TRYPANOSOMA CRUZI INFECTIONS IN BLOOD BANKS OF 12 CHILEAN HOSPITALS[1]

Myriam Lorca,[2] Antonio Atías,[3] Berbeli Astorga,[2] Patricia Muñoz,[2] and Ivette Carrere[2]

A survey of Chilean blood banks in areas endemic for Chagas' disease indicates that the risk of transmitting the disease in a blood transfusion ranges from roughly 1 to 7.5 per cent. This finding, consistent with other surveys in Chile, underlines the urgent need to devise a rational policy capable of controlling such transmissions.

Introduction

Trypanosoma cruzi, the agent of Chagas' disease, is highly persistent in blood collected and stored in blood banks *(1, 2).* Indeed, transfusions constitute the second most important avenue of Chagas' disease transmission in Chile, the most important being provided by the man-biting insect vector *Triatoma infestans.*

The specific area endemic for Chagas' disease in Chile covers roughly the northern half of the country, extending from latitude 18° South (Region I) in the extreme north, through the metropolitan areas of Santiago and Valparaiso, and down to about latitude 34° South (Region VI). The number of infected persons in this territory has been estimated at 350,000 *(3).*

An earlier article by the authors *(4)* reported the presence of blood donors serologically positive for Chagas' disease in two parts of Chile with differing prevalences of infection; and investigators in other Latin American countries—including Brazil, Venezuela, and Argentina—have reported prevalences of infection similar to or higher than those found in our country. Apart from a few isolated efforts, however, no steps have been taken to formulate a policy for Latin America that would help close this avenue of iatrogenic *T. cruzi* transmission *(5).* Therefore, in seeking to provide a more thoroughgoing base of information for coping with this problem, we felt it would be appropriate to conduct a serologic survey of *T. cruzi* infections at diverse blood banks in Chile's endemic area and to assess the risk of infection through blood transfusions in different geographic zones.

Materials and Methods

From 1978 to 1980 a total of 1,332 blood samples were collected at random from individual donors at the time they gave blood in 12 hospitals serving areas with both high and low levels of Chagas' endemicity. Men provided 1,081 of these samples and women provided 251. The donors ranged in age from 18 to 58 years, most of them (85 per cent) being between 20 and 40 years old. Seven of the blood banks were classified as "urban" and five as "rural" on the basis of their hospitals' locations and their donors' areas of residence (Table 1).

The serologic method used to examine the specimens was the indirect immunofluorescent technique described by Camargo *(6).*

---

[1]Also appearing in Spanish in the *Boletín de la Oficina Sanitaria Panamericana,* 95(4) 1983.

[2]Instructors and researchers, Parasitology Unit of the Department of Preclinical Medicine, Faculty of Medicine, Western Medical Sciences Division, University of Chile, Box 10455, Santiago, Chile.

[3]Professor and Director, Parasitology Unit, Department of Preclinical Medicine, Faculty of Medicine, Western Medical Sciences Division, University of Chile.

Table 1. Hospitals included in the survey, by urban or rural location, showing the number of samples obtained in each case.

Urban hospitals		Rural hospitals	
Locality	No. of samples	Locality	No. of samples
Areas of high endemicity			
La Serena	120	Chuquicamata	99
Coquimbo	95	Vicuña	62
		Ovalle	215
Areas of low endemicity			
Valparaiso	148		
San Antonio	77	Melipilla	104
Santiago:	314	Rancagua	98
(San Juan de Dios)	(100)		
(Salvador)	(112)		
(Universidad Católica)	(102)		
Total	754	Total	578

The specificity of this procedure has been established. Highly sensitive, it detects *T. cruzi* antibodies in subjects with early acute cases of Chagas' disease and yields a positive response with 100 per cent of the serum specimens from patients with prolonged infections (it has been demonstrated that the antibodies involved may remain at detectable levels for months and even years—*7*).

In applying this method, the serum specimens were inactivated at 56°C for 30 minutes, diluted 1:20 in PBS (0.1M, pH 7.2), and applied to an antigen consisting of fixed cultured epimastigotes. The resulting slides were incubated for 45 minutes in a humid chamber, washed twice in phosphate buffer for 10 minutes, and allowed to dry. Subsequently, a drop of human antigammaglobulin labeled with fluorescein isothiocyanate at 1:20 and prepared with Evans blue at a concentration of 1:10,000 was added to each dried slide. The slides were then incubated for 45 minutes at 37°C and were again washed twice in phosphate buffer for 10 minutes. Following this preparation, the slides were read using an incident-light fluorescent microscope, an Hb050 Hg lamp, and BG 12, KP490, and TK 510/K 515 filters.

Results

The results obtained with the tested specimens are shown in Table 2 and Figure 1. Of the 1,332 serologic samples, 80 (6 per cent) yielded positive results, the positive percentages at the various facilities ranging from 2.0 to 14.5 per cent. Averages of the findings for facilities with rural and urban donors in zones of relatively high and low endemicity are shown in Table 3. Overall, 8.3 per cent of the samples from zones deemed highly endemic yielded positive results, as did 4.2 per cent of the specimens from areas of relatively low endemicity. In areas of both high and low

Table 2. Percentages of serologic specimens yielding positive results.

Hospital locality	No. of samples tested	No. positive	% positive
Chuquicamata	99	10	10.1
La Serena	120	10	8.3
Coquimbo	95	5	5.3
Vicuña	62	9	14.5
Ovalle	215	15	6.9
Valparaiso	148	5	3.4
San Antonio	77	3	3.9
Santiago:			
San Juan de Dios	100	2	2.0
Salvador	112	4	3.6
Universidad Católica	102	4	3.9
Melipilla[a]	104	5	4.8
Rancagua	98	8	8.1
Total	1,332	80	6.0

[a]Melipilla is a rural locality in the Santiago area.

Table 3. Average prevalence of specimens yielding positive results at urban and rural localities considered to be of relatively high and low endemicity, within Chile's endemic zone.

Origin of specimens	Localities of high endemicity			Localities of low endemicity		
	No. of specimens tested	No. positive	% positive	No. of specimens tested	No. positive	% positive
Urban	215	15	7.0	539	18	3.4
Rural	376	34	9.0	202	13	6.4
Total	591	49	8.3	741	31	4.2

endemicity, the infection was found to be more prevalent among rural people than it was among urban dwellers.

Discussion

Taken together, these prevalence figures of 2 to 14.5 per cent yield an average overall prevalence for endemic parts of the country on the order of 6 per cent. As indicated in a recent review by Pinto Díaz *(8)*, these values are in agreement with those found by similar surveys in other Latin American countries.

The higher prevalence of infection in the blood banks of rural hospitals probably arose from their donors' greater contact with triatomid bugs. (Transmission is easier in rural areas, partly because it is more difficult to carry out successful triatomid control measures in rural areas.) However, residents of large cities are by no means exempt from the risk of transfusion-transmitted Chagas' disease, especially in view of the disease's increasing urbanization in Chile and other parts of Latin America as a result of the steady migration of infected individuals to the cities.

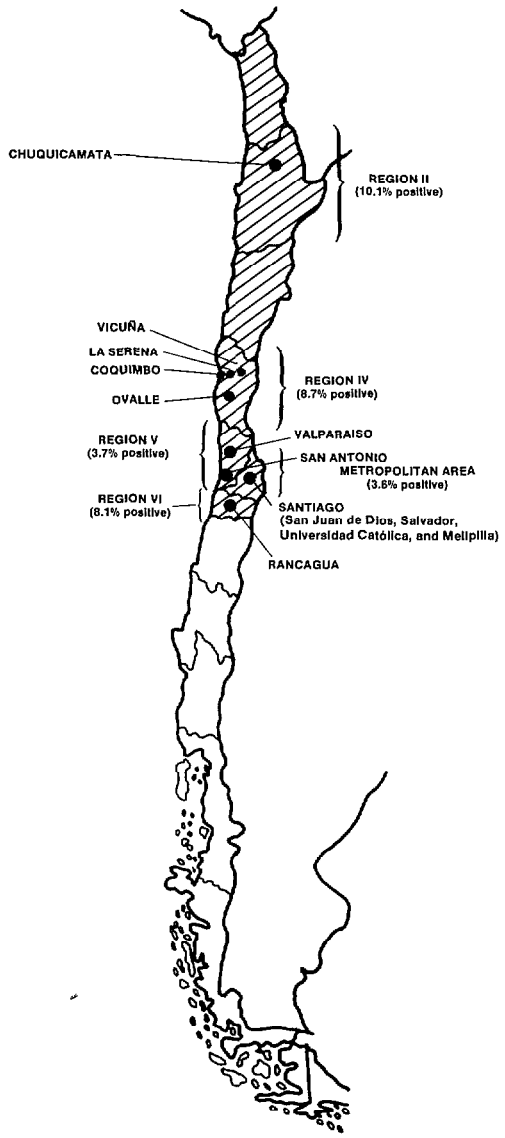
Various investigators have conducted surveys on the prevalence of Chagas' disease in Santiago. As Table 4 indicates, the prevalences found have varied considerably from one survey to another. However, the prevalences of 2, 3.6, and 3.9 per cent found by our survey of three Santiago blood banks appear similar to the results reported by Howard et al. *(9)* and Schenone et al. *(10)*, while being higher than those obtained by Apt et al. *(11)*. It is possible that the differences in these results obtained at different Santiago blood banks depended to a significant degree on variations in the proportions of donors previously residing in endemic rural areas.

Xenodiagnosis has demonstrated *T. cruzi* trypomastigotes circulating in the blood of about half the individuals infected with chronic Chagas' disease *(12)*. So it is reason-

Table 4. Comparison of the results of serologic surveys for Chagas' disease conducted by other investigators and by the authors at various blood banks in the city of Santiago.

Authors	Year of survey	Hospital surveyed	No. of samples tested	% positive
Howard et al. *(9)*	1962	Luis Calvo Mackenna	311	7.3
Schenone et al. *(10)*	1968	José J. Aguirre	305	3
Lorca et al.	1979	San Juan de Dios	100	2
Apt et al. *(11)*	1980	Barros Luco-Trudeau	325	0.3
Lorca et al.	1980	Salvador	112	3.6
Lorca et al.	1980	Clínica, Universidad Católica	102	3.9

Figure 1. A map of Chile showing the locations of the blood banks surveyed and the percentages of serologic samples testing positively for *T. cruzi* infection.



able to expect that at least half the chronic carriers of the parasite will transmit the infection when they give blood. The risk of such transmission can also be expected to increase with the number of transfusions provided by an infected donor *(13, 14)*. Overall, therefore, we should expect that roughly 2.1 per cent of the donors in the area of low endemicity and 4.2 per cent of those in the area of high endemicity would transmit the disease in Chile (Table 5).

Policies for controlling *T. cruzi* contamination of blood bank resources can focus on several approaches: (1) serologic testing of prospective donors and rejection of those found to be infected; (2) addition of a trypanosomicidal agent such as gentian violet or crystal violet (diluted 1:4,000) to the anticoagulant solution; and (3) screening donated blood and treating all seropositive donations with trypanosomicides or suitable physical processes.

The first method (testing prospective donors with highly sensitive serologic techniques and rejecting those found positive) might seem ideal. However, it would incur great expenses—for the required infrastructure, technical equipment, and specialized personnel—and would result in the loss of a considerable volume of usable blood.

The second method (adding a trypanosomicide to the anticoagulant) would permit full use of the blood and would be innocuous to blood recipients *(15-18)*. But it would impart a blue color to the blood, and could therefore provoke rejection of the blood by some recipients.

For these reasons, given the situation that prevails in our country, we feel it appropriate to propose controlling the blood after it has

Table 5. Approximate risk of *T. cruzi* infection being transmitted in the blood of a given donor to the blood banks surveyed, in areas of "high" and "low" endemicity.

	Areas of high endemicity %	Areas of low endemicity %
% of survey samples yielding positive results	8.3	4.2
Approximate risk of of infection (%)	4.2	2.1

been donated. This should be accomplished by means of a quick and simple diagnostic test that requires no extensive infrastructure and that can be performed in any laboratory. Once identified, blood donations yielding seropositive results would be given appropriate physical or chemical treatment to neutralize the infection, after which they could be used in any way desired.

Regardless of the best course to pursue, however, we believe the accumulated evidence is now sufficient to show iatrogenic transmission of *T. cruzi* through blood transfusions. There is thus an urgent need to chart a rational policy capable of controlling such transmission in our country.

SUMMARY

A total of 1,332 serologic specimens from 12 Chilean blood banks in areas endemic for Chagas' disease were tested by the indirect immunofluorescence method for evidence of *T. cruzi* infection. The endemic area involved, encompassing about half the country, includes the major cities of Valparaiso and Santiago. The results indicated that between 2 and 14.5 per cent of the donors whose blood was tested were infected with *T. cruzi,* the observed prevalences generally being higher at facilities receiving blood principally from rural donors.

Other evidence suggests that roughly 50 per cent of those people with chronic Chagas' disease have infective trypomastigotes circulating in their blood. Therefore, it seems reasonable to assume that the risk of transmitting *T. cruzi* via a blood transfusion in Chile's endemic areas ranges from roughly 1 to roughly 7 per cent. There is thus an urgent need to devise a rational policy capable of controlling such transmission in both Chile and other affected parts of the Americas.

REFERENCES

(1) Sullivan, T. D. Viability of *Trypanosoma cruzi* in citrated blood stored at room temperature. *J Parasitol* 30:200, 1944.

(2) Maekelt, G. A. Estudio sobre el tiempo de sobrevivencia del *Schizotrypanum cruzi* a temperaturas bajas. *Bol Venez Lab Clin* 3(1-4):17-28, 1958.

(3) Atías, A., and A. Neghme. *Parasitología Clínica.* Intermédica, Buenos Aires, 1979.

(4) Lorca, M., B. Astorga, A. Atías, P. Garriga, and P. Muñoz. Investigación de la enfermedad de Chagas mediante la reacción de immunofluorescencia indirecta en diversos bancos de sangre. *Rev Med Chil* 107:6, 1979.

(5) Rassi, A., and J. M. de Rezende. Prevention of transmission of *T. cruzi* by blood transfusion. In Pan American Health Organization. *New Approaches in American Trypanosomiasis Research.* PAHO Scientific Publication No. 318. Washington, D.C., 1975, pp. 273-278.

(6) Camargo, M. E. Fluorescent antibody test for the serodiagnosis of American trypanosomiasis: Technical modification employing preserved culture forms of *Trypanosoma cruzi* in a slide test. *Rev Inst Med Trop São Paulo* 8:227-234, 1966.

(7) Cerisola, J. A., M. Alvarez, and A. M. de Rissio. Inmunodiagnóstico da doença de Chagas. Evolução sorológica de pacientes com doença da Chagas. *Rev Inst Med Trop São Paulo* 12:403-411, 1970.

(8) Pinto Díaz, J. C. Mecanismos de Transmissão. In Z. Brener and Z. Andrade. Trypanosoma cruzi *e doença de Chagas.* Guanabara Koogan, Rio de Janeiro, 1979.

(9) Howard, J., M. Rubio, F. Knierim, and J. García Huidobro. Investigación de infección tripanosómica *(T. cruzi)* en dadores del banco de sangre del hospital de niños "Luis Calvo Mackenna." *Bol Chil Parasitol* 17:29, 1962.

(10) Schenone, H., P. Rubinstein, F. Knierim, J. Sandoval, and A. Rojas. Infección por *Trypanosoma cruzi* en dadores de sangre en un hospital de Santiago, Chile. *Bol Chil Parasitol* 23:83, 1968.

(11) Apt, W., C. Pérez, and J. Sandoval. Prevalencia de la infección chagásica en 4 bancos de sangre de diferentes zonas del país. *Rev Med Chil* 108:112, 1980.

(12) Schenone, H., E. Alfaro, H. Reyes, and E. Taucher. Valor del xenodiagnóstico en la infección Chagásica crónica. *Bol Chil Parasitol* 23:149, 1968.

(13) Amato Neto, V., H. E. Molinari, A. F. Siqueira, and R. S. Lucas. Análise, por melo de reação de fixação do complemento, do risco de aqui-

sição de doença de Chagas a través de hemoterapia, por parte de pacientes politransfundidos. *Rev Goiania Med* 21:1, 1975.

(14) Cerisola, J. A., A. Rabinovich, M. Alvarez, C. A. Di Corleto, and J. Pruneda. Enfermedad de Chagas y la transfusión de sangre. *Bol Of Sanit Panam* 73:203-221, 1972.

(15) Kloetzel, J. Estudo comparativo da ação tripanossomicida "in vitro" de violeta de genciana e do cristal violeta: primeiros ensaios com o emprêgo do verde de metila. *Rev Inst Med Trop São Paulo* 3:254, 1961.

(16) Nussenzweig, V., R. Sonntag, A. Biancalana, J. L. Pedreira de Fritas, V. Amato Neto, and J. Kloetzel. Ação de corantes trifenil-metánicos sobre o *Trypanosoma cruzi* "in vitro": Emprego da violeta de genciana profilaxia da transmissão de molestia de Chagas por transfussão de sangue. *Hospital (Río de Janeiro)* 44:731, 1953.

(17) Nussenzweig, V., V. Amato Neto, and O. Mellone. Novos dados sobre o emprego de violeta de genciana na profilaxia da transmissão da doença de Chagas por transfussão de sangue. *Hospital (Río de Janeiro)* 55:183, 1959.

(18) Vilaseca, G. C., J. A. Cerisola, J. A. Olarte, et al. The use of crystal violet in the prevention of the transfusional transmission of Chagas-Mazza disease (South American trypanosomiasis). *Vox Sang* 11:711, 1966.

LISTERIOSIS SURVEILLANCE

Between 1 March and 1 September 1981 the largest outbreak of listeriosis (seven adult cases and 34 perinatal cases) recorded in North America occurred in Canada's Maritime provinces. Investigation showed *Listeria monocytogenes* to be the etiologic agent. The outbreak was unique in that the source (contaminated coleslaw) was determined by detection of the epidemic strain in the implicated food. This identification led to a cabbage grower who maintained a flock of sheep, two of which were known to have died of listeriosis in 1979 and 1981. The cabbage was harvested and kept in cold storage during the winter and early sping, a practice which would favor the multiplication of *L. monocytogenes.*

The definitive report of this outbreak was published in the *New England Journal of Medicine,* Vol. 308, No. 4, 1983. In addition, a *Listeria* workshop was held in Halifax, Nova Scotia, on 1-2 June 1983, and its proceedings will be published by Canada's Ministry of Health and Welfare.

Source: World Health Organization, *Weekly Epidemiological Record* 58(37):287, 1983.