ORIGINAL ARTICLES

Seroprevalence of *Trypanosoma cruzi* in Blood Donors at the National Blood Transfusion Services – Guyana

PT Bwititi, J Browne

ABSTRACT

Introduction: *Blood transfusion is an important transmission route of* Trypanosoma cruzi (T cruzi), *a major parasitic infection in Central and South America. The limited treatment options are most effective in acute Chagas' infection. At present, there is no current data on the prevalence of* T cruzi *in the blood donor population of Guyana. This information is necessary to protect the supply of the blood donation programme.*

This study sought to determine the prevalence of T cruzi *in the blood supply at the National Blood Transfusion Services of Guyana with the hope of providing knowledge to the on-going surveillance for Chagas' disease worldwide and therefore address the risk of its spread by blood transfusion.*

Methods: *Two commercialized ELISAs utilizing crude or recombinant* T cruzi *antigens were used to study 2000 blood samples voluntarily donated for the purpose of altruistic or family replacement donation retrospectively.*

Results: *The results showed that approximately 1 in 286 donations tested positive for antibodies to T cruzi.*

Conclusion: *These results indicate that* T cruzi *continues to be a risk in Guyana and there is a need to continue screening donated blood.* Trypanosoma cruzi *is a life-long infection and infected persons may be asymptomatic chronic carriers of the disease. Education, housing improvement, and controlled use of insecticides should be introduced to contain Chagas' disease.*

Keywords: Chagas' disease, blood donor screening, enzyme-linked immunosorbent assay (ELISA), transfusion-transmission

Seroprevalencia del *Trypanosoma cruzi* en los Donantes de Sangre en el Servicio Nacional de Transfusión de Sangre en Guyana

PT Bwititi, J Browne

RESUMEN

Introducción: *La transfusión de Sangre es una vía de transmisión importante del* Trypanosoma cruzi (T cruzi), *una infección parasitaria mayor en América Central y América del Sur. Las opciones de tratamiento limitadas son más eficaces en los casos de la enfermedad de Chagas aguda. En el presente, no existen datos actualizados acerca de la prevalencia del* T cruzi *en la población de donantes de sangre en Guyana. Esta información es necesaria para proteger el suministro del programa de donación de sangre.*

Este estudio se propuso determinar la prevalencia de T cruzi *en el suministro de sangre de los Servicios Nacionales de Transfusión de Sangre en Guyana, con la esperanza de aportar conocimientos a la vigilancia que tiene lugar en relación con la enfermedad de Chagas a nivel mundial, y por consiguiente aborda el riesgo de la difusión de esta última mediante la transfusión de sangre.*

From: School of Biomedical Sciences, Faculty of Science, Charles Sturt University, Locked Bag 588, Boorooma Street, Wagga Wagga, New South Wales 2678, Australia.

Correspondence: Dr J Browne, c/o School of Biomedical Sciences, Faculty of Science, Charles Sturt University, Locked Bag 588, Boorooma Street, Wagga Wagga, New South Wales 2678, Australia. E-mail: junitabrowne@gmail.com,

Métodos: *Dos inmunoensayos ELISA con antígenos de* T cruzi *crudos o recombinantes, fueron utilizados a fin de estudiar 2000 muestras de sangre donadas voluntariamente a modo de donaciones altruistas o de reposición familiar, retrospectivamente.*
Resultados: *Los resultados mostraron que aproximadamente 1 de 286 donaciones daban positivo a anticuerpos frente al* T cruzi.
Conclusión: *Estos resultados indican que el* T cruzi *sigue siendo un riesgo en Guyana, y hay necesidad de continuar tamizando la sangre donada. El* Trypanosoma cruzi *es una infección crónica, y las personas infectadas pueden ser portadores asintomáticos crónicos de la enfermedad. Deben introducirse medidas en cuanto a educación, mejoramiento de las viviendas, y uso controlado de insecticidas, a fin de detener la enfermedad de Chagas.*

Palabras claves: Enfermedad de Chagas, tamizaje de donantes de sangre, ensayo por inmunoabsorción ligado a enzimas (ELISA), transfusión-transmisión

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INTRODUCTION

Chagas' disease (CD) also known as Trypanosomiasis is a chronic infection by the protozoan parasite *Trypanosoma cruzi* [*T cruzi*] (1, 2). This disease is insidious, potentially fatal, and occurs in Central and South America, from Mexico in the north to Argentina and Chile in the south, affecting 21 countries (3−5). Due to international travel and immigration patterns, Chagas' disease has also garnered epidemiologic concern in countries such as the United States of America, Canada, Spain and Australia (6).

Vectorial means is the most common form of transmission (1, 4, 5, 7). However, it may be congenital [pregnant woman to her baby] (5, 9), contaminated food or food borne (8). Blood transfusion (5, 10−12) and organ transplantation (5, 7, 10) are documented modes of transmission.

Transmission by blood transfusion has become a significant mode of *T cruzi* infection because of the success in reducing vector borne transmission and the migration of chronic carriers (5, 7). Human infection with the protozoan pathogen *T cruzi* signals the start of a decade-long interaction between a diverse host immune response and an intracellular pathogen capable of resisting complete immune clearance that establishes life-long blood and tissue infection (13, 14). Not only does the primary infection endangers lives, but cardiopathy, megacolon, and/or mega-oesophagus can occur (1, 2). This chronic latency of Chagas' disease, and the ability of the parasite to survive labile storage conditions (4°C, 22°C), freezing and thawing pose a threat to haemotherapy, as all blood and blood products (packed red blood cells, platelets, leukocytes, frozen fresh plasma and cryoprecipitate) with the exception of plasmatic haemoderivative are capable of transmitting the disease (4, 12). The potential risk for contaminated blood products necessitates screening all blood for this parasite, with a view to preventing blood transfusion as a route of transmission in Guyana.

The prevalence of the disease, as well as related mortality in the world is changing and this is evidenced by a decline in estimates of infected individuals from approximately 16 million in the mid-1980s to 7.6 million in 2006 (6). The number of consequential deaths also decreased from more than 40 000 in the 1980s (6) to 14 000 in 2001 (15). These estimates, while elevated, show a significant reduction in transmission which can be attributed mainly to the strengthening of vector control programmes in several endemic countries such as Argentina, Brazil, Chile and Uruguay (4, 5). However, restrictions to the control of Chagas transmission exist. The disease is primarily enzootic which prevents elimination, and limited clinical appearances of most acute cases make routine epidemiological surveillance difficult. In addition, there is no vaccine that permits the immunization of the population at risk or cheap and effective drugs that are free of side effects.

In a 2008 report, the World Health Organization estimated the economic loss due to early mortality and morbidity due to Chagas' disease to be US$ 8.156 million per year (3). The Americas cannot afford such costs in addition to the increasing expenditures on health. Therefore it is important to maintain constant surveillance for this disease.

Laboratory methods applicable to blood banks are immunological because, following the transmission of *T cruzi*, Chagas' disease progresses through the chronological production of specific antibody classes during the development of the disease.

The choice of a Chagas' disease screening assay is far from straightforward. Given the variability in sensitivity and specificity of serological assays, the Pan American Health Organization advocated the parallel use of at least two different serological assays in blood bank screening (16). Serological assays detecting antibodies to *T cruzi* are well-suited for fast and inexpensive diagnosis of the disease and it supports therapeutic decision-making and blood-bank screening. Current data are lacking on Chagas' disease within Guyana (17, 18) and epidemiological data on blood transmission of Chagas' disease in Guyana is restricted to the isolated serosurveillance study carried out about 10 years ago, which reported that none of the 500 donor samples

analysed were positive for antibodies against *T cruzi* but it was determined to be in 1.5% of Guyana's Amerindian population (17).

Guyana has seen the introduction of the National Blood Programmes, the prohibition of paid donations, the recent implementation of screening of all blood and blood components for *T cruzi* in government managed institutions. However, the outcome of these measures to improve transfusion safety should be assessed by evaluating the prevalence of *T cruzi* among Guyana's healthy blood donor population. From these data, achievement can be measured, as it offers an important index for changes in seroprevalence as control programmes are normally ongoing and highlights the support needed to maintain or expand the screening to ensure blood safety.

MATERIALS AND METHODS

Guyana is divided into ten administrative regions (Fig. 1). The National Blood Transfusion Service (NBTS), the main collection, screening and distribution facility of blood for haemotherapy in both public and private institutions is located in Region 4 – Demerara Mahaica.

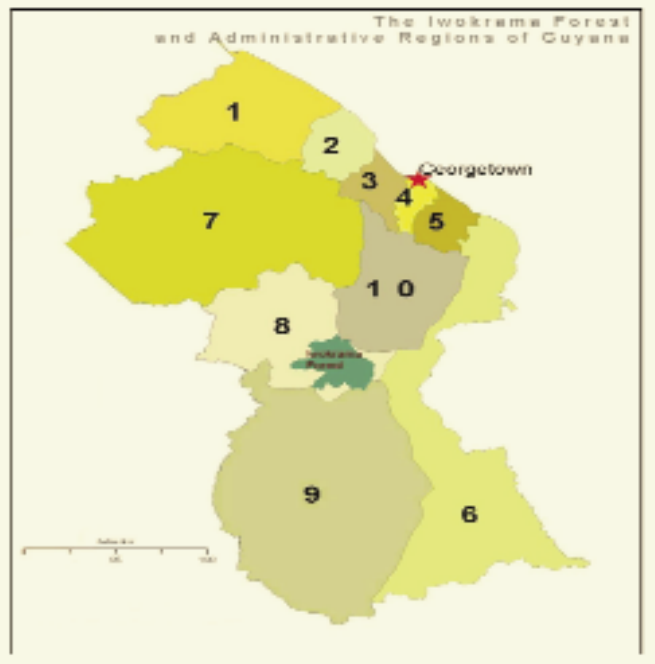

Fig. 1: Location of administrative regions of Guyana.

Region 1: Barima-Waini; Region 2: Pomeroon-Supenaam; Region 3: Essequibo Islands-West Demerara; Region 4: Demerara-Mahaica; Region 5: Mahaica-Berbice; Region 6: East Berbice Corentyne; Region 7: Cuyuni-Mazaruni; Region 8: Potaro-Siparuni; Region 9: Upper Takutu-Upper Essequibo; Region 10: Upper Demerara-Berbice

The total number of donations during the period January 2007–August 2008 was approximately 15 000 units, with altruistic donations accounting for approximately 30%. This retrospective study was undertaken in un-linked and un-identified donor samples that had been previously cleared for transfusion. Every seventh sample was selected from blood stored at -70°C and 2000 samples were tested. The sample size (n_o) was adjusted to take into consideration the population size where the sample size (n) = $n_o/1+ (n_o/N)$ where n_o was calculated as $n_o = t^2 (p (1-p)/d^2)$ (19).

Where: n_o = sample size, t^2 = confidence interval at 95% (standard value of 1.96), d^2 = margin of error, p = estimated prevalence, n = adjusted population size.

For each sample analysed, the donor's age, gender and the region from where the donor resided was ascertained.

Two commercialized enzyme-linked immunosorbent assay (ELISA)-based serological assays (Chagatek-BIOMERIEUX, Argentina SA, Bioelisa Chagas-Biokit, Spain), which detected antibodies against crude and recombinant *T cruzi* antigens, respectively, were performed according to manufacturers' instructions. Chagas' disease was diagnosed when both tests were positive. Samples with discrepant results between assays were repeated in duplicate. All positives were also repeated. Reproducibility of each assay consisted of the inclusion of characterized materials (positive and negative) within each run.

Samples were considered positive if absorbance/optical density of the assays were greater than the cut-off values for both ELISA methods and these were calculated as per manufacturer instructions. Frequencies were used to analyse the variables and their distribution within the study population. In addition to comparing the encountered prevalence with that reported in the literature, averages and proportions were calculated and the demographic findings described.

This study received the ethical approval from the Charles Strut University's Ethics in Human Research Committee and the Ministry of Health, Guyana to screen stored, un-linked and un-identified donor samples for antibodies against *T cruzi*. Blood donations are voluntary and donors were made aware through counselling and information that their blood would be screened for potential infectious diseases.

RESULTS

During the period January 2007 to August 2008, of the 2000 donor samples tested, 96.5% (1931/2000) were collected in Region 4 and the remaining 3.5% represented Regions 2, 3, 6 and 9 (Table). The mean age of all donors included in this study was 35.94 ± 10.93 (SD) with an age range of 17−75 years and a male predominance of 69.7% (Fig. 2). Ten (0.5%) samples were initially reactive for antibodies against *T cruzi*, but repeatedly positive in only seven samples, all of which were males of Region 4 between the ages 22 and 62 years. These seven males were not repeat donors during this period. This indicates that blood units that tested positive for *T cruzi* antibodies have a prevalence of 0.35% (Table). There was no significance between the age of males and females ($p < 0.0035$).

Table: Geographical location of *T cruzi* test results of blood donors in Guyana

Location	Test Results			
	Negative	Positive	Discordant	Total
Region 2	7 (0.35)	0 (0.0)	0 (0.0)	7 (0.35)
Region 3	10 (0.50)	0 (0.0)	0 (0.0)	10 (0.50)
Region 4	1921 (96.05)	7 (0.35)	3 (0.15)	1931 (96.5)
Region 6	43 (2.2)	0 (0.0)	0 (0.0)	43 (2.2)
Region 9	9 (0.45)	0 (0.0)	0 (0.0)	9 (0.45)
Total	**1990 (99.5)**	**7 (0.35)**	**3 (0.15)**	**2000 (100)**

n = 2000 samples

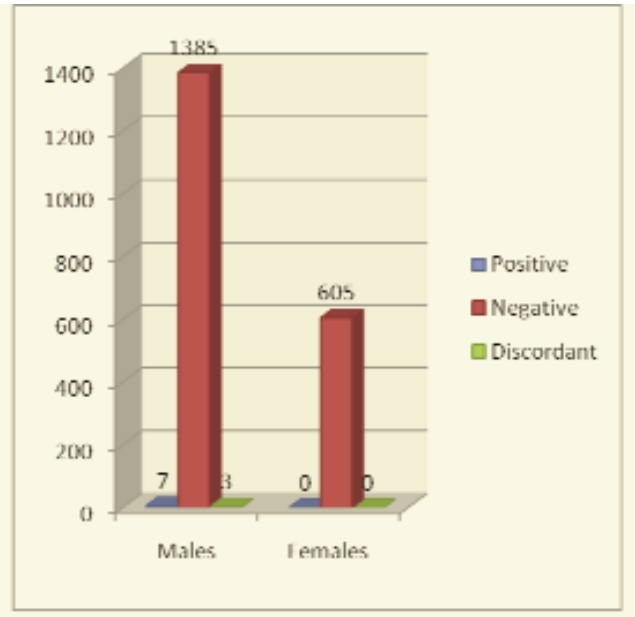

Fig. 2: Distribution of *T cruzi* study population of blood donors in Guyana by gender and test results.

DISCUSSION

The results of this study confirmed that Guyana's blood supply is tainted by *T cruzi* and perhaps there is need to revise the country's blood banking policies. Prior to this study, there could have been the belief that the detection of this parasite was very low or absent in blood donated in Guyana. These seroprevalance data are comparable to those of neighbouring countries of Venezuela (20) and Brazil (10) where the prevalence of Chagas' disease transmission by infected donors is reported to be below 1% by the systematic screening of donated blood.

Approximately 42% of Guyana's 749 190 persons reside in Region 4 (21) thereby making Region 4 the most populous region and this perhaps is responsible for the increased probability of finding infected donors in this region. One should note that the distribution of the sample population was not representative of all regions of Guyana. Therefore it cannot be concluded from this data that the probability of a donor being positive for antibodies against *T cruzi* was greater for Region 4. However, the intensification of programmes aimed at reducing the transmission of the parasite in Region 4 is recommended. Additionally, blood donor drives could be intensified throughout the rest of the country.

Enzyme-linked immunosorbent assay tests based on crude lysates seem to have better sensitivity, whereas those produced from recombinant antigens and/or synthetic peptides demonstrate better specificity, thus, blood bank authorities must give precedence to sensitivity to eliminate any asymptomatic carriers (22, 23).

Noteworthy in the assessment of serology results is that there is no licensed confirmatory test to define discrepant samples (24). Discrepant samples can be defined by supplementary testing and blood banks, after considering the important limitations for use, can opt for one of the many supplementary tests (25, 26). However, a positive result in two ELISAs with different antigenic compositions is sufficient to corroborate a result and define an infected donor (5, 27, 28). Further, donors who test positive for *T cruzi* should be rejected and notified of their rejection after re-testing of a new sample. These facts, however, do not diminish the public health relevance of the problem presented here, and the real numbers of potentially infected units/infected persons may be higher than indicated in this study. Previous studies show that laboratory testing and rejection of infected blood donors are reasonable ways to prevent transfusion-transmitted Chagas' disease (24, 26).

The screening of blood donors is a good strategy in the maintenance of the balance between eligible donors and transfusion safety.

Blood screening was instituted in all government managed blood banks from January 2009 in Guyana and such measures will no doubt reduce the risk for *T cruzi* infection. Guyana has joined some of its Latin American neighbours, Venezuela, Argentina, Uruguay and Brazil among others (20, 21), in testing its blood and blood products with a view to reducing the prevalence and therefore the incidences of transfusional–transmission of *T cruzi*. The cost of screening blood and the potential loss of units of blood are a deterrent to screening but given the chronic latency of Chagas' disease and the rising costs for medical treatment, it is our view that blood screening programmes are necessary. It is expected that through such programmes, Guyana's seroprevalence of 0.35% can also be reduced with continued systematic screening of blood for transfusion. The presence of seropositive blood donors suggests the need to undertake a pilot study to define the epidemiology of this disease in the various other regions of Guyana and to extend this study to include pregnant women with a view to gain insight into the transplacental route of transmission of *T cruzi*.

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