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Evaluation of the performance of two Chagas disease antibody tests in patients from the Chaco region (Argentina)

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Abstract

Chagas disease (CD) is caused by the protozoan *Trypanosoma cruzi* and is a significant zoonotic disease affecting millions worldwide. The disease progresses from an often asymptomatic acute phase to a chronic phase, which can lead to severe cardiac or digestive issues in about 30% of untreated individuals after 20 to 30 years. Early diagnosis and treatment are crucial to managing the disease and preventing further transmission. Diagnosis typically involves serological tests to detect anti-*T. cruzi* antibodies, with the Indirect Hemagglutination Assay (IHA) and ELISA being the most common methods. Given the antigenic diversity of the parasite, the Pan American Health Organization (PAHO) recommends the use of two different tests for a reliable diagnosis. The objective of the present study was to evaluate the performance of two commercial diagnostic assays for chronic CD in the Chaco Region. Blood samples were collected from 388 patients from the Chaco Region between November 2019 and November 2023, and the results showed a high degree of agreement between the IHA and Lysate ELISA commercial tests. However, Lysate ELISA was found to produce more false negatives compared to the PAHO diagnostic algorithm, which could leave patients untreated and contribute to ongoing transmission. The study demonstrated very good performance of the commercial tests evaluated. However, the presence of some false results underscores the importance of continuing to use the PAHO algorithm based on two serological tests for reliable diagnosis in the region.

Introduction

Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*), is one of the leading zoonotic diseases worldwide (1). It can be transmitted by the triatomine bug (vector-borne), *Pastrongilus*, *Rhodnius*, or

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Triatoma, as well as orally (food-borne), from mother to child (congenital), through blood/blood products, or organ transplantation. The World Health Organization estimates that more than 7 million people are currently infected with *T. cruzi* worldwide, including CD, with the neglected tropical diseases. In the Americas, the disease is endemic in 21 countries, with approximately 70 million people at risk of the infection. At least 30,000 new cases and approximately 12,000 deaths occur each year (2). The Chaco Region is particularly affected due to its social and ecological context, which hampers effective control efforts, especially in rural and remote areas (3, 4). CD progresses from an initial acute phase, often asymptomatic or oligosymptomatic, to chronic Chagas disease (CCD) if left untreated. After 20 to 30 years of asymptomatic progression, one-third of affected individuals will develop significant cardiac or digestive problems, which can lead to death. Therefore, key strategies to prevent CCD are timely diagnosis and treatment (5).

In the acute phase, parasitemia can be detected by direct parasitological examinations or by molecular tests. However, in the absence of clinical suspicion at this stage, most diagnoses are made during the chronic phase (5). In CCD, parasitemia drops to undetectable levels, with generally negative results in parasitological, cultural, antigenic, and even molecular tests. In this chronic phase, serological tests must be used for diagnosis (5). For the diagnosis of CCD, the most frequently used tests include indirect hemagglutination (IHA) and enzyme-linked immunosorbent assay (ELISA), the latter varying according to the type of antigens: total lysate antigens or recombinant antigens. There are seven *T. cruzi* variants, called Discrete Typing Units (DTUs), that generate different antigenic responses. The distribution of these DTUs varies by geographic region. Therefore, the accuracy and precision of the serological diagnostic tests vary depending on the antigens used in each assay and the geographic origin of the infection (6, 7). Since no test achieves 100% sensitivity and specificity, and no test is considered the gold standard, the PAHO recommends the use of at least two different tests for a reliable diagnosis (8). The present study provides an updated assessment of two commercial diagnostic tests for CCD conducted among patients in the Chaco Region of Argentina. The objective of the present study was to evaluate the performance of two commercial diagnostic assays for chronic CD in the Chaco Region, and to analyze their use within the diagnostic algorithm.

Material and methods

Study Area

Data were collected over ten three-day campaigns in Huanqueros (30°00'49.8"S 61°13'12.8" W) and Fortín Olmos (29°03'00.7"S 60°25'13.3"W), between November 2019 and November 2023. Huanqueros and Fortín Olmos are two semi-rural localities located in the province of Santa Fe (Argentina), in the southern Chaco Region (Figure 1). The environmental conditions in the region constitute the natural habitat of triatomine insects. The precarious housing conditions in the area, and the adaptation of *Triatoma infestans* to human habitation, increase the risk of *T. cruzi* infection.

Participants

A non-probability convenience sampling method was used, including individuals aged over one year. Patients who had previously received treatment with anti-*T. cruzi* drugs were excluded from the study, as the serological test results in these individuals can be unreliable. Clinical evaluation of the patients was not part of the present study.

Sample collection

Approximately 5 mL of blood samples were collected through venipuncture with sterile equipment and placed in sterile tubes containing a coagulation activator and separator gel. After allowing the samples to clot for 15 minutes at room temperature, they were centrifuged on-site at 3500 rpm for 10 minutes with a Rolco centrifuge

(Buenos Aires, Argentina). The samples were transported to the laboratory under refrigerated conditions at 4–8 °C and processed within a week.

Laboratory analysis

Each sample underwent analysis using the Indirect Hemagglutination Assay (IHA, Chagatest HAI, Wiener Lab, Rosario, Argentina) and the Lysate ELISA (Chagatest Lysate ELISA, Wiener Lab, Rosario, Argentina), adhering to the manufacturer's protocols. In cases where the test results were discordant, a recombinant ELISA (Chagatest recombinant ELISA v. 3. 0, Wiener Lab, Rosario, Argentina) was conducted. Micropar Washer (Rosario, Argentina) and Mindray MR-96A reader (Shenzhen, China) were employed for processing the ELISA tests. A positive result was determined when at least two of the tests returned positive outcomes.

Statistical methods

The total percentage agreement, positive percent agreement, and Kappa index were calculated to assess the agreement between the tests.

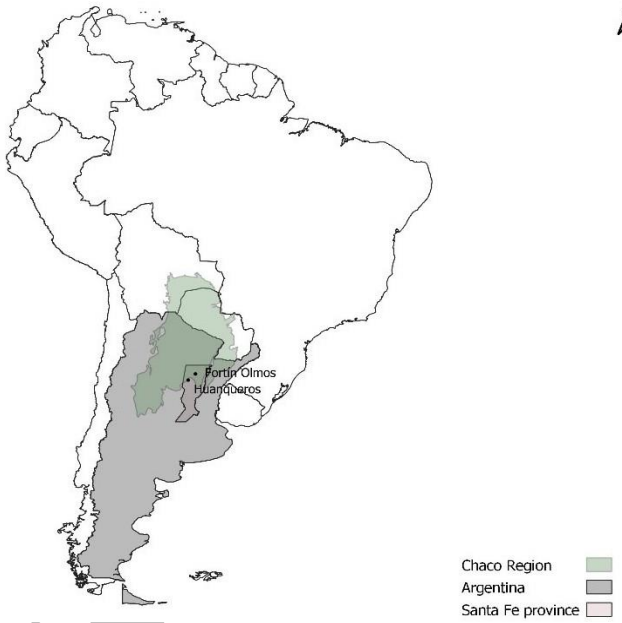

Fig. 1. Study area in Argentina. The green area shows the Chaco Region, Huanqueros, and Fortin Olmos are areas where samples were collected.

Results

A total of 283 blood samples were collected in Huanqueros and 105 in Fortín Olmos, encompassing 388 patients aged between 1 and 83 years (62% female and 38% male). Out of the total, 24 of the 283 samples (8.48%) were seropositive in Huanqueros, while 39 of the 105 samples (37.14%) tested positive in Fortín Olmos. The results of IHA and Lysate ELISA revealed a high degree of concordance between the two assays (Table 1).

The results of the comparison between IHA and Lysate ELISA with the PAHO algorithm, are presented in Table 2.

Table 1. Concordance between Indirect Hemagglutination and lysate ELISA.

		IHA		
		Positive	Negative	Total
Lysate ELISA	Positive	58	9	67
	Negative	3	318	321
	Total	61	326	388

Global percentage of agreement= 96.90%
Positive percentage of agreement= 82.86%
Kappa index= 0.889

Table 2. IHA and ELISA lysate results compared with the official PAHO.

		IHA		Lysate ELISA		
		Positive	Negative	Positive	Negative	Total
Algorithm result	Positive	61	2	61	6	67
	Negative	1	324	2	319	321

Discussion

A high seroprevalence of CCD was found in the studied populations, although this was not the objective of the study, which highlights the importance of having adequate diagnostic tests in the region. Controlling Chagas disease in the region requires ensuring access to available diagnostics; however, it is estimated that only 10% of those infected people receive a diagnosis (9). The high degree of concordance between IHA and lysate ELISA indicates that both tests could be used interchangeably. This result does not differ from other studies, despite the different origins of the patients (10, 11). However, the absence of a gold standard for CCD diagnosis limits the analysis to concordance statistics between the assays and prevents calculation of their sensitivity and specificity.

Compared with the PAHO diagnostic algorithm, the lysate ELISA tends to generate a higher number of false negatives, which could leave many patients without the necessary care. This situation could contribute to many patients with CCD remaining undiagnosed, allowing mother-to-child transmission and clinical progression that could affect cardiac and digestive health.

Discrepancies in serological test results can be caused by various reasons: differences in the host immune response, heterogeneity in T. cruzi strains, or factors dependent on the test used. In our case, since the tests were evaluated on samples from the same patients, differences in immune response would be ruled out. Furthermore, both HAI and lysate ELISA use the same antigens (total parasite culture lysate) from the same manufacturer, i.e., the same strain. Therefore, strain variability could also be ruled out. Therefore, the higher number of false negatives observed in lysate ELISA results would be due to factors specific to the test itself (12).

Although some studies suggest relying on a single test for serological diagnosis (13, 14), this alternative should be discarded for the moment since both tests leave patients undiagnosed. For resource-limited and difficult-to-access settings, previous publications recommend combining immunochromatographic testing (rapid test) with conventional serology (15), or direct combined use of two different rapid tests (16). This could be the best alternative for the diagnosis of CCD in the affected population.

Conclusion

The PAHO diagnostic algorithm, which uses both IHA and ELISA assays, remains the most reliable option for diagnosing Chagas disease in the Chaco Region.

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Ethical approval
The study protocol was approved by the Advisory Committee on Ethics and Safety Research of the Biochemistry and Biological Sciences Faculty, National University of Littoral in Argentina (CAESI Acta 04.16, CE2018-71-C). Participants signed an informed consent form before sample collection. For those under 18 years of age, their parents signed the informed consent form. All test results were provided to each patient individually.

Conflict of interest statements
The authors declare that there is no conflict of interest.

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