIDEMIOLOGICAL SURVEY OF Trypanosoma cruzi INFECTON IN NORTH-EASTERN BRAZIL USING DIFFERENT DIAGNOSTIC METHODS(1)

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SUMMARY

A survey of the prevalence of **Trypanosoma cruzi** infection was carried out in Oitis, a small community in the State of Piaui, Brazil. Two hundred and sixty five individuals were screened by microscopic examination, hemoculture, indirect immunofluorescence (IFA), enzyme-linked immunosorbent assay (ELISA), and competitive enzyme-linked immunosorbent assay (C-ELISA) using the monoclonal antibody TCF87 against to a 25kd **T.cruzi** antigen. Seropositivity was 14.3% by the IFA test, 14.7% by ELISA, and 13.2% by C-ELISA. The C-ELISA using the TCF87 monoclonal antibody seems to be applicable in serodiagnosis of Chagas' disease.

KEY WORDS: Trypanosoma cruzi; Epidemiological survey; Serodiagnosis; Indirect immunofluorescence; Enzyme-linked immunosorbent assay; Competitive enzyme-linked immunosorbent assay.

INTRODUCTION

Chagas' disease, caused by the infection with **Trypanosoma cruzi,** is restricted to the American continent and affects about 10-12 million inhabitants[2]. In Brazil, including the Northeastern region Chagas' disease is still a serious problem of public health. Since only a few population-based surveys have been conducted in that region, an epidemiological survey was carried out in a rural community of the Piaui State, using several serological and parasitologic diagnostic methods. Serology is very useful for population surveys and epidemiological purposes[7]. In this study, we employed several immunological methods, including a competition enzyme-linked immunosorbent assay (C-ELISA) using a monoclonal antibody against **T.cruzi**-specific 25kd antigen[19]. On the basis of our results, we estimated the prevalence of **T. cruzi** infection to be about 14% in this small community in Northeastern Brazil.

MATERIALS AND METHODS

Subjects

Two hundred and sixty five individuals were selected for this study in Oitis, a small, community located on central part of Piaui State and about 250 km apart from Teresina, the capital of the State. The population of this community is nearly 1,000 and farming and stock raising are the main economic activities.

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Parasitological methods

From each individual, blood smears and hemocultures were prepared. The smears were microscopically examined after staining with Giemsa solution. One or two drops of the blood were immediately inoculated in Novy-MacNeal-Nicoll culture system (NNN). NNN culture was performed accoording to the modified procedure by KANEDA [14];the solid phase consisted of 1.5% agar containing 12.5% defibrinated rabbit blood and liver-infusion-tryptose medium was employed as the overlay. The cultures were examined at 14,30,60 and 90 days post-inoculation.

Xenodiagnosis was applied to 9 subjects, some of them had been examined 12 years ago in a prior survey[1]. Procedure of xenodiagnosis was as previously described[1] and the following triatomids were used: **Triatoma infestans, T. brasiliensis, Panstrongylus megistus**, and **Dipetalogaster maximus.**

Serological tests

Serum samples obtained from each individual were examined by using immunofluorescence antibody test (IFA), enzyme-linked immunosorbent assay (ELISA) and a competitive ELISA (C-ELISA). In IFA test, anti-**T. cruzi** IgG and IgM were examined with formaldehyde-fixed epimastigotes. Anti-**Leishmania** IgG was also examined by using **Leishmania donovani** promastigotes. A titer of 1:20 or greater was judged as positive. ELISA method was essentially performed according to the procedure previously described[19]. Briefly, wells of a polyvinyl chloride microtiter plates were treated with 4 ug protein of sonicated epimastigotes of **T. cruzi**. After addition of 400-fold diluted test serum, the mixture was incubated for 1 hr at 23°C. The plates were then washed with PBS containing 0.05% of Tween 20, and incubated with a 1:1,000 dilution of horseradish peroxidase-labeled anti-human IgG goat antibody for 1 hr at 23°C. The reaction was quantified at 492 nm in the presence of ophenylenedianine and H_2O_2. C-ELISA test using the TCF87 monoclonal antibody was done as previously reported[20]. Briefly, wells of microtiter plates were treated with **T. cruzi** epimastigotes as described above. To the wells were added simultaneously 50 ul test serum diluted 1:10 in PBS containing 1% ski milk and 50 ul horseradish peroxidase-labeled monoclonal antibody (TCF87) diluted in PBS-Tween, and the plates were incubated for 3 hr. After washing with PBS-Tween, the reaction was quantified as above. The cut-off values were the mean O.D. of negative controls plus 3 SD for ELISA, and minus 3 SD for C-ELISA. Normal serum of health japanese who had never been in endemic areas were used as negative controls for the serological tests.

RESULTS

A total of 265 individuals were examined. The age distribution of subjects and the number of IFA and C-ELISA positives summarized in Table 1. Thirty-eight individuals were positive by IFA, with only one, an 11 year-old boy, under the age of 24. Sex differences in seropositivity were not observed. The prevalence of the positives in the 265 individuals was 14.3% by IFA and 13.2% by C-ELISA. Limited clinical informations were obtained from all individuals; for children under 5 years, the mother was interviewed. The commonest complaints were: cardiac problems, 18 individuals; hypertension, 7; thoracic ache, 5; fever, 2. However, these complaints could not be correlated with IFA and C-ELISA positive titers.

Table 1 - Correlation between age and seropositivity for **T. cruzi** infection. Results are based on indirect immunofluorescence test (IFA) and competition enzyme-linked immuno sorbent assay (C-ELISA).

		IFA positive		C-ELISA positive	
Age	No.	No.	Prevalence (%)	No.	Prevalence (%)
0- 4	21	0	0	0	0
5- 9	56	0	0	0	0
10-14	60	1	1.7	1	1.7
15-19	27	0	0	0	0
20-24	6	0	0	0	0
25-29	17	2	11.8	2	11.8
30-34	16	6	37.5	6	37.5
35-39	14	4	28.6	4	28.6
40-44	13	5	38.5	5	38.5
45-49	11	6	54.5	5	45.5
50-54	7	3	42.9	2	28.6
55-59	5	3	60.0	2	40.0
60-64	2	1	50.0	1	50.0
65-69	7	4	57.1	4	57.1
70-74	2	2	100.0	2	100.0
75<	1	1	100.0	1	100.0
Total	265	38	14.3	35	13.2

Table 2- Comparison of 6 **T. cruzi** diagnostic procedures used to screen 265 individuals. The 41 subjects listed below tested positive by at least 1 of the serological procedures

Sujects		IFA titers		ELISA	C-ELISA	Xeno-diag-nosis	Anti-leishmania IgG titers
Age	Sex	IgG	IgM				
52	m	1280	N	P	P	nd	160
48	m	640	N	P	P	nd	160
30	f	2560	20	P	P	N	160
73	m	640	N	P	P	N	80
53	f	1280	10	P	P	N	80
49	f	320	N	P	P	N	40
36	f	320	N	P	P	N	80
29	m	1280	N	P	P	P	160
66	f	2560	N	P	P	N	320
32	f	160	N	P	P	nd	20
48	f	1280	10	P	P	nd	320
27	f	N	N	P	N	nd	N
60	m	320	10	P	P	nd	80
39	f	80	N	P	P	nd	N
49	f	1280	10	P	P	nd	320
58	m	640	N	P	P	nd	160
70	m	2560	10	P	P	N	320
32	f	320	N	P	P	nd	40
11	m	N	N	P	N	nd	N
76	f	640	N	P	P	nd	160
58	m	320	N	P	P	P	40
55	f	40	N	N	N	nd	N
11	m	640	10	P	P	nd	160
20	f	N	N	P	N	nd	N
41	f	1280	20	P	P	nd	160
27	f	1280	20	P	P	nd	160
32	f	1280	N	P	P	nd	80
49	f	320	10	P	N	nd	20
32	f	1280	N	P	P	nd	80
65	f	320	N	P	P	nd	80
37	f	1280	10	P	P	nd	80
34	f	640	N	P	P	nd	80
49	m	640	N	P	P	nd	40
69	f	640	N	P	P	nd	160
44	f	640	N	P	P	nd	80
42	m	2560	10	P	P	nd	160
50	f	20	N	N	N	nd	N
66	m	320	N	P	P	nd	80
40	f	1280	10	P	P	nd	80
41	m	640	N	P	P	nd	80
39	m	2560	20	P	P	nd	80

N: negative; nd: not done; P: positive

The IFA and/or ELISA seropositive findings are listed in Table 2 and compared with several other diagnostic methods. Anti-**T.cruzi** specific IgM was also detected by the IFA, generally in individuals with high IgG titers. Xenodiagnosis was performed on nine individuals, and parasitemia was confirmed in two individuals who were seropositive in all tests, but not in the IFA. Hemoculture and blood smears were negative in all individuals.

Of the 265 surveyed subjects, 39 were ELISA-positive and 38 were IFA-positive; 36 were positive by both tests (Table 3). Of the positive sera in IFA, two cases were negative in both ELISA and C-ELISA tests. These cases also had the lowest IFA titers. It is therefore reasonable to assume that these individuals are not infected with **T. cruzi.**

Anti-**Leishmania** IgG was also monitored in the 265 subjects. Of these, 35 were positive. Their titers correlated with anti-**T. cruzi** IgG titer.

Table 3- Comparison of results with ELISA, C-ELISA and IFA tests in the diagnosis of **T.cruzi** infection

		IFA	
		Positive	Negative
ELISA	Positive (39)	36	3
	Negative (226)	2	224
C-ELISA	Positive (35)	35	0
	Negative (230)	3	227
Total (IFA)		38	227

DISCUSSION

A number of parasitological and serological procedures are available for the diagnosis of Chagas' disease, such as microscopic examination, hemoculture, xenodiagnosis, IFA, ELISA and C-ELISA. An absolute diagnose is based on identifying the parasite in patient specimens. However, parasitological procedures are impractical for use in mass survey or in most chronic patients, because of the excessive amount of time and effort for obtaining results. For example, xenodiagnosis could not be performed on all the subjects in this survey due to an insufficient number of triatomines. In addition, the sensitivity of these procedures is much lower than that of the serologic test, as is shown in the present study. BROFEN et al.[3] and CHIARI et all. [9] reported hemoculture and xenodiagnosis data with a higher incidence of positives than the present survey. These differences may be due to the very large number of triatomines and volume of blood they used: 30 ml of blood and 40 triatomine nymphs per patient.

For the diagnosis and large scale screening of Chagas' disease, as stated by CAMARGO [4,6], serologic procedures are recommended because of their simplicity, specificity and sensitivity. The IFA test, in particular, turns positive early in the infection[8]. The test is practical for routine purposes since freeze-dried antigens can be prepared and there are excellent, readily available, commercially prepared conjugates. Besides, through the use of specific anti-IgM conjugates, this test is useful for the demonstration of IgM antibodies to **T. cruzi.** CAMARGO & AMATO NETO[5] demonstrated that anti-**T. cruzi** IgM was found in most acute cases, although seldom detected at the chronic stage of Chagas' disease. We also measured specific IgM levels with the IFA test, but were unable to detect high IgM titers, even in two cases where parasites were isolated by xenodiagnosis.

Generally, the sensitivity of serological tests is higher than that of parasitological procedures, and ELISA tests seems to be the most sensitive. In the present study, three of 39 ELISA-positive sera may be false positive, because they were not confirmed by the other tests. Therefore, there may be a problem with the specificity. In order to promote specificity, serological tests using purified glycoproteins of **T. cruzi** were reported by SCHARFSTEIN et al.[17] and SCHECHTER et al.[18]. However, these tests are very expensive and require purified antigens, precluding large-field applications. The C-ELISA test using a monoclonal antibody against maturational products of **T. cruzi**-specific glycoprotein (GP72) was developed for serodiagnosis of Chagas' disease[11,15]. We also developed a C-ELISA using monoclonal antibody (TCF87) against **T. cruzi** specific 25kd antigen [20]. The use of TCF87 monoclonal antibody in C-ELISA allows precise immunodiagnosis for Chagas' disease, and was used to detect chagasic patients throughout South America, and to discriminate between Chagas' disease and leishmaniasis. In the

present survey, the C-ELISA procedure was further evaluated by this field application. Based in our findings, this test seems useful for serodiagnosis of Chagas' disease.

The occurrence of Chagas' disease in the Piaui State was first mentioned in 1916 by NEIVA & PENNA [16]. Thereafter, the disease was rarely reported in this region, undoubtedly due to the lack of organized surveys. In 1975, the first reliable autochthonous cases were described [12], and the distribution of 7 triatomine species was reported[13]. CORREIA-LIMA et al.[10] determined that prevalence of **T.cruzi** infection, by the IFA test, was 18.6% in Oitis. In the present study, the prevalence was 14.3% by the IFA test. In a geographically wider survey, CAMARGO et al.[7] estimated the prevalence of seropositivity in Piaui state at 4.04%, on the basis of random sampling. Follow-up surveys are necessary to obtain a truly reliable indication of the prevalence of Chagas' disease in this endemic area.

RESUMO

Pesquisa epidemiologica da infecção por *Trypanosoma cruzi* no nordeste brasileiro utilizando-se diferentes métodos diagnósticos

A prevalência da infecção pelo **Trypanosoma cruzi** foi pesquisada em Oitis, uma pequena comunidade do Estado do Piauí. Duzentos e sessenta e cinco indivíduos foram investigados pelos seguintes métodos: pesquisa direta, hemocultura, imunofluorescência indireta (IFA), teste imunoabsorvente ligado a enzima (ELISA), e ELISA de competição (C-ELISA) com a utilização do anticorpo monoclonal TCF87 contra um antígeno do **T. cruzi** com 25kd. A IFA foi positiva em 14,3% dos indivíduos, ELISA em 14,7% e C-ELISA em 13,2%. Este último teste mostrou-se aplicável no diagnóstico sorológico da Doença de Chagas.

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