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SEROPOSITIVITY FOR ANTI-*TRYPANOSOMA CRUZI* ANTIBODIES AMONG BLOOD DONORS OF THE "HOSPITAL UNIVERSITÁRIO REGIONAL DO NORTE DO PARANÁ", LONDRINA, BRAZIL (1)

Edna Maria Vissoci REICHE (2), Marta Mutsumi Zaha INOUYE (2), Rubens PONTELLO (2), Helena Kaminami MORIMOTO (3), Shiduca ITOW JANKEVICIUS (4), Tiemi MATSUO (5) & José Vitor JANKEVICIUS (6)

SUMMARY

The most frequent form of acquisition of Chagas' disease in endemic areas was the transmission through the feces of contaminated triatominae. However, special attention should be paid in urban areas to transmission by blood transfusion, justifying the compulsory screening of blood donors. Early investigations at blood banks in the town of Londrina, Brazil, demonstrated that the seroprevalence of anti-*Trypanosoma cruzi* antibodies among blood donors was approximately 7.0% in the fifties[9, 34]. Further studies demonstrated pratically the same seroprevalence until the eighties[4, 32, 41]. In an attempt to obtain data about the real dimension of the seropositivity for anti-*Trypasonoma cruzi* antibodies in the region, the authors carried out a large-scale study on 45,774 serum samples from blood donors of the Hemocentro of Hospital Universitário Regional do Norte do Paraná (HURNP), Universidade Estadual de Londrina. The immunological tests were done at the Division of Clinical Immunology of HURNP from May 1990 to December 1994. The serum samples were studied by the indirect hemagglutination assay (IHA, using kits commercially obtained from EBRAM) and by indirect immunofluorescence (IFI, using kits from LIO SERUM) with anti-human IgG conjugate (LABORCLIN). The results demonstrated that 643 serum samples were positive in both assay corresponding to a seroprevalence of 1.4%, i.e., a significant decrease in anti-*Trypanosoma cruzi* antibodies in the region in comparison with the previously mentioned rates. Data correlating sex and age of seropositive blood donors are presented, as well as the possible factors that may have contributed to the results observed.

KEYWORDS: Blood donors; Chagas' disease; *Trypanosoma cruzi*.

INTRODUCTION

Chagas' disease (CD), caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) is still a major Public Health problem in rural, suburban and urban areas of Central and South America, where 16 to 18 million people are infected, from Mexico in North America to Argentina and Chile in South America[39]. There are more than 100,000 infected persons in the United States and that number should increase because of the immigrants from endemic countries[22]. Although most cases of human *T. cruzi* infection have been acquired through contact with contaminated feces of infected triatomine bugs in rural areas, other ways of acquiring the infection are becoming relevant. While the classical transmission is being controlled by sanitary programs to eradicate the vectors in endemic areas, blood transfusion has

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(2) Professor Adjunto de Imunologia Clínica, Centro de Ciências da Saúde, Universidade Estadual de Londrina. Londrina, Paraná, Brazil.
(3) Professor Auxiliar de Imunologia Clínica, Centro de Ciências da Saúde, Universidade Estadual de Londrina. Londrina, Paraná, Brazil.
(4) Professor Titular de Microbiologia Geral, Centro de Ciências Biológicas, Universidade Estadual de Londrina. Londrina, Paraná, Brazil.
(5) Professor Adjunto de Estatística, Centro de Ciências Exatas, Universidade Estadual de Londrina. Londrina, Paraná, Brazil.
(6) Professor Adjunto de Microbiologia Geral, Centro de Ciências Biológicas, Universidade Estadual de Londrina. Londrina, Paraná, Brazil.
Correspondence to: E.M.V. Reiche, Centro de Ciências da Saúde, Universidade Estadual de Londrina. Rua Robert Koch 60. 86038-440 Londrina, Paraná, Brazil.

become the most frequent way of transmission, mainly in urban areas. The seroprevalence of CD among blood donors in some American countries is highly variable, ranging from negative to 62%, (Table 1) and from negative to 19.10% in Brazil (Table 2). A review of the incidence of transfusion associated with *T. cruzi* infection cited in literature was published in 1992[43]. Reports of 10,000 to 20,000 new cases of CD occurring yearly in Brazil alone were published in the seventies[39].

Previous investigations among donor of blood banks in Londrina, Paraná state, Brazil, showed rates of seroprevalence around 7.0% in the fifties[9, 34]. Further studies demonstrated practically the same rates until the eighties[4, 7, 32, 37, 41]. In an attempt to obtain data about the real dimension of the seropositivity of anti-*T. cruzi* antibodies among blood donors in the region, a serological survey was proposed with the results of CD among blood donors of Hemocentro of Hospital Universitário Regional do Norte do Paraná (HURNP), Londrina, Paraná, Brazil.

MATERIAL AND METHODS

The authors reviewed the results of serological tests performed on 45,774 serum samples from blood donors seen at Hemocentro of Hospital Universitário Regional do Norte do Paraná, Londrina, Brazil, collected during the period from May 1990 to December 1994. The sample size could be considered representative for the purpose, due to the characteristics of the city and of the hospital. Londrina is located in the north of Paraná state, in a metropolitan region and the Hemocentro of the HURNP attends the population of the greater region. The serological tests were performed at the Division of Clinical Immunology of HURNP, Universidade Estadual de Londrina (UEL). Two method were employed: qualitative indirect hemagglutination assay (IHA) with commercially available reagents (EBRAM) with initial serum dilution of 1:32 and qualitative indirect immunofluorescence (IFI) carried out with fixed *T. cruzi* antigen on microscope slides, also commercially provided by LIO SERUM, with initial serum dilution of 1:40 and anti-human IgG conjugate supplied by LABORCLIN.

The results of IHA and IFI were scored as positive, negative or dubious according to standard procedures[11, 12]. To calculate the rate of prevalence of CD in the sample analyzed, the dubious results were scored as negative and results were considered positive only when both tests (HAI and IFI) were positive. Samples with discordant results were also considered to be negative[13]. The cut-off values were

TABLE 1
Seroprevalence of anti-*Trypanosoma cruzi* antibodies among blood donors in various American countries.

Author	Year	Country	Sample	Seroprevalence (%)
Rassi & Rezende	1976	Eight Latin American Countries	125,505	5.42-28.00
Raby et al.	1989	Valparaiso, Viña del Mar (Chile)	16,358	0.48
Schmunis	1991	Argentina	256,463	1.70-23.90
		Bolivia	1,477	4.90-62.00
		Colombia	491	7.50
		Equador	26,167	0.00-0.83
		Venezuela	972,599	1.33-2.08
		Costa Rica	2,574	0.80-1.60
		Guatemala	1,260	5.00
		Honduras	1,225	11.60
		Mexico	200	17.50
		USA	1,227	0.00-0.097
		Brazil	156,862	0.09-14.60
		Chile	17,233	0.03-29.00
		Uruguai	84,369	0.92-7.70
Landivar et al.	1992	Santa Cruz, Bolivia	316	48.7
Pan et al.	1992	United States of America	13,309	0.166
Ache	1993	Venezuela	961,933	1.20
Contreras et al.	1993	Jalisco Mexico	3,419	1.28
Guevara et al.	1994	Endemic Peruvian areas (South States)	2,174	3.5-7.2

TABLE 2
Seroprevalence of anti-*Trypanosoma cruzi* antibodies among blood donors in Brazil, reported by various authors.

Author	Year	Place	Sample	Prevalence (%)
Biancalana et al.	1953	Araguari (MG)	233	19.10
Biancalana et al.	1953	S.J. Rio Preto (SP)	134	14.90
Queiroz & Pascual	1958	Londrina (PR)	1,330	6.90
Brofman	1958	Londrina (PR)	NR	7.00
Jatene & Jacomo	1959	Uberaba (MG)	640	15.00
Freitas & Siqueira	1959	Ribeirão Preto (SP)	3,055	14.40
Freitas & Siqueira	1959	Ribeirão Preto (SP)	6,405	10.80
Pellegrino	1959	Belo Horizonte (MG)	10,982	6.79
Mellone & Pagenotto	1965	São Paulo (SP)	62,575	1.45
Coura	1966	Rio de Janeiro (RJ)	4,595	1.26
Cotarelli et al.	1972	Londrina (PR)	2,110	6.44
Campos et al.	1975	Goiânia (GO)	4,372	10.43
Amato Neto et al.	1977	São Paulo (SP)	4,714	1.01-2.50
Baldy et al.	1978	Londrina (PR)	3,744	7.90
Baldy et al.	1978	Londrina (PR)	726	5.20
Takaoka et al.	1990/1980	Londrina (PR)	3,000	7.40
Buainain et al.	1981	Araraquara (SP)	6,236	1.18
Reiche et al.	1982	Londrina (PR)	35,968	4.91
Pontello et al.	1985	Northern Paraná (PR)	1,977	7.20
Zicker et al.	1990	Goiânia (GO)	1,358	3.50
Ling et al.	1992	Londrina (PR)	1,196	0.00-1.82
Ling et al.	1992	Londrina (PR)	13,429	1.22
Blood Epidemiological Bulletin	Jan-Jun 1993	Northern Region (BR)	43,502	0.20
		North-East (BR)	151,971	0.48
		South-East (BR)	408,352	0.45
		South (BR)	75,511	1.31
		Center-West (BR)	56,552	1.47
Beck et al.	1993	Santiago (RS)	1,140	9.20
Osaida et al.	1993	Santa Maria (RS)	14,036	3.31
Wanderley et al.	1993	São Paulo (SP)	74,930	1.30
Moraes-Souza et al.	1994	850 Brazilian cities	622,985	0.97

NR – not reported

1:32 for HAI and 1:40 for IFI according to the standardization for serodiagnosis for CD recommended by the manufacturers.

RESULTS

The frequency of all the possible results obtained with both tests (IHA and IFI) is presented in Table 3. The results were in agreement in 97.02% of the samples and in disagreement in 2.46%. Some samples (0.48%) showed interference in both tests, probably because they were excessively lipemic.

Table 4 shows the serological positivity to anti-*T. cruzi* antibodies in 45,774 serum samples from blood donors of Hemocentro of HURNP, Londrina, Brazil, from 1990 to 1994. Total seropositivity was 1.4%, corresponding to 643 samples with positive results to both methods employed. No wide variability was detected since the seropositivity for anti-*T. cruzi* antibodies obtained in the period studied was not statistically significant, ranging from 1.13% to 1.44%, except for 1991, when the rate was 2.03% ($x^2 = 24.36$, $p < 0.00002$). As far as the age of the blood donors is concerned, the higher rates of seropositivity were obtained in older individuals: 6.36% for subjects 61 to 70 years old

and 7.14% for subjects older than 70 years, in contrast to no seropositivity for subjects less than 20 years old. Table 5 presents the seroprevalence of anti-*T. cruzi* antibodies according to blood donor sex and age. Of 45,774 samples studied, 31,162 (68.07%) were from males and 6,771 (14.79%) from females, and no information was available for 7,841 (17.12%) samples. Among the samples with sex identification, positive results were obtained for 393 samples from males and 134 from females, with respective rates of 1.26% and 1.97%. Statistical analysis showed that the rate for males was significantly higher ($x^2 = 20.40$, $p < 0.0001$). Higher rates were obtained for older individuals: 5.45% for 61 to 70 year old males ($x^2 = 243,616$, $p < 0.0001$) and 16.67% for females older than 70 years ($x^2 = 105,682$, $p < 0.0001$). Statistical analysis of trends showed that prevalence rates increased with donor age ($x^2 = 215,638$, $p < 0.001$ for males and $x^2 = 90,354$, $p < 0.0001$ for females. Statistical analysis by the Chi-square or Fisher's Exact test showed significant differences in seroprevalence between males and females and between age ranges, with significantly higher values for females in the 31 to 40 year and 41 to 50 year age ranges and in the group without age registration.

TABLE 3

Frequency of all possible results obtained with IHA an IFI tests performed in 45,774 serum samples of blood donors of Hemocentro of HURNP, Londrina, Paraná, Brazil, 1990-1994.

IHA	IFI	n	%
Negative	Negative	43,651	95.36
Positive	Positive	643	1.40
Negative	Positive	428	0.93
Positive	Negative	12	0.02
Negative	Dubious	354	0.77
Dubious	Negative	182	0.39
Dubious	Dubious	121	0.26
Positive	Dubious	20	0.04
Dubious	Positive	142	0.31
Interference (*)	Interference (*)	221	0.48
Total	45,774	100.00	(*)

(*) = Lipemic samples.

DISCUSSION

The serological indicator is a useful tool for monitoring the index of exposure to *T. cruzi* in human populations and serologic screening at blood banks has been chosen for evaluating the possibility of transmission of CD in endemic areas[40]. Brazilian policy[14] requires the use of two tests with different serological techniques for CD screening among blood donors, chosen among available tests such as immunofluorescence, hemagglutination, direct agglutination, complement fixation and enzyme-linked immunosorbent assay (ELISA). The results of the two techniques used in the present study (Table 3) indicate high agreement between the IHA and the IFI (97.02%), justifying their use in serological screenings, mainly in those laboratories unable to routinely use other techniques that are more sensitive and specific such as ELISA or Western Blot. With the low prevalence of infected donors (< 2%) detected at most blood collecting facilities (Table 2), one has to expect a low positive predictive value, leading to a high rate of false-positive results that must be confirmed by other methods[44].

In addition, the IHA test employed here detects *T. cruzi* antibodies by the agglutination of red blood cells from different animal species (sheep, chicken, humans) sensitized

TABLE 4

Seropositivity to anti-*T. cruzi* antibodies according to blood donors age groups, from 1990 to 1994 at Hemocentro of HURNP, Londrina, Paraná, Brazil.

Age groups (Years)	1990	1991	1992	1993	1994	Sample	Prevalence %
	+ %	+ %	+ %	+ %	+ %		
10-20	ND	0	0	0	0	888	0
21-30	ND	0.28	0.27	0.19	0.30	13,238	0.26
31-40	ND	1.48	1.13	1.09	1.16	11,346	1.19
41-50	ND	3.90	2.98	2.72	2.09	6,788	2.81
51-60	ND	3.63	2.91	1.56	2.39	2,730	2.60
61-70	ND	5.61	9.41	2.08	6.67	267	6.37
> 70	ND	16.67	0	0	0	14	7.14
Not determined	1.35	2.95	2.85	3.95	2.71	10,503	1.83
Total	1.35	2.03	1.44	1.20	1.13	45,774	1.40

ND - Not Determined.

TABLE 5

Positivity of serologic tests of anti-*T. cruzi* antibodies according to blood donor sex and age from 1990 to 1994 at Hemocentro of HURNP, Londrina, Paraná, Brazil.

AGE (Year)										p
	(+)	n	%	(+)	n	%	(+)	n	%	
10-20	0	740	0	0	148	0	0	888	0	NS
21-30	29	10,856	0.27	6	2,382	0.25	35	13,238	0.26	NS
31-40	103	9,400	1.10	32	1,946	1.64	135	11,346	1.19	0.0422
41-50	143	5,551	2.58	48	1,237	3.88	191	6,788	2.81	0.0121
51-60	54	2,267	2.38	17	463	3.67	71	2,730	2.60	NS
61-70	12	220	5.45	5	47	10.64	17	267	6.36	NS
> 70	0	8	0	1	6	16.67	1	14	7.14	NS
ND	52	2,120	2.45	25	542	4.61	77	2,662	2.89	0.0113
Total	393	31,162	1.26	134	6,771	1.97	527	37,933(a)	1.40	

ND = Not determined
NS = Not significant
(a) = 7,841 samples with no sex identification

with extracts of the parasite. Since sera from infected and noninfected individuals may have nonspecific antibodies, especially heterophilic antibodies, which could promote direct red blood cell agglutination, false-positive results may be expected. This could be circumvented by controls with unsensitized cells, usually not supplied in commercial kits.

Routinely, the serological diagnosis of parasitic diseases lacks specific antigens, stardardization and quality control of the reagents, resulting in divergent results among different laboratories, as previously shown[33]. Depending on the antigens used, different degrees of cross-reactivity with other diseases occur, mainly mucocutaneous or visceral leishmaniasis, because of common epitopes between *T. cruzi* and *Leishmania* sp. Cross-reactions have been observed with antibodies to African trypanosomes and to a lesser extent, to *T. rangeli*. Antigenic structures shared with several insect trypanosomatids, such as the genus *Herpetomonas* and *Crithidia* have also been observed. An antigenic relationship also exists between *T. cruzi* and several strains of *Phytomonas serpens*, a parasite of edible fruits such as tomatoes, whose antibodies may interfere with the serological tests for CD[8]. Common antigenic determinants may be found in very different structures such as sheep erythrocytes or human tissues, such as myocardium and nerves. False-positive results due to natural IgM antibodies are frequently observed, mainly in patients of low socio-economic background, probably reacting with phosphocholine, an antigenic fraction common to many parasites such as *T. cruzi, Leishmania, Toxoplasma, Filaria, Toxocara* and bacteria[14]. Thus, positive serological results may not represent actual clinical CD cases and prevalence studies may overestimate the actual prevalence of the disease and must be carefully considered.

The hypothesis previously discussed could also explain the rate of 2.46% of contradictory and dubious results obtained in the sample analyzed, as shown in Table 3. Contradictory and dubious results also could be explained by the fluctuation of the antibody levels of the chagasic patients[14]. Although the serological techniques for CD have high sensitivity, from 95 to 100% in the chronic phase of the disease, as determined in the present study (97.02%), the main problem of the serological screening for CD patients at blood banks is the variable percentage of samples showing reactivity in the "gray-zone" around the cut-off values[14]. Confirmatory tests should be used routinely to elucidate these results. This is still a major problem in the diagnosis of CD at blood banks, while no standard confirmatory tests of CD are available, in contrast to other infectious agents[44].

The present 1.4% rate of seropositivity for *T. cruzi* antibodies shows a considerable reduction of *T. cruzi* infection in recent years. This progressive decrease could be mainly attributed to four factors. First, the delayed effect of vector control as the result of the efficiency of the sanitary measures obtained with the National Control Program of Chagas' Disease in the 1980's, which eradicated the vectors in 85% of the endemic areas[19]. A highly efficient vector control program started in the 1970's has led to a virtual elimination of natural transmission from large areas of *Triatoma infestans* distribution, resulting in a relevant decrease of the number of cases and deaths attributed to CD[40]. This factor may explain the data presented in Tables 4 and 5. As expected, the rates of seropositivity for anti-*T. cruzi* antibodies increased with the age of the blood donors studied and were concentrated among older subjects in the age groups from 41 to 50 years old (2.81%), 61 to 70 (6.37%) and above 70 years old (7.14%) in contrast to 0% in subjects

less than 20 years old. Similar results were obtained with evaluation by serological tests of the young population aged 7 to 14 years. The positive rate for these groups in selected municipalities of some Brazilian states was 0.09%[40].

The second factor could be intrinsic to the transfusional system and its improvement. In other words, the quality of the hemotherapy services carried out in our country has improved significantly. In 1990, 92.9% of the hemotherapy services in the State of São Paulo, Brazil, utilized two or more serological techniques of high sensitivity, such as IFI and ELISA, in the routine serodiagnosis of CD at blood banks[42]. This fact certainly contributed to a safer and adequate serological screening of blood donors.

A third factor is that a previous positive serodiagnosis reduces the chance of seropositive blood donors returning to the hemotherapy services[28].

A fourth factor could be the pattern of sociological development in endemic areas[43]. The area of Londrina used to be primarily rural but has recently become more developed, with an increased population migration to urban areas. Therefore, it is possible that presently our region has more blood donors born in urban areas than was the case 20 years ago, with a reduced chance of natural transmission of CD.

The significantly higher prevalence of anti-*T. cruzi* antibodies among female than male blood donors should be carefully analyzed because the heterogeneity of the sample may influence the results obtained. This fact may be relevant in terms of the prevalence of the disease, due to the possibility of congenital transmission and should be better investigated.

Although the rates obtained demonstrated a decrease of the prevalence of *T. cruzi* infection in Northern Paraná state, these findings could still be considered alarming because these persons were thought to be healthy and potential blood donors. These data support the continuous need for a rigorous serological screening of blood donors, using much more sensitive and accurate techniques for the prevention of CD transmission by blood transfusion.

When positive, contradictory or dubious results are obtained in the serum samples tested at the hemotherapy service of HURNP, the blood is rejected, the blood donor is considered "unfit" and a clinical and laboratory investigation is carried out by a multidisciplinary professional team of the Universidade Estadual de Londrina. The blood donor should be regularly submitted to follow-up examinations with repeated serological reactions for CD and confirmatory tests for CD using commercially available or homemade Western Blots.

The need for better laboratory techniques justifies the continuous efforts in the development of an ideal standard confirmatory test for CD.

RESUMO

Soropositividade para anticorpos anti-*Trypanosoma cruzi* em doadores de sangue do Hospital Universitário Regional do Norte do Paraná, Londrina, Brasil.

A forma de aquisição mais freqüente da Doença de Chagas em áreas endêmicas foi a transmissão clássica, através das fezes de triatomíneos contaminados. Entretanto, atualmente deve-se dar especial atenção nos centros urbanos para a transmissão por transfusão de sangue, justificando a necessidade de rigorosa triagem sorológica dos doadores. As primeiras investigações em bancos de sangue de Londrina mostraram uma soroprevalência de anticorpos anti-*T. cruzi* de aproximadamente 7,0% nos anos 50[9, 34]. Estudos posteriores praticamente registraram a manutenção desta soroprevalência até a década de 80[4, 32, 41]. Com o objetivo de obter subsídios quanto a dimensão atual da soropositividade de anticorpos anti-*T. cruzi* em nossa região, foi feito um levantamento dos resultados das reações sorológicas para doença de Chagas em 45.770 amostras de soro de doadores de sangue do Hemocentro do HURNP, realizadas no período de maio de 1990 a dezembro de 1994. As amostras foram analisadas empregando-se a técnica de hemaglutinação indireta (HAI, comercialmente obtida da EBRAM) e imunofluorescência indireta (IFI, obtidos da LIO SERUM) usando-se conjugados anti-IgG humano (fornecidos pela LABORCLIN). Destas, 643 (1,4%) amostras apresentaram resultado reagente para ambas as técnicas. Pode-se observar uma alteração significativa nos índices de positividade de anticorpos anti-*T. cruzi* em doadores de sangue de Londrina. Dados correlacionando sexo e idade dos doadores soropositivos são apresentados, bem como os possíveis fatores que contribuíram para os resultados observados.

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