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Screening for *Trypanosoma cruzi* in patients living with the human immunodeficiency virus (PWH) in the Peruvian Amazon

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ABSTRACT

Introduction: Chagas disease (CD) - *Trypanosoma cruzi* infection - in people living with HIV (PWH), particularly those with advanced CD4 T cell depletion, can lead to severe syndromes affecting the central nervous system and the heart.

Methods: We performed a cross-sectional study to screen for CD among PWH in Iquitos, Peru, between October 2023 and May 2024, with the objective of understanding the frequency of infection in this population. Adults with confirmed HIV attending outpatient services at two regional hospitals were enrolled. Two ELISA tests (lysate and recombinant) were used, and discordant results were adjudicated by indirect chemiluminescence immunoassay (CLIA).

Result: Of 534 PWH, the median age was 41 years (IQR 32–49), 66.1 % were male, the median current $CD4^+$ count was 443/μL, and 75.8 % had an undetectable viral load. Two discordant serologic results were resolved with a negative CLIA, resulting in no confirmed CD cases.

Conclusion: The prevalence of *T. cruzi* infection is likely very low in urban and peri-urban areas of Iquitos. *Continued epidemiological surveillance is essential to monitor potential changes over time.*

1. Introduction

Trypanosoma cruzi, the protozoan etiological agent of Chagas disease (CD), is responsible for the highest burden of parasitic disease in the Americas, affecting an estimated 6–8 million people [1]. It is primarily transmitted by infected hematophagous triatomine insects, although congenital exposure, blood transfusion, and ingestion of contaminated food or beverages are also possible routes of transmission [2].

An acute phase of circulating parasitemia develops 1–2 weeks after exposure to *T. cruzi* and can last up to 3 months. Most cases are asymptomatic or present as a non-specific, self-limiting febrile illness. The acute phase ends when the host immune system suppresses parasite replication, causing parasitemia to disappear, however residual parasites often persist in deep muscle and nerve tissues. So begins the chronic phase, which is lifelong in most untreated individuals. Unfortunately, 20–30 % of those with chronic CD eventually develop irreversible and potentially fatal end-organ disease, famously manifested as Chagas cardiomyopathy and/or gastrointestinal megasyndromes [1,3]. These complications usually occur after decades of infection and are often associated with significant disability, loss of productivity, and elevated healthcare costs, making CD not only a medical but also a social and economic problem [2].

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Immunocompromised individuals have a significantly higher risk of morbidity and mortality from CD. In people with chronic CD, advanced CD4 T cell depletion caused by HIV may permit *T. cruzi* reactivation, leading to high levels of circulating parasitemia and a wide range of clinical manifestations, with the most severe syndromes involving the central nervous system and heart [4,5]. The mortality rate of *T. cruzi* reactivation disease in people with HIV (PWH) is greater than 75 %. Beyond HIV, reactivation has also been described in patients undergoing chemotherapy, hematopoietic stem cell transplantation, and immunosuppressive therapies, highlighting the importance of monitoring diverse vulnerable populations [2].

PAHO as well as U.S. and Spanish guidelines recommend that all people with CD risk factors be screened particularly immunocompromised populations like PWH [6,7]. Several studies have evaluated *T. cruzi* epidemiology among PWH in non-endemic and endemic settings [8]. In the UK, Ahmed et al. [9] screened 86 PWH and found no positive cases. In Italy, Rodari et al. [10] evaluated 389 patients, reporting a prevalence of 0.5 %–1.29 % depending on the confirmatory technique. In Spain, Salvador et al. [11] identified a prevalence of 3.9 % among 141 Latin American patients, while Llenas-García et al. [12] reported 1.9 % in a cohort of 155 patients. In a U.S. study, Hayon et al. measured a CD frequency of 2/294 (0.68 %) in PWH screened for CD with serology and PCR [13]. In endemic areas, Stauffert et al. [14] screened 200 PWH in southern Brazil (Rio Grande do Sul) and found a coinfection prevalence of 5 %. In Bolivia, Reimer-McAtee et al. [15] evaluated 116 PWH, reporting a coinfection prevalence of 27.6 %. However, no studies to date have assessed the prevalence of *T. cruzi* infection among PWH in Perú.

In Perú, *T. cruzi* is endemic in several southwestern regions, with a prevalence of around 0.4 % in Arequipa, Moquegua, Tacna, Ayacucho, and Apurímac [16]. Although Loreto has traditionally been considered non-endemic, sporadic acute cases have been reported [17]. In Iquitos, isolated cases (1 of 300) were identified among pregnant women [18], but none in a general population study (n = 394) [19]. To date, no studies have assessed the risk of *T. cruzi* infection or reactivation in PWH, underscoring the need for region-specific research. The objective of study was to perform a serological screening of CD among PWH in the Peruvian Amazon.

2. Material and methods

This was a prospective, cross-sectional study of PWH receiving care between October 20, 2023, and May 20, 2024, at one of two hospitals in Iquitos, Peru: (1) the Regional Hospital of Loreto "Felipe Santiago Arriola Iglesias" and (2) Hospital de Iquitos. Adults aged ≥18 years with confirmed HIV attending outpatient care appointments at either hospital were eligible for enrollment. After providing informed consent, participants underwent an interview detailing their sociodemographic characteristics and awareness of CD. The questionnaire applied is in **annex 1**. They submitted blood samples for *T. cruzi* serological testing.

We used *T. cruzi* IgG antibody assays: Chagatest ELISA lysate (Wiener, Rosario, Argentina) and Chagatest ELISA recombinant v4.0 (Wiener, Rosario, Argentina). We performed the assays according to the manufacturer's instructions, with the positivity threshold set at 0.200 OD units above the mean of two negative controls included per plate). For discordant results, we performed a third serological assay, an indirect chemiluminescence immunoassay (CLIA) for the qualitative detection of IgG antibodies against *T. cruzi* (MAGLUMI Chagas™, Snibe Diagnostics, Peru), following the manufacturer's instructions. We defined a confirmed diagnosis of CD as positive results by two tests. We extracted $CD4^+$ T cell counts and viral loads from medical records within approximately two months of the screening. This study was approved by the Ethics Committee of the Regional Hospital of Loreto (EXP: ID-018-CIEI-2023).

3. Results

We enrolled 534 PWH. The median age was 41 years (IQR: 32–49) and 66.1 % were male, 33.1 % lived in rural areas, 47.9 % lived in houses made of wood or leaves, and 20.4 % reported a history of blood transfusion. The median nadir $CD4^+$ was 238/μL (IQR 117–375), the median $CD4^+$ was 443/μL (IQR, 281–615), and the proportion with an undetectable HIV viral load (<20 copies/ml) was 75.8 % (Table 1). All but five participants were receiving antiretroviral therapy (ART), with more than 95 % adherence in 85.6 % of them. These sociodemographic data reflect the socioeconomic vulnerability of this population, where poor housing and limited access to healthcare may further increase their risk of neglected tropical infections.

Two patients tested positive by either Chagatest ELISA lysate or Chagatest ELISA recombinant; both had discordant results. CLIA was negative for both patients (Table 2), thus we identified no cases *of T. cruzi* infection. Although prevalence was zero in this sample, the possibility of false negatives cannot be excluded, and the findings should be interpreted with caution.

Table 1
Epidemiological characteristics of patients with human immunodeficiency virus (HIV) included in the study.

	Overall (N = 534)
Sex, Male, n (%)	353 (66.1 %)
Age	
Age, median (IQR), years	41 (32–49)
Age ≥50, n (%)	67 (12.5 %)
Hospital attended, n (%)	
Regional Hospital of Loreto	416 (77.9 %)
Hospital of Iquitos	118 (22.1 %)
Residence, n (%)	
Iquitos district	171 (32.0 %)
Punchana district	134 (25.1 %)
San Juan district	109 (20.4 %)
Belen district	87 (16.3 %)
Outside of Iquitos metropolitan area	33 (6.2 %)
Occupation, n (%)	
Unemployed or student	215 (40.3 %)
Self-employment	152 (28.5 %)
Cattle, agriculture or construction	97 (18.2 %)
Intellectual work	45 (8.4 %)
Craft work	25 (4.7 %)
Education, n (%)	
None and attended primary school	113 (21.2 %)
Attended secondary school or university	421 (78.8 %)
Epidemiological risk factors, n (%) φ	
Resides in rural location	177 (33.1 %)
Lives in a house made of wood or leaves	256 (47.9 %)
Blood transfusion	109 (20.4 %)
Risk group, n (%)φ	
Heterosexual	374 (75.4 %)
Non-heterosexual	97 (19.4 %)
HIV acquisition, n (%) φ	
Sexual	463 (86.7 %)
Vertical	3 (0.6 %)
Parenteral	1 (0.2 %)
Unknown	62 (11.6 %)
Antiretroviral treatment	
Yes	529 (99 %)
Adherence[a] < 90 %	33 (7.6 %)
Adherence[a] 90–95 %	6.9 (6.9 %)
Adherence[a] >95 %	374 (85.6 %
Immunology and virology, n (%)	
Nadir $CD4^+$, median (IQR),/uL[b], median (IQR)	238 (117–375)
Current $CD4^+$, median (IQR),/uL[b], median (IQR)	443 (281–615)
Current $CD4^+$ < 200/ml[b], n (%)	52 (13.9 %)
Current undetectable HIV viral load[c], (<20 copies/mL), n (%)	330 (65.8 %)

IQR: interquartile range.
φ Epidemiological risk factors, Risk group, and HIV acquisition may include more than one response per participant.
Data availability varies across variables: [a] information available for 437 participants, [b] for 303 participants; [c] for 374 participants; [d] for 501 participants.

Table 2
Clinical and serological findings in two patients with discordant serological tests.

Patient	Age/ Sex	CD4 cell/ μL	Chagatest ELISA lysate[a] (optical density)	Chagatest ELISA recombinant v4.0[a] (optical density	Indirect chemiluminescence immunoassay[b]
1	46 year/ female	928	0.320, positive	0.08, negative	0.024, negative
2	28 year/ male	444	0.087, negative	0.447, positive	0.179, negative

[a] Results were considered positive when the optical density (OD) value exceeded that of the negative control by 0.200, in accordance with the manufacturer's instructions.
[b] Results were considered positive when the value was ≥1.0 index/ml, in accordance with the manufacturer's instructions.

4. Discussion

Our study identified no cases of *T. cruzi* infection among 534 PWH enrolled in Iquitos, Peru. The prevalence of CD among PWH in Latin American countries varies widely by region, population, and diagnostic method, but is generally reported between 1 % and 28 % in endemic areas. In Bolivia, a cross-sectional study in Cochabamba found a prevalence of 27.6 % among PWH, reflecting the hyperendemic status of Chagas disease in that country [15]. In Brazil, studies using highly specific diagnostic methods report prevalence rates of 0.8 %–2 % among urban HIV cohorts, though older literature and less specific assays have reported rates up to 5 % [20,21]. In Argentina, a retrospective review of PWH in Buenos Aires found that 80 out of 1200 HIV patients (6.7 %) were coinfected with T. cruzi, though this cohort was enriched for individuals with epidemiologic risk factors [22]. Studies of Latin American immigrants with HIV in non-endemic countries (e.g., Spain) report prevalence rates of 1.9 % overall, but much higher rates (up to 21.5 %) among Bolivian migrants [9].

The sensitivity of serological tests is lower in PWH. With very low $CD4^+$ counts, B cells may not function properly, making the humoral response unreliable. Several publications have reported cases of PWH who tested seronegative but positive by PCR [15,23]. Therefore, if PCR had been used, a positive case might have been identified. However, as the average CD4 count of our study population was high (above 200) and with good adherence of treatment, their serologic response was likely intact. Another consideration is the use of rapid diagnostic tests, which, although less sensitive, could play a role in screening at primary care level in endemic areas [24].

This study has several limitations. First, the sample size was not powered to accurately estimate the prevalence of CD in this population; when the expected prevalence is low (e.g., around 1 %), at least 1000 participants would be needed for precise estimates. Second, we did not include a comparison group of HIV-seronegative individuals from the same area, which would have provided additional epidemiological context. Third, PWH with central nervous system manifestations such as meningoencephalitis or cerebral mass lesions were not included, although this population may be at higher risk of *T. cruzi* reactivation. Fourth, parasitological methods to assess parasitemia were not performed, as the protocol focused on serological screening.

Larger studies are therefore required to determine the true prevalence of CD among PWH living in Iquitos and surrounding areas. Future research should also explore the cost-effectiveness of routine screening in HIV programs, integration with existing laboratory infrastructure, and the development of targeted clinical algorithms for immunocompromised patients.

In conclusion, the prevalence of CD is likely very low in urban and peri-urban areas of Iquitos. However, given the high mortality of *T. cruzi* reactivation disease, continued epidemiological surveillance among PWH is essential to detect potential changes over time and to prevent future severe reactivation cases in this region, rather than recommending systematic screening at entry-to-care.

CRediT authorship contribution statement

Silvia Otero-Rodriguez: Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Martin Casapia-Morales:** Writing – review & editing, Methodology, Investigation. **Lilia-Lorena Pinedo-Ramirez:** Writing – review & editing, Investigation, Data curation. **Esperanza Merino:** Writing – review & editing, Methodology. **Eva H. Clark:** Writing – review & editing, Supervision. **José-Manuel Ramos-Rincón:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jose Manuel Ramos Rincon reports article publishing charges and equipment, drugs, or supplies were provided by Miguel Hernandez University of Elche Faculty of Medicine. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tmaid.2025.102927.

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