

Blood transfusion and iatrogenic risks in Mexico city. Anti-*Trypanosoma cruzi* seroprevalence in 43,048 blood donors, evaluation of parasitemia, and electrocardiogram findings in seropositive

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Iatrogenous transmission of Trypanosoma cruzi by blood transfusion was suggested as a potential risk by Pellegrino (1949). Seropositive blood donors in Mexico were first reported in 1978, however, limited information is available due to small sampling, the use of heterogeneous serologic assays, and geographically limited studies. A wide survey carried out in 18 out of the 32 states of Mexico, showed a national mean of 1.6% seropositive among 64,969 donors, ranging from 0.2 to 2.8%.

In the present study, we have screened 43,048 voluntary blood donors in a period of five years at the Instituto Nacional de Cardiología I. Chávez, a concentration hospital located in Mexico city which serves mainly the metropolitan area and accepts from all over the country. Standardized ELISA and IIF were used to identify seropositive individuals in addition to hemoculture, PCR and standard 12 lead ECG tests that were applied to a group of seropositive patients (29/161). The result showed a seropositivity of 0.37% (161/43,048). From the group of seropositive individuals 40% (12/29) were potential carriers of T. cruzi at the donation time and 5/29 had subclinical ECG abnormalities. Parasitological tests performed in 70 erythrocyte and platelet fractions from seropositive units (70/161) showed negative results. Our findings strongly support T. cruzi screening in the transfusion medicine practice and identify subclinical heart disease among seropositive blood donors.

Key words: *Trypanosoma cruzi* - Chagas disease - blood bank - seroprevalence - Mexico

Blood transfusion is the second most important mode of transmission of Chagas disease in Latinoamerica (Pinto & Brener 1984, Schmunis 1991). Nearly 800 cases of blood borne infection with *Trypanosoma cruzi* caused by iatrogenic mechanism related to transfusion medicine practice had been collected in South America and the risk is covered by normative regulations in many countries including Mexico. In addition, iatrogenic infection surveillance was included by the international initiatives to control Chagas disease in the Americas.

Almost thirty years after the first report predicting transfusion linked to *T. cruzi* infection (Pellegrino 1949), seropositive blood donors were recognized in Oaxaca, Mexico (Goldsmith & Kagan 1978), and several studies since then have shown a seropositive incidence varying between 0.2 to 17% in different regions of the country (Monteón et al. 1987, Ramos-Echevarria et al. 1993, Rodríguez-Felix et al. 1995, Trujillo-Contreras et al. 1995, Rangel et al. 1998, Guzmán-Bracho et al. 1998, Monteón-

Padilla et al 1999, Sánchez-Guillen et al. 2002). A wide survey was carried out in the national blood bank network, which included 64,969 samples collected in 18 Mexican states showed a seroprevalence varying between 0.2 and 2.8%, with a general mean of 1.6% (Guzman-Bracho et al. 1998).

In Mexico, Chagas serological screening in blood bank units is not routinely done in all the endemic areas (Schmunis 1999), and the potential transfusion transmission of parasites together with the clinical status of seropositive subjects have been poorly studied (Monteón-Padilla et al. 1999).

We screened, in a period of five years (1999-2003), all voluntary blood donors attended in the National Institute of Cardiology I. Chávez in Mexico city. Additionally, seropositive individuals were investigated in order to identify circulating parasite and/or parasite DNA, ECG was also performed to identify subclinical abnormalities in these presumptively infected individuals.

MATERIALS AND METHODS

Studied population - In a period of five years (1999-2003) we invited consecutive blood donors attending the blood bank service at the Instituto Nacional Cardiología "I. Chávez" in Mexico city, to participate in a survey which included an epidemiological questionnaire and serological screening for anti-*T. cruzi* antibodies.

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Routinely, donated blood is leucoreduced, and separated in three fractions: plasma, erythrocytes, and platelets.

Serological tests for anti-T. cruzi antibodies - The home-made ELISA test previously validated (Monteón et al. 1995, Sánchez et al. 2001) was used as the screening assay. In brief, polystyrene plates were coated with 10 µg/ml of *T. cruzi* protein extract (INC-1 Mexican strain, isolated from chronic cardiomyopathy chagasic case) in alkaline-buffered solution. Human serum was diluted at 1:200 and anti-human IgG-peroxidase conjugate (Zymed laboratories) was used at 1:15,000. The reaction was revealed by the addition of O-phenilen-diamine and read at 490 nm.

Sera from 30 healthy cases individually tested were used to establish the cut off. The mean OD plus 5 SD classified all healthy individuals as negative.

Indirect immunofluorescence (IIF) was used as the second test. All the ELISA-*T. cruzi* positive sera were submitted to the IIF test. In brief, IIF was performed in the *T. cruzi* epimastigotes phase, fixed on a microscope slide. Human serum was diluted at 1:32 and the fluorescein conjugated goat anti-human IgG was used at 1:100 dilution. Positive and negative controls were always included, and the slide was read under epifluorescence microscope.

Blood supply from blood donor reactive only in the ELISA-*T. cruzi* screening test was discarded. A confirmative positive blood donor for *T. cruzi* infection should have both ELISA and IIF tests positive.

Parasitologic studies of T. cruzi-infected human blood - Seventy out of 161 serological *T. cruzi* positive blood supplies, in particular platelet and erythrocyte fractions were submitted to hemoculture and PCR.

A subgroup of 29 out of 161 seropositive donors that accepted to participate in a second round were resubmitted for hemoculture, PCR and ECG tests.

(i) Hemoculture: we followed the technique previously described (Luz et al. 1994) with minor modifications. From platelet and erythrocyte fractions approximately 50 ml were centrifuged at 3500 g for 15 min. Platelet sediment was resuspended in 10 ml of liver infusion tryptose (LIT) media supplemented with 10% fetal calf serum. In the case of the erythrocyte fraction, after centrifugation, approximately 10 ml of the upper layer was divided into four tubes and equal amount of LIT medium was added. The tubes were incubated at 28°C for four months. Monthly evaluation of cultures was performed by taking out an aliquot to be observed under light microscope (250×).

In the subgroup of 29 seropositive blood donors 20 ml of blood sample was obtained for hemoculture. In brief, the blood sample was centrifuged as mention above, the supernatant was discarded and the sediment washed twice with LIT medium. The sediment was divided into four tubes and resuspended with equal amount of LIT medium.

(ii) PCR for *T. cruzi* detection - From platelet and erythrocyte fractions and whole blood as second sample from seropositive subjects, 1 ml aliquot was used for DNA extraction as previously reported (Monteón-Padilla et al.

1999). Lysis buffer was added to each sample (SDS final concentration 1% and proteinase K 0.2 mg/ml). The mixture was incubated for 3 h at 42°C and extracted twice with phenol/chloroform. Subsequently, sodium acetate (final concentration 0.3M) and ethanol were added for DNA precipitation. DNA pellet was re-suspended in 20 µl of sterile water.

Amplification reaction was performed in 50 µl volume containing 1 µl of DNA sample, 300 ng of each minicircle specific primers KNS1 and KNS2 (Monteón-Padilla et al. 1999), 0.2 mM of each dNTPs, and 2.5 U of Taq polymerase (Invitrogen), Tris-HCl 10 mM, pH 8.8, KCl 50 mM, MgCl 1.5 mM. The reaction was subjected to 35 cycles: 94°C for 1 min, 56°C for 45 s, and 72°C for 1 min. The amplified product was submitted to a 2% agarose gel electrophoresis and visualized after ethidium bromide staining under UV exposure. Positive and negative controls were always included.

(iii) ECG: all blood donor seropositive volunteers that accepted to participate in this second phase of the protocol (29 out of 161 seropositive), a standard 12 leads ECG was recorded at a paper speed of 25 mm/s with a portable Fukuda Denshi Model FX-2111 electrocardiograph (Fokuda Co.). The tracing was blind analyzed independently by two cardiologists. The criteria for the ECG interpretation were those utilized by the Department of Electrocardiography and Electrophysiology of the Instituto Nacional de Cardiología. Special attention was given to the presence of arrhythmias, A-V heart block, bundle branch blocks and ventricular hypertrophy and repolarization abnormalities with changes in the ventricular gradient.

Ethical consideration - This work was done in accordance to "Reglamento de la Ley General de Salud en Materia de Investigación" which stated forearm venipuncture as a minimal risk procedure and requires oral consent when illiterate participants are invited to a clinical or epidemiological study. A written informed consent letter was used. The Ethics Committee of the Institute (Instituto Nacional Cardiología "I. Chávez") approved the study protocol.

The subgroup of 29 seropositive individuals were delivered for medical attention and follow-up study.

Statistical analysis - The descriptive section consisted only in determination of the mean, standard deviation and percentage.

RESULTS

In a period of five years (1999-2003) 43,048 blood donors were serological screened. When a positive serum was identified this was confirmed at the CNTS (Reference Center) for HIV, HVB, and HVC, since *T. cruzi* serology is not mandatory for urban areas such as Mexico city. All positive cases were re-tested and confirmed by using a second validated test in our Institution (Monteón et al. 1995, Sánchez et al. 2001). In Table I we can observe that seroprevalence for anti-*T. cruzi* antibodies, by at least two different tests, showed similar values along the different years that were analyzed with a significant increase in the year of 2003 with a 0.6% seroprevalence, obtaining

a 0.37% of seroprevalence in the total population. Similar results were observed for HVC showing seroprevalence between 0.16% to 0.44% for the period analyzed and 0.28% in the total population. In the cases of HIV and HVB a higher seroprevalence was detected in 2002 and 2000 respectively, with a global seroprevalence of 0.15% for HIV and 0.07% for HVB.

Out of the 161 *T. cruzi* positive confirmed cases, only in a group of 29 of them were able to carry out ECG exam, hemoculture and PCR. We found in this group a median age of 32 ± 10 years old with predominance of male over female (72.4% male) (Table II).

Those 29 individuals came from 12 different states of the 32 states of Mexico, and the geographic localization is pointed out in Fig. 1 and Table II. From the central part of Mexico there were 20 donors out of 29 (68.9%), from costal Gulf of Mexico 2 (6.9%) and from costal Pacific Ocean 7 (24.1%).

Fifty nine percent (25,398/43,048) of blood donors screened were born and raised in the urban area of Mexico city, and 37% (15,928/43,048) in the Central part of Mexico and only 2.6% (1,119/43,048) in the costal Pacific Ocean and 1.4% (603/43,048) in the costal Gulf of Mexico. This data mean that 96% of blood donors seen in our Institu-

TABLE I
Seroprevalence in blood donors at the Instituto Nacional Cardiología "I. Chávez" in a period of five years (1999-2003)

Year	<i>T. cruzi</i> (%)	HVC (%)	HIV (%)	HVB (%)
1999 n = 7673	25 ^a (0.32)	22 (0.28)	15 (0.19)	4 (0.05)
2000 n = 8756	28 (0.31)	24 (0.27)	10 (0.11)	9 (0.1)
2001 n = 8932	20 (0.22)	22 (0.24)	6 (0.06)	5 (0.05)
2002 n = 8230	30 (0.36)	37 (0.44)	31 (0.37)	7 (0.08)
2003 n = 9457	58 (0.6)	16 (0.16)	4 (0.04)	9 (0.09)
Total 43,048	161 (0.37)	121 (0.28)	66 (0.15)	34 (0.07)

a: number of positive blood donors confirmed by at least two different tests. In the case of *Trypanosoma cruzi* by ELISA and IIF, for HIV, HVB and HVC by ELISA and Western blot and RIBA in exceptional cases (genome amplification).

TABLE II
Demographic, electrocardiographic and parasitological findings in *Trypanosoma cruzi* seropositive blood donors

Case	Age/Sex	Place of birth	Place of residence	ECG	PCR	Hemoculture
1	20/M	Puebla	Puebla	Normal	Positive	Negative
2	26/F	DF	DF	Normal	Positive	Negative
3	22/M	Hidalgo	Hidalgo	LBBB	Negative	Negative
4	47/M	Veracruz	Veracruz	Normal	Positive	Negative
5	44/M	Hidalgo	Hidalgo	Normal	Negative	Negative
6	26/F	Mexico State	Mexico State	Normal	Negative	Negative
7	27/F	DF	DF	Normal	Positive	Negative
8	19/M	Hidalgo	DF	RBBB	Negative	Negative
9	43/M	Puebla	Puebla	Normal	Negative	Negative
10	25/F	DF	DF	Normal	Positive	Negative
11	54/M	Oaxaca	Oaxaca	Normal	Negative	Negative
12	21/M	Mexico State	Mexico State	Normal	Positive	Negative
13	43/F	Oaxaca	DF	Normal	Negative	Negative
14	25/M	DF	DF	Normal	Negative	Negative
15	28/M	Guanajuato	Guanajuato	Normal	Positive	Positive
16	36/M	Guanajuato	Guanajuato	Normal	Positive	Negative
17	22/F	Michoacan	Michoacan	VE	Positive	Negative
18	43/M	Puebla	DF	Normal	Negative	Negative
19	30/M	Hidalgo	Mexico State	Normal	Positive	Positive
20	42/M	Oaxaca	Veracruz	Normal	Negative	Negative
21	40/M	Sinaloa	Sinaloa	Normal	Positive	Negative
22	35/M	Oaxaca	Oaxaca	Normal	Negative	Negative
23	20/F	Guerrero	DF	Short P-R	Negative	Negative
24	59/M	Hidalgo	DF	Normal	Negative	Negative
25	34/M	Morelos	Morelos	RBBB, VE	Positive	Positive
26	26/F	DF	DF	Normal	Negative	Negative
27	39/M	Veracruz	Veracruz	AVB	Negative	Negative
28	35/M	Tlaxcala	DF	Normal	Negative	Negative
29	23/M	Guanajuato	Guanajuato	Normal	Negative	Negative
n = 29	Mean = 32 ± 10 years old			5/29 (17%)	12/29 (41%)	3/29 (10%)

LBBB: left bundle-branch blockage; RBBB: righth bundle-branch blockage; VE: ventricular extrasystole



Fig. 1: geographic localization of seropositive blood donors. Dark zone indicate the place of birth of the seropositive subjects.

tion came from the central part of Mexico, including Mexico city (data not shown).

T. cruzi DNA was detected in 12 out of 29 subgroup of seropositive blood donors (41%) by PCR (Fig. 2, Table II) and positive hemoculture in only 3 out 29 (10%). Although, hemoculture and PCR were also carried out in 70 out of 161 red blood and platelet fractions from seropositive units, none gave positive results for *T. cruzi* parasites. This negative result may be explained by the fact that leukocyte rich fraction is eliminated throughout the fractioning process and must likely eliminates inclusive parasites.

Abnormal ECG compatible with Chagas disease was found in 5 out of 29 (17.2%), that means seropositive blood

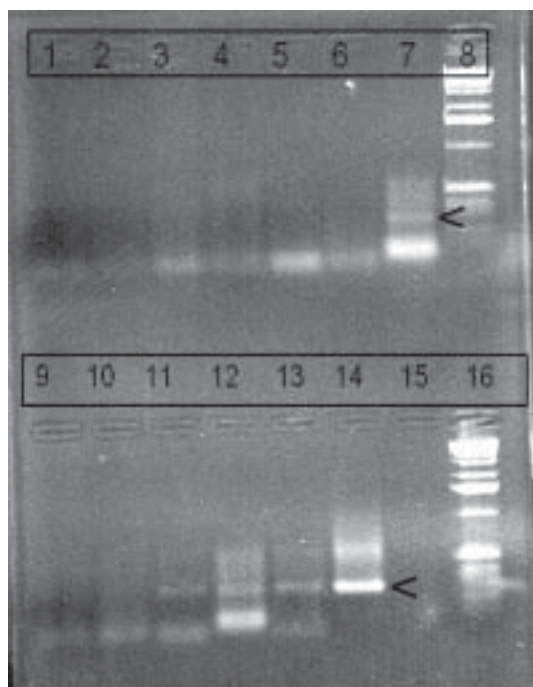


Fig. 2: representative PCR products. Lines - 1 and 15 blanks (without DNA); 2: negative control (DNA from a seronegative individual); 14: positive control (*Trypanosoma cruzi* DNA); 3 to 6 and 9-10: negative blood donors; 7, 11, 12, and 13: positive blood donors; 8 and 16: molecular markers (100 bp ladder).

donors represents subclinical chagasic subjects. Three of them came from the central part of Mexico (Hidalgo and Morelos State), one from Veracruz State (costal Golf of Mexico) and one more from Michoacan State (costal Pacific Ocean). The ECG findings were LBBB (left bundle branch blockage) in one subject, RBBB (right bundle branch blockage) in two, VE (ventricular extra systole) in two and one AVB (auricular-ventricular blockage) in one of them (Table II).

DISCUSSION

This study was carried out in an urban blood bank center located in Mexico city that is ruled out of triatomines contamination, but migrant people from endemic areas may disseminate this antropozoonosis, mainly related to blood transfusions.

Mexico city is an ideal model for this condition mentioned above due to the influx of permanent migrants who have steadily increased the city's population from the late 1930s to present.

In the present work we screened 43,048 blood donors, being this, the first study in Mexico with such large sampling in a single blood bank unit. This size sample provided us confidence on the obtained results. The seroprevalence observed for *T. cruzi* was 0.37%, being higher than the values obtained for HIV and HVB, and only comparable to HVC (0.28%) on which routine serological testing was performed (Table I).

Although, minor differences in seroprevalence for each period analyzed was observed that may indicate that similar unchanged risk factors predominated in the period studied. A marked increase was observed for Chagas disease in 2003 (0.6%), as well as observed for HVC and HIV in 2002 (0.44 and 0.37% respectively), this finding may be linked to increasing poverty in the country side that obligate the people to move from endemic chagasic regions to a large cities as Mexico city, having as a consequence crowd and high risk for viral transmission infections.

The seroprevalence of 0.37% was similar to that previously reported seven years ago for the same blood bank center in a smaller sampling (Monteón-Padilla et al. 1999) and almost half of that reported in a sentinel survey conducted in 1994 which reported 1% seroprevalence in Mexico city in 2300 blood donors (Guzmán-Bracho et al. 1998). This discrepancy may be attributed to methodological procedures, namely the two subjective tests employed in that work: Indirect hemagglutination (IHA) and (IIF). Since, in a previous study carried out by these two laboratories they observed a *Kappa* index close to 0.8 (Monteón et al. 1995).

In this context we can suggest that seroprevalence for *T. cruzi* in Mexico city can be established between 0.3 up to 1%.

For the blood donors screened at the Instituto Nacional Cardiología I. Chávez, 59% were born and raised in Mexico city, and the remaining 41% in the country side. We identified, in a group of 29 seropositive *T. cruzi* blood donors (Table II) that 96% of them came from the central part of Mexico bordering Mexico city, a distance that was no more than 500 km away or 8 h travel. In this group 15

out of 29 seropositive (51%) were born in this central part of Mexico an area considered of low endemicity, including 5 (17%) that were born and raised in Mexico city. Only 9 out of 29 seropositive (31%) came from well known endemic areas located in the Pacific coast and Mexico Gulf.

Fortuneless we could not collect information about the total number of donors originated from each state in order to provide relative prevalences, however, for Mexico city we registered 0.1% of relative seroprevalence (27/23,398), for central part of Mexico excluding Mexico city was 0.5% (82/15,884), for Pacific coast 3% (38/1235) and for Mexico Gulf 0.55% (14/2531). This data indicate that areas considered of low prevalence for *T. cruzi* infection as the central part of Mexico including Mexico City may represent a serious potentially risk in blood banking practice. In this sense our data support previously reported findings (Goldsmith & Kagan 1978, Monteón et al. 1987, Trujillo-Contreras et al. 1993, Rodríguez-Felix et al. 1995, Rangel et al. 1998, Guzmán-Bracho et al. 1998, Sánchez-Guillen et al. 2002).

Considering all the data, these studies encourage the need to search anti-*T. cruzi* antibodies in blood donors, even in urban locations in Mexico.

Current strategies to prevent transfusion-associated Chagas disease include the identification of *T. cruzi*-infected blood donors by questionnaire, serological tests, and in areas of high endemicity, the treatment of collected blood with gentian violet (Appleman et al. 1993, Carvalho et al. 1993, Ramírez et al. 1995). Recently, white cell-reduction filters have been evaluated in artificially *T. cruzi* infected blood to remove the infectious agent showing that the efficiency depends on the concentration of parasites (Moraes-Souza et al. 1995).

Circulating parasites or parasite DNA in blood seropositive donors were achieved in 12 out of 29 seropositive subjects (41%) by PCR and in 3 out of 29 (10%) by hemoculture. These findings confirm that some seropositive subjects are potentially active transmitters of *T. cruzi* infection at the moment of blood donation. Accordingly to this data we can suggest that *T. cruzi* infection risk by seropositive blood donors should be at least between 10 up to 40%, but positive leukoreduced supplies may represent a reduced risk of transmission of *T. cruzi* parasites, as was seen in the seventy red blood and platelet fractions from seropositive units tested, since hemoculture and PCR tests gave negative results, however, we consider it is necessary to carry out experimental test in order to verify this observation.

Seroepidemiological surveys carried out in subjects living in high and low endemic regions for *T. cruzi* infection in Mexico have showed that electrocardiographic alteration is present in 18 up to 22% of seropositive subjects (Rangel-Flores et al. 2001, Sosa-Jurado et al. 2003). In this work we found that 5 out of the 29 seropositive volunteer (17%) blood donors were subclinic subjects presenting ECG alterations compatible with Chagas disease. The main ECG alterations were right bundle-branch blockage, left bundle-branch blockage and ventricular extrasystole. We excluded for this purpose one subject with short P-R.

In conclusion we could observe that 17% of anti-*T. cruzi* seropositive blood donors represents subclinic chagasic subjects with rhythm or conduction defects; furthermore at least 40% of them are potential carriers of *T. cruzi* parasites at the moment of blood donation, but use of leukoreduction process may be useful to prevent *T. cruzi* transmission and finally blood banking practice in central part of Mexico considering of low prevalence for *T. cruzi* human infection, may represent a potential risk factor for spreading of the disease.

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