ARTIGO

DETECTION OF ANTIBODIES AGAINST *Trypanosoma cruzi* IN BLOOD DONORS IN THE GENERAL HOSPITAL OF MEXICO CITY

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ABSTRACT

Chagas disease is an important problem of public health in Mexico, where recently a seroprevalence of 540.000 people infected by *T. cruzi* was estimated. Seroreactivity to *T. cruzi* in 2,210 blood donors (1,858 men and 352 woman, aged 19-60 years), during 1998-2000, from 28/31 states of Mexico and Mexico City is reported. All samples were tested by indirect hemagglutination (IHA) and enzime linked immunosorbent assay (ELISA). A questionnaire to obtain socio-demographic data, visual identification of vector (*Triatoma pallidipennis*) and a letter of informed consent were applied. From the 2,210 individuals, 151 (6,8%) (IC 95% 5.8 to 7.9), were positive in both tests. They were from 12 states and from Mexico City. One hundred and twenty nine (85.4%) were male and 22 (14.6%) female, and only 4% (6/151) identified the vector. This high seroprevalence was attributed to the source (Hospital General de Mexico) which is a national health care center that receives the population from all over the country and, also, because of the high migration of population from endemic areas toward Mexico City.

KEY WORDS: Chagas disease. *Trypanosoma cruzi.* Blood donor. Blood bank. Transfusion. Mexico.

INTRODUCTION

Carlos Chagas, in Minas Gerais, Brazil (5), discovered *Trypanosoma cruzi,* the etiologic agent of Chagas disease. Schofield (31), based on the

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serological prevalence data published by Guzmán Bracho in 1998 (9), estimated an annual incidence of 10,854 and a seroprevalence of 540,000 people infected by *T. cruzi* in Mexico.

The disease can be transmitted either by triatomine bugs, especially in rural areas, and through blood transfusions in urban areas which is considered the second mechanism of infection with *T. cruzi* (urban Chagas). Mazza, in Argentina (39), first suspected the possibility of transmission through blood transfusion. The first study in blood donors was performed in Brazil by Pellegrino (1949) using complement fixation test (19); Pedreira de Freitas (1952) reported in Brazil the first transfusionally transmitted case (38). Other countries reported data with different prevalence for blood donors: Argentina 7% in 1989 (20) and 2.66% in 2001 (2), Bolivia 50% (41), Venezuela 4% (14), Brazil 7% in 1985 and 3.2% in 1995 (17), Colombia 3.3% in endemic areas and 1.3% in non endemic-areas (8), Honduras 5.2% in 1987 and 1.2% in 1998 (21) and United States 4.9% in immigrants from Nicaragua and Salvador (13).

In Mexico, the states with high prevalence are Chiapas 5.0%, Oaxaca 4.5%, Hidalgo 3.2%, Veracruz 3.0%, Baja California 2.8%, San Luis Potosi 2.5%, Zacatecas 2.4%, Quintana Roo 2.4%, Tabasco 2.3% and Durango 2.2% (Velasco, 1992) (37). Guzmán-Bracho (2001) reported more than 400 confirmed acute cases from 23 states located at the south of the Tropic of Cancer, with a seroprevalence in the total population of 1.6% in urban areas and 1.7% in rural areas, with 1.5% prevalence among blood donors (10). Salazar-Schettino in 1989 (25) reported the first case of transmission through blood transfusion. On the literature, the prevalence of 0.28 to 17% is reported, which may be due to the use of different serological techniques and to the origin and number of blood donors studied (Table 1) (7, 9, 15, 16, 22, 23, 24, 29, 36). Evidences for the risk of transmission in blood banks was poor until ten years ago, when some seroprevalence studies in blood donors showed the importance of this route of transmission, with consequent official approval in 2000 of the *Norma Oficial Mexicana* for use of human blood for therapeutics purposes (10).

Table 1. Prevalence of infected blood donors in México (1978-2002)

City	Author	Year	Tests[a]	Donors	%
Oaxaca	Goldsmith	1978	CF, IHA, DA	298	4.40
Mexico City	Monteón	1987	IIF, CIE	265	1.10
Mexico City	Ramos	1993	ELISA, dot-ELISA, Wb	1,076	0.28
Jalisco	Trujillo	1993	IHA	3,419	1.28
Yucatán	Rodríguez	1995	IIF	215	5.60
Cuernavaca	Rangel	1998	ELISA	318	17.00
Mexico City	Guzmán	1998	IHA, IIF	64,969	1.50
Mexico City	Monteón	1999	IIF, ELISA, Wb	3,300	0.30
Puebla	Sánchez	2002	IHA, ELISA	2140	7.70

CF = Complement Fixation, IHA = Indirect Hemagglutination, DA = Direct Agglutination, IIF = Indirect Immunofluorescence, CIE = Contraimmunoelectrophoresis, ELISA = Enzyme-linked Immunosorbent Assay, Wb = Western-blot.

The present study aimed the detection of anti-*T. cruzi* antibodies in blood donors in order to evaluate the importance of parasite transmission by this route.

MATERIALS AND METHODS

Population studied

Healthy blood donors from the blood bank of the Hospital General de Mexico, Mexico City, during 1998-2000 were studied. Inclusion criteria were: age 18-65, weight above 50 kg, clinically healthy and seronegative to the following infectious agents – malaria, HIV, hepatitis, and syphilis. They also should have no history of immunization, transplantation, menstruation, pregnancy or lactation as well as tatoos, according to the Technical Norms for Blood Banks protocol (TNBB) (18). A signed letter of informed consent was obtained from all the participants.

Interview

Interviews were conducted applying a questionnaire to collect information on age, gender and location of residence. At the same time, specimens of triatomines (*Triatoma pallidipennis*, complete biological cycle) were shown to the interviewed population for their identification. In this case, the common name by which the bug was known was recorded.

Sera

Five milliliters of peripheral blood were obtained by venous puncture using sterile non-reusable syringes; after clot formation, blood samples were centrifuged at 550 g for 5 min, and sera were separated for analysis.

Antigen

The antigens and guidelines for both tests were given by the Institute Dr. Mario Fatala Chaben (INDIECH) from Argentina. Hemagglutination and ELISA reagents were prepared from epimastigotes of *T. cruzi* (11). These reagents were broadly used previously in epidemiological surveys and blood bank studies (6, 30, 32, 33).

Indirect hemagglutination

The modified Boyden technique was employed (INDIECH 1995) (11). Briefly, 25 µl of stabilizing saline solution, 25 µl of sera and 25 µl of the antigenic

suspension were mixed in U bottom 96 polystyrene plates, with the aid of multichannel micropipettes. Plates were left to rest for 60 min until reading. Positive and negative control sera with known titers were processed in the same way as the tested sera. The cut-off titer was established by the INDIECH with previously tittered panel sera (11, 32, 33). Sera with titers equal or higher than 1:8 dilutions were considered positive.

ELISA

Voller's technique, modified by INDIECH (6, 11, 33) was used. Briefly, after resuspending the lyophilized antigen, it was adsorbed on flat bottom polystyrene plates, placing 70 µl in each well. To block unspecific sites, 100 µl of a 5% phosphate-milk buffer was added to each well for 1 h at room temperature. Test (in duplicate) and control sera were used at 1:200 dilutions with 70 µl per well, incubated for 30 min at room temperature (22-25°C); 70 µl of human anti-IgG conjugated with peroxidase (Sigma Chemical Co, St. Louis, MO, USA), at 1:2000 dilution, was added to each well for 30 min at room temperature. Reaction was revealed with 70 µl of the enzymatic substrate, and left in darkness at room temperature for 15 min when the reaction was stopped with 70 µl of 1 N sulfuric acid. Readings were made with 490 nm filters (Dynatech Laboratories Inc. MR 650). INDIECH established the cut-off titer (6, 32) at the mean value (optical density O.D., 490 nm) of the values obtained in reactive and non-reactive sera plus 3 SD (S n-1). Positive values were those with readings higher than 0.200 O.D., and a gray zone was established when values obtained were between 0.160 to 0.199. O. D.

Statistical analysis

Descriptive analysis was used to assess mean age, percentage of seroreactivity, gender, bug identification and CI 95% for the prevalence.

RESULTS

The blood donors were from 28 states and from Mexico City. The mean age was 31 ± 8, of the 2.210 serum samples analyzed. IHA showed antibodies against *T. cruzi* in 238 (11%). In ELISA, 161 (7.3%) sera were positive. However positivity with both tests was found in 151 sera (6.8%, CI 95% 5.8 to 7.9). From them, 129 belonged to male and 22 to female donors (Table 2).

The true infected blood donors, those with both positive tests (IHA and ELISA), came from 12 states and from Mexico City, distributed as follows: Mexico City 7.6% (93/1217); Mexico State, 7.5% (20/266); Guanajuato, 12.5% (7/

56); Oaxaca, 8.3% (7/84); Hidalgo, 5.6% (6/106); Puebla, 4.0% (5/125); Veracruz, 4.0% (4/98); Tlaxcala, 14.2% (3/21); Guerrero, 3.2% (2/61); Chiapas, 7.1% (1/14); Jalisco, 6.2% (1/16); Sinaloa, 12.5% (1/8) and Zacatecas, 16.6% (1/6) Figure 1.

Identification of triatomines was positive in 5.9% (131/2210). From the true seropositive individuals, only 4% (6/151) identified the triatomine bug; from true seronegatives, 6% (125/1962) identified it as well. It was possible to list 46 different common names for the bug, among them *chinche besucona* (kissing bug), *chinche trompuda* (big-snouted bug), *chinche voladora* (flying bug), *chupasangre* (blood-sucking bug) and *chinche con pistola* (bug with gun).

Table 2. Distribution of blood donors according to seroreactivity to *T. cruzi* with IHA and ELISA test, geographical origin, gender and mean age

State origin	Sample	Sex F/M	Mean age	IHA +	ELISA +	IHA/ ELISA +	IHA/ ELISA –	Discordant
Aguascalientes	5	5 / 0	38 ± 10	0	0	0	5	0
Baja California	1	1 / 0	20	0	0	0	1	0
Campeche	1	0 / 1	42	0	0	0	1	0
Coahuila	1	1 / 0	38	0	0	0	1	0
Colima	1	1 / 0	40	0	0	0	1	0
Chiapas	14	11 / 3	33 ± 11	3	1	1	11	2
Chihuahua	2	2 / 0	35 ± 1	1	0	0	1	1
Durango	1	1 / 0	27	0	0	0	1	0
Mexico City	1217	1.005/212	30 ± 8	131	96	93	1083	41
Mexico state	266	226/40	32 ± 9	28	21	20	237	9
Guanajuato	56	47/9	34 ± 8	9	7	7	47	2
Guerrero	61	46/15	31 ± 8	9	2	2	52	7
Hidalgo	106	93/13	33 ± 8	10	9	6	93	7
Jalisco	16	13/3	36 ±10	3	1	1	13	2
Michoacán	65	51/14	35 ± 9	2	1	0	62	3
Morelos	21	19/2	32 ± 9	0	1	0	20	1
Nayarit	2	2/0	30 ± 7	0	0	0	2	0
Nuevo Leon	4	3/1	40 ± 5	0	0	0	4	0
Oaxaca	84	79/5	35 ± 9	10	7	7	74	3
Puebla	125	111/14	32 ± 8	8	6	5	116	4
Queretaro	11	8/3	42 ± 10	1	0	0	10	1
SanLuis Potosi	9	7/2	30 ± 9	0	0	0	9	0
Sinaloa	8	7/1	33 ± 13	2	1	1	6	1
Tabasco	1	1/0	32	0	0	0	1	0
Tamaulipas	1	1/0	34	0	0	0	1	0
Tlaxcala	21	19/2	36 ±10	3	3	3	18	0
Veracruz	98	87/11	32 ± 8	15	4	4	83	11
Yucatán	6	6/0	37 ± 5	2	0	0	4	2
Zacatecas	6	5/1	36 ± 9	1	1	1	5	0
TOTAL	2210	1858/352	31 ± 8	238	160	151	1962	97

State origin: Place of origin of blood donors (29/32); sample: number of individuals and processed samples; mean age: mean age of blood donors from 29 states; IHA +: positive samples with indirect hemagglutination (> 1:8 dilution); ELISA+: positive samples with enzyme linked immunosorbent assay (> 0.200 O.D.); IHA/ELISA +: true positives using both assays; IHA/ELISA –: true negatives using both assays; discordant: different results between the serological tests analysis.

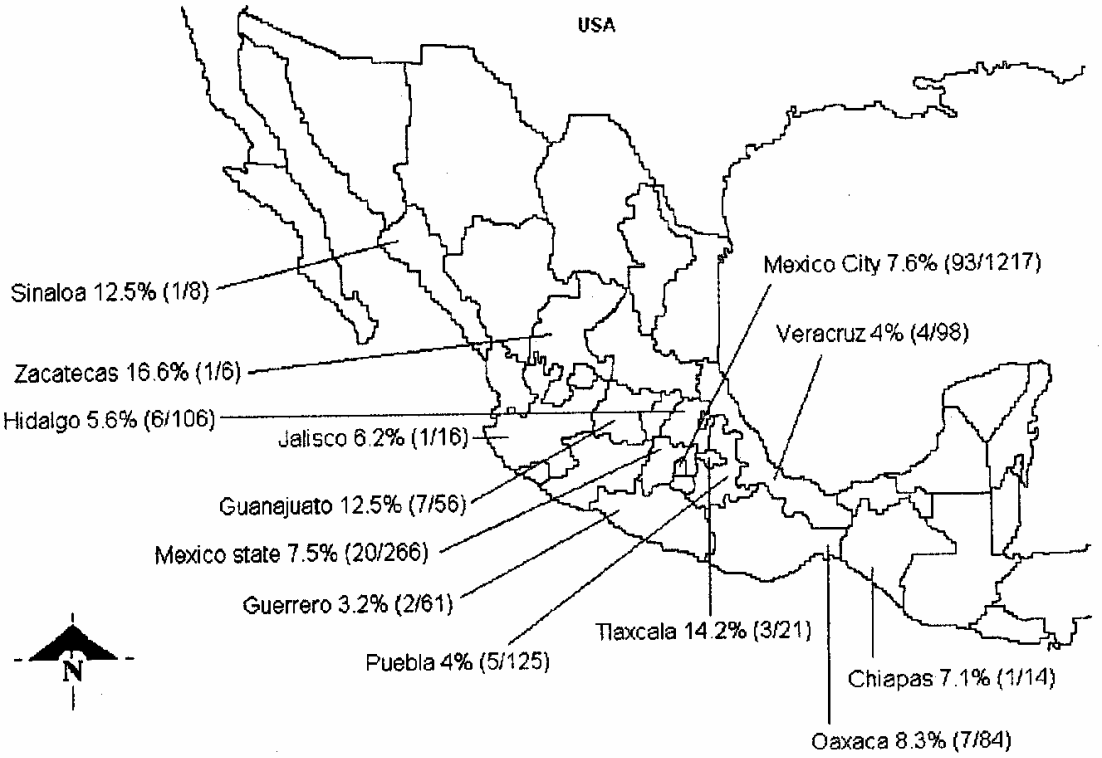

Figure 1. Map of Mexico and states with seropositives (percentage, positive blood donors/total samples)

DISCUSSION

Seroprevalence studies with *T.cruzi* infected blood donors are scarce in Mexico, where only nine studies have been published up to now (Table 1). These studies show prevalence data varying broadly, between 0.28 and 17%, a fact that could be explained because of the use of different serological techniques and the origin and number of blood donors studied. In this study, a prevalence of 6.8% with IHA and ELISA tests simultaneously performed was found. These data are in agreement with those published by Sánchez et al. (2002), which showed a prevalence of 7,7% in Puebla, even considering that in the present study the antigens employed were from a different country (Argentina), with non-autoctonous strains.

The high prevalence of *T.cruzi* infected donors in Mexico City could be explained because of the characteristics of the Hospital General de México, a national health care center that receives the population from all over the country. Mexico City gives conditions for the permanent wave of migrants, so that many infected blood donors may have been exposed to *T. cruzi* infection in their birth place, before they move to México City.

A relationship between states with seropositive blood donors and the presence of triatomines was found in Chiapas, Guanajuato, Guerrero, Hidalgo,

Jalisco, Oaxaca and Veracruz. In Guanajuato, Hidalgo, Oaxaca and Veracruz, the vector was reported positive to *Trypanosoma cruzi* (38).

The presence of *T. cruzi* has been confirmed through other studies, as seroepidemiological, seroprevalence on blood donors, presence of vectors or reservoirs, or reports of human cases (9, 7, 15, 22, 24, 23, 16, 29, 12, 34, 28, 27, 35, 26, 3, 37) in all the 12 states and Mexico City of the seropositive individuals found in this study.

A possible explanation for the low identification of triatomines by the donors could be that the only one species showed was *Triatoma pallidipennis.* It is worthwhile mentioning that seven genera and twenty four species of genus *Triatoma* have been described in Mexico (3).

The amount of components used per blood unit in Mexico, is from 2.6 to 3.5 (9) and 1.116.000 blood donations per year are recorded (40). Due to this figures, it is possible to estimate 3.48×10^5 as the number of receptors. Among them, 236.640 blood products from infected donors are included. Thus, considering the 15% transmission risk established by Cerisola (4) and the *T. cruzi* prevalence, the estimated prevented infections were 15% of 236,640, a total of 35,496 considering that each receptor received one single transfusion.

If the recommendations made by Appleman in 1993 (1) regarding the relevance of applying specific and adequately directed questionnaires to identify possibly infected donors were followed together with serological testing, the urban Chagas disease cycle could be controlled. However in this study, the data from interview and the recognition of triatomine is not related with the presence of the infection. Other social or anthropological factor could be influencing the data. The only way to identify infected blood donors in Mexico is the use of serological tests.

The relevance of this study is the identification of the high prevalence of *T. cruzi* infection, even in non endemic states. At present, health authorities in Mexico are very interested in this issue and the National Transfusion Center is in charge of a nation wide blood control against the transmission of *T. cruzi* by this route, initially through a serologial screening in all blood centers.

Until recently Chagas disease was restricted to Latin America, but with human migration towards developed countries, transfusion transmission has exceeded geographic boundaries and is becoming a worldwide risk.

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RESUMO

Detecção de anticorpos anti-*Trypanosoma cruzi* em doadores de sangue do Hospital Geral da Cidade do México

A doença de Chagas é um problema importante de saúde pública no México, onde estimativas recentes demonstraram a prevalência de 540 mil indivíduos infectados com o *Trypanosoma cruzi*. Neste trabalho determinou-se a sororreatividade ao *T. cruzi* em 2.210 doadores de sangue (1.858 homens e 352 mulheres, com idades entre 19 e 60 anos), no período 1998-2000, de 28/31 estados de México, bem como da cidade do México. Todos os soros foram testados por meio da hemaglutinação indireta e do imunoensaio enzimático (ELISA). Aplicou-se, na ocasião, um questionário para obter dados sociodemográficos. Em seguida, os indivíduos foram levados a identificar visualmente o vetor (*Triatoma pallidipennis*) e a assinar uma carta de consentimento. Dos 2.210 soros, 151 (6,8%) (IC 95% 5,8 a 7,9) foram positivos em ambos os testes. Os indivíduos eram provenientes de 12 estados e da cidade do México, sendo 85,4% homens e 14,6% mulheres. Somente 4% (6/151) identificaram o vetor. Essa elevada soroprevalência foi atribuída não só ao local onde foi realizado o estudo, Hospital General de México, um centro nacional de atenção à saúde, que assiste a população de todo o país, mas também ao elevado movimento migratório das áreas endêmicas para a cidade do México.

DESCRITORES: Doença de Chagas. *Trypanosoma cruzi*. Doadores de sangue. Banco de sangue. Transfusão de sangue.

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