doi: 10.5216/rpt.v46i1.46298

SHORT REPORT

EVALUATION OF CROSS REACTIVITY BETWEEN *Trypanosoma cruzi* AND *Leishmania infantum* IN SEROLOGICALLY INELIGIBLE BLOOD DONORS DUE TO CHAGAS DISEASE

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ABSTRACT

Inconclusive serological screening for *Trypanosoma cruzi* has been a problem for blood banks. This study examined the performance of serological techniques for Chagas disease in reagent samples from blood bank screenings and verified the possibilities of cross reactivity with visceral leishmaniasis. 68 samples of reagent donors tested with ELISA for Chagas disease were evaluated by other techniques and for the detection of anti-*Leishmania* antibodies. Four donors (5.9%) with positive results for *T. cruzi* were positive for ELISA Kalazar Detect (visceral leishmaniasis),three of which were confirmed by Western blot. This study confirms the specificity of the tests for Chagas disease in blood banks and reinforces the urgent adoption of measures to assess the real risk of transfusion transmission of visceral leishmaniasis.

KEY WORDS: Chagas disease; blood donors; visceral leishmaniasis; cross reactivity.

According to estimates based on 2010 data, 5,742,167 people were infected with *Trypanosoma cruzi* in 21 Latin American countries, of which 62.4% (3,581,423 people) were from countries of the Southern Cone Initiative. Argentina, Brazil and Mexico were the 3 countries with the highest estimated number of infected people (1 505 235, 1 156 821 and 876 458, respectively) (WHO, 2015).

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Received for publication: 4/10/2016. Reviewed: 23/3/20176. Accepted: 23/3/2017.

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Despite the fact that the overall prevalence rate of the infection among blood donors in the country has gradually fallen from around 7.0% in the 1970s to 0.2% currently, it is estimated that approximately 7,000 Brazilian donors are seropositive for *T. cruzi*. The study by Lunardelli et al., 2007, showed there is a 12% to 48% risk of transmission via contaminated blood transfusion, and the challenge for blood banks is to identify and exclude asymptomatic and chronic carriers of the parasite (Brasil, 2011; Schmunis, 1997).

Similarly to Chagas disease, leishmaniasis is an important endemic zoonotic disease in Brazil with significant organic disorders in humans. Visceral leishmaniasis (also known as kalazar) is endemic in 62 countries, totaling 200 million people at risk of contracting the disease. *Leishmania chagasi* is the species that most often causes kalazar in the New World, particularly in South America (Brazil, 2010). It is considered a neglected endemic disease in this country, present in 23 of the 27 states. It is also susceptible to transfusion transmission in endemic regions its prevalence among blood donors ranges from 5.5% to 9.0% (Guerin et al, 2002; Singh & Sehgal, 2010; Urias et al, 2009; Luz et al., 1997).

There are endemic areas for leishmaniasis and for Chagas disease in many parts of South America, including Brazil. *Leishmania* sp and *Trypanosoma cruzi*, the causative agents of these parasitoses, respectively, belong to the Trypanosomatidae family and share many antigens that cause cross reactivity in serological diagnosis when mixtures of antigenic complexes are used.

In order to increase safety levels for the blood receptor and blood products, high sensitivity laboratory screening tests for Chagas disease are required. As for leishmaniasis, there is no legislation on screening in blood banks.

Although the tests currently available present levels of sensibility equal to or higher than 99%, we do not have, so far, a gold standard test and the challenge in our blood banks has been the specificity of the anti-*T cruzi* tests (Ferreira-Silva et al., 2010).

Various studies have shown that most of these results derive from false-positive reactions due to cross reactivity with other agents (*Leishmania* sp, *Plasmodium* sp, mycobacteria and others) (Ferreira-Silva et al., 2010; Sabino et al., 2010).

The purpose of this study, therefore, was to analyze the performance of serological techniques for Chagas disease in reagent samples from the Hemominas Foundation blood banks, as well as to identify possible cross-reactions with visceral leishmaniasis caused by *L.* (L) *chagasi*.

68 serum samples were analyzed by ELISA test (Gold ELISA Chagas REM®, Brazil) from reagent donors for Chagas disease (59 positive and 9 indeterminate) from blood centers in Belo Horizonte, Montes Claros and Uberaba. These samples were processed at the Federal University of

the Triângulo Mineiro and São Paulo Institute of Tropical Medicine serology laboratories, using other techniques for the detection of *T. cruzi*, including ELISA to detect IgG1 antibody (IgG1 ELISA), Wiener ELISA®, bioMérieux ELISA® and TESA-blot® (Umezawa et al., 1996). In the Western blotting immune-enzymatic assay, the 24 strains sensitized with T. cruzi TESA antigen proteins, were immune-electrically separated according to their molecular mass and transferred to a pre-prepared nitrocellulose membrane, then stored in the appropriate channels, adding serum diluted 1:100. After two hours of incubation on a shaker, the contents of the 120 channels were aspirated and four/five minute washes were performed with appropriate solution; anti-human IgG conjugate was added, with a further one hour incubation on the same shaker and repetition of the five minute wash sequences. The incubation was carried out on the shaker for ten minutes with a chromogenic solution (4-chloro-alpha-naphthol) diluted 1:5 by aspirating the contents of each plate well and performing two washes. Next, the nitrocellulose strips were transferred with the aid of an elastic clamp to filter paper and after complete drying in the open air, the following reactions were performed: positive reactions with presence of bands in the region of molecular mass of 120-200 kDa and negative reactions with absence of these (Umezawa et al., 1996). The samples were also tested for visceral leishmaniasis by InBios' Kalazar Detect™ ELISA test®. As for the positive samples in Kalazar Detect ELISA, the Western blotting technique was performed for *Leishmania infantum*, with a pre-stained marker whose molecular weight ranged from 10 to 250 kDa (BioRad Laboratories, USA). The serum samples that reacted against antigens from 14 to 16 kDa were considered positive, described in the study by Mary et al. (1992) as fractions of high sensitivity and specificity in the diagnosis of visceral leishmaniasis.

In the 59 seropositive samples the screening tests for Chagas disease bioMérieux ELISA, 93.2%; Wiener ELISA, 91.5%, IgG1 ELISA, 91.5% reached a positivity higher than the confirmatory TESA-blot test, 86.4%; while the TESA-blot technique had the highest reagent results rates (13.6%). Among these 59 samples, the false-positive rate was lower than that reported in the literature by Furuchó et al., 2008, which can be explained by the high specificity of the screening kit used (Silveira-Lacerda, 2004; Furuchó, 2008). As for the nine inconclusive samples, good concordance was observed among the four tests, with TESA-blot and ELISA bioMerieux presenting 44.4% seropositivity and Wiener and IgG1 presenting 55.5%. There were no inconclusive cases, since these were considered seropositive when three or more tests were reagent, while the reverse was considered non-reagent and no sample proved different. Therefore, the reactivity rate among indeterminate cases also proved lower than that reported in the literature, which may be due to the good specificity of the kit used for donor screening (Table 1).

Table 1. Performance of tests for Chagas disease diagnosis in 68 samples from reagents donors.

TEST		POS (n=59)		INC (n=09)	
		No.	%	No.	%
IgG 1-ELISA	Pos	53	89.8	05	55.5
	Neg	05	8.5	04	44.5
	Inc	01	1.7	0	0.0
ELISA-Bio	Pos	55	93.2	04	44.4
	Neg	04	6.8	05	55.6
	Inc	0	0.0	0	0.0
ELISA-W	Pos	54	91.5	05	55.6
	Neg	05	8.5	04	44.4
	Inc	0	0.0	0	0,0
TESA-blot	Pos	51	86.4	04	44.4
	Neg	08	13.6	05	55.6
	Inc	0	0,0	0	0,0

Pos: Positive; Neg: Non-reagent, Inc: Inconclusive; ELISA-Bio: ELISA-bioMériéux; ELISA-W: ELISA-Wiener; TESA-blot: Trypomastigote Excreted-Secreted Antigens.

Among the 9 donor samples with inconclusive serology in this study, 4 samples (44.4%) were positive by TESA-blot. A study carried out by Silveira-Lacerda et al (2004) showed that TESA-blot detected non-reagent responses in 97% (338/348) of the serum samples previously diagnosed as inconclusive. When studying 60 patients during the chronic phase of Chagas disease and 73 donors with conventional inconclusive serology, Furuchó et al (2008) found that only 20.5% of the inconclusive samples reacted positively to TESA-blot, less than the results in this study totaling 44.4%. Furuchó et al (2008) also found that 67.1% of the inconclusive serological results selected were non-reagent with TESA-blot for Chagas disease, a slightly higher result compared to those found in this study (55.6%).

The detection of anti-*Leishmania* antibodies in the 68 reagent samples for Chagas disease was performed using the Kalazar Detect™ ELISA technique. Among these, only 4 samples were positive (5.9%) and all of them were amongst the previously positive samples for *T. cruzi* infection (Table 2). The samples from these four donors were also processed by Western blotting technique, and *Leishmania* infection was confirmed in three samples (75%). All four samples were repeatedly positive for all other high performance techniques carried out

for *T. cruzi* and presented at least three epidemiological indicators of Chagas disease (they lived in rural areas, had seen the kissing bug inside or outside their houses and had family members with Chagas disease).

Table 2. Serological results of the four tests for Chagas disease among reactive samples for visceral leishmaniasis (E- Kalazar detect).

Sample	IgG1 (DO/CO)	E-bio Merieux (DO/CO)	E-Wiener (DO/CO)	Tesa-Blot	E-Kalazar Detect (DO/CO)	W. Blotting *Leishmania*
10.011	Pos (1.47)	Pos (3.02)	Pos (3.03)	Pos	Pos (1.13)	Pos
10.023	Ind (0.93)	Pos (4.63)	Pos (4.88)	Pos	Pos (1.22)	Pos
10.032	Ind (0.89)	Pos (7.59)	Pos (2.15)	Pos	Pos (0.90)	Neg
10.059	Pos (1.20)	Pos (2.25)	Pos (1.96)	Pos	Pos (0.82)	Pos

Pos: Positive ; E: ELISA; W: Western

Many authors agree that the interpretation of diagnostic test results in endemic areas for Chagas disease and leishmaniasis, should be cautious, especially those coming from epimastigote cultures, which often present cross reactions with other species of the Trypanosomatidae family. However, as observed in this study, of the four cases in which the ELISA results for visceral leishmaniasis were positive, three confirmed the presence of visceral leishmaniasis by Western blotting. Furthermore, the conclusion was reached that, at least in these three cases, both trypanosomiases coexisted considering the positive epidemiological data and the concordance of all tests for Chagas disease.

In general, this study confirmed the good performance of the techniques and kits currently available, as well as that of the in-house IgG1 ELISA for blood donor screening and the concordance among the tests, especially bioMérieux and Wiener which presented a positivity of 93.2% and 91.5% respectively in the previously positive samples; similar data was found by Caballero et al (2007). In a study carried out by Silveira-Lacerda et al (2004), the results of three serological tests were compared using TESA-blot to analyze the occurrence of cross reactivity between *Trypanosoma cruzi* and *Leishmania* sp. They noted that the TESA-blot was 100% sensitive and specific, and the test was non-reagent for 80% of the patients in the indeterminate group. Moreover, the absence of seropositivity for leishmaniasis among nine tested inconclusive for *T. cruzi*, demonstrates that the tests currently used in the serological screening of donors for Chagas disease, at least in the laboratory staff screening and the

four in this study, showed no cross-reactivity with leishmaniasis. Additionally, the occurrence of four positive results for visceral leishmaniasis, three of which concordant in the Western blotting technique, indicates that the donors had both trypanosomiases.

These results endorse the requirements of the Brazilian legislation for the use of high sensitivity methodologies in the serological screening for Chagas disease in donors (ELISA). These techniques differ in sensitivity and specificity according to the antigenic fraction used. The use of parasite lysate makes these techniques more sensitive and the use of recombinant protein leaves them more specific, mainly for leishmania. However, the high prevalence of donors positive for leishmaniasis, as demonstrated in several studies, requires the urgent adoption of measures to assess the real risk of transfusion transmission, as well as alternatives for its effective control.

ACKNOWLEDGMENTS

We offer our deepest thanks to the Institution FAPEMIG (BIP 00135/13 e 00188/14). Financial support: Agência Nacional de Vigilância Sanitária (ANVISA). Coordenação de Aperfeiçoamento De Pessoal de Ensino Superior (CAPES).

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